

How Is the Monitoring of the Health Status of Laboratory Animals Conducted in Our Country and Is the Awareness of Researchers on This Issue Sufficient?*

Ülkemizde Deney Hayvanlarının Sağlık Durumu İzlemi Nasıl Yürütülüyor ve Bu Konu Hakkında Araştırmacıların Farkındalığı Yeterli mi?*

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*The data from this study were presented as an oral presentation at the 5th National Congress of Laboratory Animal Science (September 14–16, 2023 – Istanbul).

*Bu çalışmaya ait veriler 5. Ulusal Laboratuvar Hayvanları Bilimi Kongresinde (14-16 Eylül 2023 – İstanbul) sözlü sunum olarak sunulmuştur.

Geliş Tarihi/Received :14.02.2025
Kabul Tarihi/Accepted :03.07.2025
Yayın Tarihi/Publication Date :19.09.2025

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Cite this article: Sevgili, A.M., Kayabaşı, B., & Tatar, Y. (2025). How Is the Monitoring of the Health Status of Laboratory Animals Conducted in Our Country and Is the Awareness of Researchers on This Issue Sufficient?. *Journal of Laboratory Animal Science and Practices*, 5(2), 92-101.
<https://doi.org/10.62425/jlasp.1639844>



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ABSTRACT

The microbiological status of laboratory animals can substantially influence research outcomes. Although legal regulations mandate the supervision of laboratory animal health, to our knowledge, no study has been conducted to examine how laboratory animal units carry out health monitoring of the animals. Similarly, researchers' interests and attitudes regarding this issue is unknown. With our study, we aimed to fill these gaps. We used two separate questionnaires. The first aimed to gather information about health screening programs in approved laboratory animal units, while the second aimed to assess researchers' knowledge and attitudes regarding animal health monitoring programs. Out of the 120 units in our country 42 (35%) contributed to our study. Over 60% of these laboratory animal units lack microbiological monitoring practices. Out of 8 units housing specific pathogen-free animals, 7 adhere to the required microbiological monitoring program. While 85% of the units conducting microbiological monitoring perform fecal examinations, only 53% serologically analyses the blood. Overall, our researchers' knowledge levels and awareness regarding the microbiological monitoring of laboratory animals are inadequate. Meanwhile, researchers who held a certificate had slightly higher awareness compared to those who did not. In summary, it has been observed that the microbiological monitoring of laboratory animals in our country is inadequate, and researchers don't request sufficient information from the units on this issue. Training programs, including undergraduate or postgraduate courses, may prove more effective than certificate programs. Additionally, it would be beneficial to incorporate provisions in our legislation that outline the methodology for microbiological monitoring of conventional animals.

Keywords: Laboratory animals, Laboratory animal units, Microbiological monitoring.

ÖZ

Deney hayvanlarının mikrobiyolojik durumu, araştırma verilerini önemli düzeyde etkileyebilir. Ülkemizde yasal düzenlemeler deney hayvan sağlığının gözetimini zorunlu tutmakla birlikte bildiğimiz kadarı ile günümüze kadar deney hayvanı ünitelerinin hayvanların sağlık gözlemini nasıl yürüttüklerini inceleyen bir çalışma yapılmamıştır. Benzer şekilde ülkemizde araştırmacıların bu konuya olan ilgi ve tutumlarını değerlendiren bir çalışma da bulunmamaktadır. Çalışmamız ile bu boşlukları doldurmayı amaçladık. Çalışmada iki ayrı anket kullandık. İlki onaylı deney hayvan ünitelerinin sağlık tarama programları hakkında bilgi toplamayı amaçlarken, ikincisi araştırmacıların yürütülmesi gereken hayvan sağlık izlem programlarıyla ilgili bilgi düzeyi ve tutumlarının değerlendirmesini amaçlamaktaydı. Ülkemizde bilimsel amaçlar ile sık kullanılan kemirgenleri bulduran 120 üniteden 42 (%35)'si çalışmamıza katkı sağlamıştır. Bu deney hayvanları ünitelerinin en az %60'ı hiçbir şekilde mikrobiyolojik gözetim yapmamaktadır. Spesifik patojen ari (Specific Pathogen Free-SPF) hayvan bulduran 8 üniteden 7'si mevzuatın öngördüğü mikrobiyolojik gözetim programını uygulamaktadır. Mikrobiyolojik izlem yapan ünitelerin %85'i gaita incelemesi yaparken ancak %53'ü kandan serolojik analiz yapmaktadır. Genel olarak araştırmacıların deney hayvanlarının mikrobiyolojik gözetimi konusundaki bilgi düzeyleri ve farkındalıklarının yeterli olmadığı söylenebilir. Diğer taraftan sertifika sahibi olan araştırmacıların olmayanlara göre farkındalıkları biraz daha yüksekti. Sonuç olarak ülkemizde deney hayvanlarının mikrobiyolojik izleminin yeterli düzeyde olmadığı ve araştırmacıların bu konuda ünitelerden yeterli bilgi talep etmedikleri görülmüştür. Bu konularda gelişme sağlanması için sertifika programlarından öte daha geniş kapsamlı lisans ya da lisans üstü ders gibi eğitimler daha verimli olabilir. Ayrıca mevzuatımıza konvansiyonel hayvanların mikrobiyolojik izleminin nasıl yapılması gerektiğini belirleyecek maddeler eklenmesinin faydası olacaktır.

Anahtar kelimeler: Deney hayvanları, Deney hayvanları üniteleri, Mikrobiyolojik izlem.

Introduction

In any scientific research, investigators bear the responsibility to adhere to ethical principles throughout the research process and to ensure the validity and reliability of the data they present. Obtaining reliable and valid data begins with the rigorous control of all known variables to the greatest extent possible, excluding the variable under investigation. The most significant of these is the standardization of experimental animals that enables highly reproducible results (Çelik et al., 2023). Initiating scientific studies involving experimental animals with healthy specimens is a fundamental principle. Consequently, health monitoring (HM) programs for laboratory rodents must serve as a foundational step in preserving animal health and ensuring the validity of biomedical research data (Burkholder et al., 2012). On the other hand, the ethical importance of health monitoring in laboratory animals should not be overlooked. The 3R principle, which constitutes the foundation of ethical standards for the use of laboratory animals, emphasizes the implementation of alternative experimental methodologies whenever feasible (Replacement), the minimization of the number of animals used in research without compromising the scientific value of the study (Reduction), and the assurance of animal welfare before, during, and after the experimental procedures (Refinement) (Çelik et al., 2023; Tüfek & Özkan, 2018). The standardization achieved through regular health monitoring of laboratory animals contributes to reducing data variability in experimental outcomes, thereby enabling a reduction in the number of animals required for research (Öbrink et al., 2000). Furthermore, ensuring optimal health is essential for the welfare of laboratory animals and represents a fundamental aspect of animal rights (Ergün, 2011).

Publications on pathogens affecting laboratory animals began in 1947 and have expanded over the years both in number and scope. The increase in these publications first led to the establishment of the Animal Care Panel in 1950, followed by the International Council for Laboratory Animal Science (ICLAS) in 1956, the American Association for Laboratory Animal Science (AALAS) in 1967, and the Federation of European Laboratory Animal Science Associations (FELASA) in 1978. In 2011 and 2014, through the collaborations established by FELASA with ICLAS and AALAS, all aspects related to the use of laboratory animals in experiments were assessed within a scientific framework, and efforts were made to develop a common language. FELASA's most recent revision of its recommendations on the microbiological health monitoring of laboratory animals was published in 2014 (Mähler et al., 2014).

Numerous groups of microorganisms are responsible in the occurrence of infections in rodents and rabbits. Most

infections do not manifest with overt clinical symptoms. Therefore, the absence of clinical signs holds only limited diagnostic value. Numerous examples demonstrate the influence of microbes on the physiology of experimental animals, encompassing behavior, growth rate, relative organ mass, and immunological response. Effects of many organisms on research outcomes are reviewed elsewhere (Baker, 1998; Connole et al., 2000; Nicklas et al., 1999). Infections, regardless of the presence of clinical symptoms, can confound scientific outcomes, increase biological and experimental variability, and lead to a greater demand for animal use. For instance, *mouse hepatitis virus*, despite causing no clinical signs in immunocompetent animals, can alter liver enzyme levels, induce anemia or leukopenia, and even reduce the incidence of diabetes (Nicklas et al., 1999). Likewise, *Trichosomoides crassicauda*, a widely prevalent urinary tract parasite in rats, often remains undetected due to the lack of overt clinical symptoms. However, its ability to induce hematuria can compromise the reliability of findings, particularly in studies focusing on the urinary system (Sevgili et al., 2010). Moreover, certain infections in laboratory animals can also be transmitted to humans (zoonoses) (Colby & Zitzow, 2018; Gül et al., 2013; Uçak, 2024).

Therefore, regular health monitoring of laboratory animals is essential and highly valuable. Our study aims to examine the current status of laboratory animal health monitoring in our country and to evaluate researchers' approaches toward this issue.

Methods

Approval for this study was obtained from the Gazi University Clinical Research Ethics Committee (27.01.2020-117). Our study was conducted using two separate surveys. The first survey aimed to assess how units approved by the Ministry of Agriculture and Forestry in our country conduct the health monitoring of laboratory animals. The survey, consisting of 24 questions, was prepared and administered via Google Forms. In designing the survey questions, the current legal framework served as the primary reference point, and detailed questions related to microbiological monitoring were incorporated (Deneyisel ve Diğer Bilimsel Amaçlar İçin Kullanılan Hayvanların Refah ve Korunmasına Dair Yönetmelik, 2011). The survey included questions assessing the conformity of the physical infrastructure of laboratory animal units with legislative requirements, the frequency, responsible personnel, and methods of general health monitoring of animals, whether these monitoring activities were conducted within the framework of the strategic plan and surveillance program mandated by the legislation, and the frequency and methodologies employed for microbiological monitoring.

As of January 2023, a total of 192 laboratory animal

facilities had been granted operational permits, with 120 of them (%62.5) housing rodent species such as mice, rats, guinea pigs, and rabbits. Rest of the facilities (72 out of 192) are housing either aquamarine species or veterinary clinics. The survey, along with the ethics committee approval for our study, was sent to all units using the email addresses provided in the list published by the Ministry of Agriculture and Forestry. One month later, units that had not yet responded, called individually. Direct contact was successfully established with 74 of the units. During the consultations, the objectives of the study were explained, and efforts were made to encourage unit managers to participate in the survey. The survey remained accessible on Google Forms for an additional three months, after which the data were retrieved from the system for analysis.

The second survey was designed to assess researchers' level of awareness regarding the subject. Comprising 15 questions, the survey was developed and administered via Google Forms. This survey included questions evaluating the demographic characteristics of the participating researchers, their research backgrounds, their awareness of general health monitoring of laboratory animals, their experiences related to health monitoring of laboratory animals in the studies they participated in, and their awareness of the standards of the training they received. The survey was disseminated through multiple channels, a total of 99 participants completed the survey.

Statistical Analysis

For the statistical evaluation, the Jamovi software was utilized. In addition to descriptive analyses, the Mann-Whitney U test was applied for pairwise group comparisons, while the Kruskal-Wallis test was used for multiple group comparisons. Furthermore, Pearson correlation analysis was conducted to examine relationships between variables. The difference was considered statistically significant when $p < .05$.

Results

Despite extensive efforts, direct contact was successfully established only with 74 out of 120 (%58) facilities housing commonly used rodents in research. Among these, a total of 42 facilities (35%) participated in the study. The majority of these facilities (71.4%) were affiliated with public universities. Six responding facilities belonged to private institutions outside universities, representing all (100%) of the private units authorized by the Ministry of Agriculture and Forestry to house laboratory animals. When evaluated in terms of the care and housing rooms required by the regulations, the physical conditions of the 42 participating facilities were generally found to be adequate. However, it was determined that 13 facilities (31%) lacked a designated room for housing sick and injured animals, 10 facilities

(24%) did not have a post-operative care room, 9 facilities (21%) were missing an operating room, and 8 facilities (19%) lacked a preparation room for procedures.

It was determined that general health monitoring of the laboratory animals was conducted at least once per day in 39 of the 42 (92.8%) participating facilities. Among the remaining units, one facility performed general health checks every three days, while two facilities conducted these assessments on a weekly basis. In 40 (95.2%) of the facilities, these evaluations were carried out either directly by a veterinarian or under veterinary supervision, whereas in two facilities, general health monitoring was solely the responsibility of veterinary technicians.

In alignment with regulatory requirements, 95.1% (39/42) of the participating facilities reported having a strategy for regular health monitoring of animals, while 73.2% (30/42) had a defined strategy for new animal intake procedures. However, only 48.8% (20/42) of the facilities had a strategy about health disorder management program in place, and merely 31.7% (13/42) had a strategy for microbiological surveillance program.

While 2 of the 13 units (15.3%) with a “microbiological surveillance program strategic plan” did not have a “microbiological surveillance program” and did not perform microbiological surveillance, it was noteworthy that there were 5 units that had and implemented a microbiological surveillance program despite not having a strategic plan, and 1 unit that performed microbiological surveillance despite not having both a strategic plan and a surveillance program (Figure 1). The total number of units conducting microbiological surveillance amounted to 17, representing only 40.5% of the survey participants.

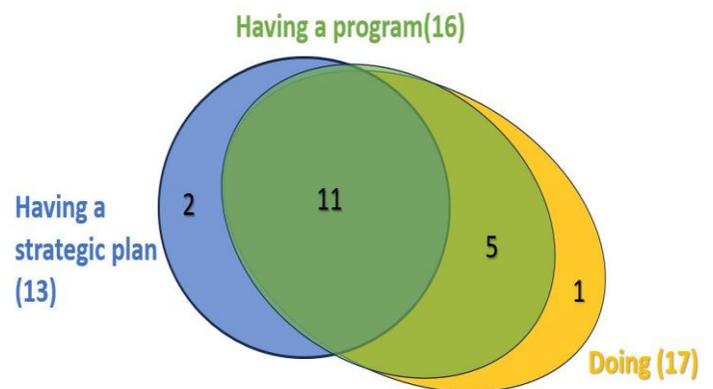


Figure 1. Microbiological surveillance status of the units.

Şekil 1. Ünitelerin mikrobiyolojik gözetim durumu.

When asked about the types of materials used for microbiological surveillance, it was observed that two units

that had previously stated they did not conduct microbiological surveillance identified material types, while one unit that had indicated performing microbiological surveillance did not specify any material type. The distribution of the 17 (40.5%) units that provided material descriptions is presented in Table 1. A particularly notable response among those provided to this question was from a unit that had previously indicated not conducting microbiological surveillance, stating: "Since we are a supplier organization, the researchers responsible for the study and the institutions providing the laboratory animals conduct the examinations."

Table 1: Distribution of Material Types Used for Microbiological Surveillance.

Tablo 1: Mikrobiyolojik gözetim amacı ile kullanılan materyal tiplerinin dağılımı.

Among the 13 (30.9) units that collected blood samples, 9 (%21.4) conducted screenings using microbiological ELISA

Blood Only	4 units
Feces Only	4 units
Feces + Saliva	1 units
Blood + Feces	3 units
Blood + Feces + Oral Mucosa + Fur Samples	1 units
Blood + Feces + Urine	2 units
Blood + Feces + Urine + Environmental Monitoring	1 units
Blood + Feces + Sentinel Animal (Blood + Feces + Fur Samples + Oral Mucosa)	1 units

or PCR, whereas the remaining four performed biochemical screenings and/or blood counts; therefore, they did not conduct a microbiological evaluation. 13 (30.9) units collecting fecal samples analyzed them for internal parasites.

While three units didn't respond to "Do you conduct microbiological monitoring during the admission process for new animals that you receive?" question, 23 out of 42 unites (55%) answered "no." Of the units that responded "yes" (38%; 16/42), 14 of them also conducted microbiological surveillance on their own animals, while two units stated that, despite the absence of routine microbiological monitoring within the facility, such monitoring was performed specifically for newly admitted animals. The final questions of our survey directed at

laboratory animal facilities were: "Does your facility house Specific Pathogen-Free (SPF) animals?" and, if so, "Are you able to properly implement the screening program specified in the regulatory guidelines for SPF animals?". Among the surveyed facilities, only 19% (8/42) reported housing SPF animals, and nearly all of these facilities (7/8) indicated that they were able to conduct the required inspections in accordance with regulatory standards.

Additionally, our study assessed researchers' awareness of the health status of laboratory animals through a second survey. A total of 99 researchers participated in the survey. Of the respondents, 70% were female, and interest from early-career researchers was notably higher (Figure 2).

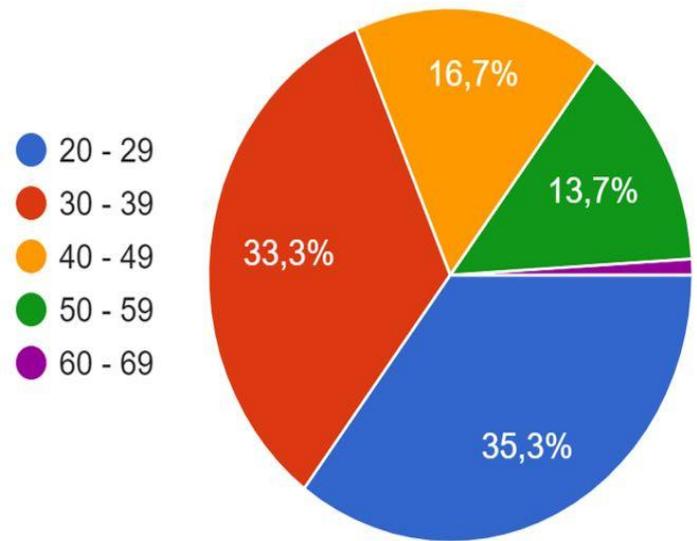


Figure 2. Age distribution of participants.

Şekil 2. Katılımcıların yaş dağılımları.

Among the participants, 86% were employed in medical faculties or research hospitals. While 68% held a laboratory animal use certificate, 8% of these individuals had never participated in any research studies. A total of 70% of all participants had been involved in studies utilizing laboratory animals, with 47% of these individuals having participated in such studies for over six years, while only 5.1% had been involved for a duration of less than six months. The responses to the question designed to assess researchers' awareness levels regarding the animals that could be used in scientific research are presented in Table 2.

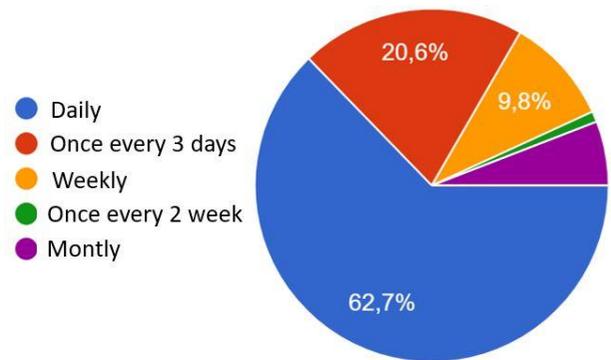
Table 2: Researchers' awareness levels regarding animals that can be used in scientific studies.**Table 2:** Arařtırmacıların bilimsel alıřmalarda kullanılabilir hayvanlarla ilgili farkındalık dzeyi.

Animal Characteristics	Usable (Number of People)	Not Usable (Number of People)
Stray animals (cats, dogs, etc.)	13	79
Owned domestic animals (cats, dogs, rabbits, etc.)	12	80
Owned farm animals (cows, sheep, chickens, etc.)	17	76
Wild-caught animals (frogs, birds, mice)	24	70
Animals obtained from experimental animal breeders	98	1
Genetically modified animals	78	15
Animals with an infectious condition	16	76
Animals that are carriers of an infectious agent	25	68
Animals infested with endoparasites	20	72
Animals infested with ectoparasites	20	73
Pathogen-free animals	80	16

* The areas highlighted with a gray background in the table indicate the usage status of animals with the specified characteristics according to our legislation.

The areas marked with a gray background in the table indicate the use of animals with the specified characteristics for experimental and other scientific purposes according to our regulations. Among the 11 characteristics defined in the question, the majority of our researchers chose the correct option according to our regulations for 5 of them, while the vast majority leaned towards the incorrect option for 6 of them. Overall, more than 70% of our participants believe that no animals, whether owned or unowned, should be used in scientific studies except for that bred by animal research units. Similarly, more than 68% of the participants believed that laboratory animals carrying pathogens could not be used in experiments. When the data were classified based on whether researchers held a laboratory animal use certificate, no significant difference was observed between the groups ($p > .05$). Likewise, prior experience in studies involving laboratory animals did not result in any significant differences between groups ($p > .05$). Analyses conducted based on age distribution also did not reveal any significant variations ($p > .05$). The only statistically significant difference we were able to identify was related to the use of owned animals, where women expressed the opinion that they were "not usable" at a higher rate than men ($p < .05$).

In response to the question "How often would you like the general health status of the animals to be checked at a minimum?" 64 participants answered "daily" (Figure 3). Forty-five of these individuals were certificate holders. Therefore, only 66% of the certified researchers deemed daily checks necessary, as required by the regulations. When the data were analyzed based on certification status, age distribution, gender, experience in laboratory animal use, and duration of laboratory animal use among experienced researchers, none of these factors created a statistically significant difference.

**Figure 3.** Researchers' expectations regarding the frequency of general health monitoring of animals.

Őekil 3. Arařtırmacıların hayvanların genel saėlık durumu izleminin sıklığı hakkında beklentisi.

In response to the question “When using laboratory animals, do you obtain information from the producer/supplier regarding the health status of the animals?”, 26 participants answered “no”. Among the respondents, two individuals answered the follow-up question, “If your answer to the previous question is YES, what type of data is provided regarding the health status of the animals?” They indicated receiving information on Age and Species/Breed. Additionally, one participant reported receiving microbiological screening data and genetic information. All 66 participants who responded “yes” to the initial question indicated that they were provided with information on Age, Sex, and Species/Breed. Among these respondents, 29 researchers reported receiving additional data on the genetic characteristics of the animals, 18 indicated that microbiological screening data were provided, and four noted that they obtained information on general health status assessments. Interestingly, 7 researchers who held a laboratory animal use certificate and had participated in studies involving animal experimentation left both questions unanswered.

To the question “Have you incidentally detected any health problems in the animals you used for experiments?”, 26 researchers answered “yes”, while 24 answered “no”, and 49 researchers left it unanswered. The conditions observed by those who responded “yes” are listed in Table 3.

Table 3: Health problems incidentally identified by researchers in experimental animals.

Table 3: Araştırmacıların deney hayvanlarında tesadüfen fark ettikleri sağlık problemleri.

Health Problem	Number of Reports
Infection	8
Parasite	5
Tumor – Cyst – Organ anomaly	8
Obesity	2
General debility	2
Pulmonary hemorrhage	1
Worse outcome in the control group	1
Sudden unexpected death	1

Only four participants (4%) answered “yes” to the questions “Did you feel the need to check whether the animals you would use carried any pathogens that could affect the study results?” and “Did you check whether the animals you would use carried any pathogens that could affect the study results?”. All of the researchers who answered “yes” were women, and three of them were over the age of 50. Considering that 26 researchers had previously incidentally detected health problems in their studies, the notably low number of affirmative responses to these questions is particularly striking. Further analyses revealed that only three of the researchers who had incidentally encountered health problems felt the need to verify the presence of potential pathogens and carried out such checks. The number of researchers who left these questions unanswered was 56 and 58, respectively. Furthermore, some of the statements used by researchers when answering these questions were noteworthy.

- I did not feel the need to check whether the animals carried any pathogens that could affect the study results,
 - “because I assume that this has already been checked”
 - “but I have significant concerns about ectoparasites”
- I checked whether the animals carried any pathogens that could affect the study results,
 - “because I think there is insufficient control”
 - “A swab and culture were taken due to conjunctivitis, treatment was administered, but it was unrelated to the experimental setup and was not excluded from the study.”
 - “I worked with conventional animals, so there was already a possibility that they could be pathogen carriers. However, during the study, they did not exhibit any changes beyond the effects of the study protocol (such as piloerection, retreating to the corner of the cage, redness around the eyes, etc.)”

While creating the options for the question “What do you think the minimum training for researchers should include?”, we used the 10 items from the 10th appendix of our regulation, “Minimum Training Standards for Those Using Animals in Procedures.” 56% of all our participants, and 63% (43/68) of our certified participants, marked all the options. 33% of all our participants and 22% (25/68) of our certified participants found training on the use of humane endpoints unnecessary. The data is summarized in Table 4.

Table 4: Researchers' view on the minimum training content.**Table 4:** Araştırmacıların asgari eğitim içeriği ile ilgili görüşleri.

	Not Necessary (Number of People)	
	Total	Certified
Animal behavior, housing, and enrichment	19	5***
Animal health management and hygiene	7	3
Anesthesia, analgesic methods, and euthanasia	8	0***
Alternative methods, reduction, and refinement requirements (3R)	21	9*
Designing Procedures and Projects Based on Appropriate Environment for Animals	23	10*
Use of humane endpoints	33	15**
Recognition of species-specific pain, distress, and suffering in common laboratory animals	11	3*
Basic knowledge of species-specific biology, anatomy, physiology, reproduction, genetics, and genetic modifications	12	7
Human-animal relationships, ethical considerations on the use of animals for scientific purposes, and ethical principles regarding the value of life	10	5
National legislation on the production, transportation, management, care, and use of animals for scientific purposes	17	8

* <0.05 ** <0.01 *** <0.001

Discussion

Perhaps the most significant finding of our study was the hesitancy of laboratory animal units and researchers to participate. Although we had obtained ethical approval and despite explicitly stating in the “voluntary consent form” that the identities of the units or participants would not be disclosed under any circumstances, we frequently encountered concerns such as “what if something happens to us for filling out this survey?” Unfortunately, the primary reason behind the limited participation—only 42 out of 120 rodent laboratory animal units—was this type of fear. Among the 74 units contacted directly, 32 units still chose not to participate. This led us to believe that, had they taken part, their responses would have further lowered the overall averages observed in our findings.

In general, the physical conditions of the units are a point that is strictly controlled according to our regulations, thus ensuring that the applying unit has sufficient infrastructure in terms of care and accommodation rooms before a work permit is granted. It was observed that all the units participating in our survey, despite some deficiencies, have sufficient physical conditions for a unit of their size. Our current regulations have also emphasized multiple times

that the health status of laboratory animals should be monitored daily by veterinarians, and according to the results of our study, it appears that this legal requirement has been largely adhered to. On the other hand, approximately 70% of our units do not have a strategy and program for microbiological oversight, which is not questioned before granting work permits. This situation, despite being included in our legal regulations, shows that our units can be deficient in matters that are not questioned before granting work permits.

In our current legislation regarding laboratory animals, it is not specified which pathogens should be screened for laboratory animals other than microbiologically defined animals (conventional animals). However, many studies conducted in our country to date have shown that some of the pathogens recommended for screening in the FELASA guidelines have been detected in laboratory animals (Beyhan et al., 2010; Biyikoglu, 1996; Çetinkaya et al., 2017; İçil & Erbaş, 2024; Polat et al., 2024). Among these, İçil and Erbaş (İçil & Erbaş, 2024) demonstrated the presence of *Helicobacter species* in feces and colon samples through their screening in experimental animal units in the Aegean region. They detected *H. Typhlonius*, which is especially recommended for screening in FELASA guidelines, in 72% of the mice.

Most of the studies conducted in Türkiye have focused on the prevalence of parasitic species such as *Aspicularis*, *Syphacia*, *Trichomonas*, and *Hymenolepis*. In the study conducted by Beyhan and colleagues (Beyhan et al., 2010) on laboratory animals, a 100% prevalence of helminth infections was observed in the feces of mice and an 81% prevalence in the feces of rats. In these animals, *Syphacia species*, *Aspicularis tetraptera*, *Hymenolepis species*, and *Trichosomoides crassicauda* were identified. The identification of these species in studies conducted on laboratory animals in our country, as recommended for screening in the FELASA guidelines, indicates the need for a standardized screening program in this regard. However, our study has shown that these types of microbiological screenings are conducted by at most 40% of licensed units. This rate is already very low considering that the need is 100%, and when considering the hesitance and the rate of participation in the survey, it can even be seen as an optimistic figure. Another striking finding in our study was that out of the 17 units that claimed to conduct screenings, 13 performed stool examinations, and among them, 4 evaluated only stool samples. This situation can probably be attributed to the fact that direct fecal examination and internal parasite evaluation are almost cost-free and accessible, whereas the high cost of blood and culture medium-required studies is a significant factor. On the other hand, the fact that the only unit in our country conducting screenings using sentinel animals is a state university indicates that sufficient resources can be found when necessary for this process.

Of the units participating in the study, 2 do not conduct regular screenings on their own animals but have stated that they do so for animals newly joining the unit from outside. This situation could be a cost-related issue, or it might stem from the misconception that if the incoming animals are regularly checked, the source that could carry pathogens into the unit is already controlled, and the unit is being protected in this way. As the literature also shows, pathogen contamination in a rodent colony can originate not only from other rodents but also from the consumables used in the unit (bedding, feed, etc.) and from the staff (Lytvynets et al., 2013). The current FELASA recommendations on the microbiological monitoring of rodent colonies include periodic serological assessments using blood samples, internal parasite screening from fecal samples, external parasite screening from skin and fur samples, and necropsies on deceased animals. According to our study, there are only 2 units that meet these recommendations.

On the other hand, the fact that units housing Specific Pathogen-Free (SPF) animals—the only group for which

microbiological monitoring requirements are explicitly defined in our legislation—are able to conduct this monitoring effectively demonstrates that microbiological surveillance can be successfully implemented when legally mandated and financial resources can be allocated when deemed necessary. In the second part of our study, at least 68% of participants indicated that they expect the animals used in their research to be “SPF” if not “pathogen free”. However, only 19% of the units participating in our study, or at most 7% of all officially approved units in the country, actually house SPF animals. Therefore, it would not be inaccurate to state that scientific research in our country is predominantly conducted using conventional animals.

A total of 99 researchers completed the second questionnaire, 68% of them were under the age of 40 suggests a high level of interest among younger researchers, which is an encouraging finding. On the other hand, the fact that 70% of the participants are women reveals that gender is also a factor in interest in this subject.

When the responses of our participants to the survey were examined, it was observed that their views on which animal species could be used in scientific studies were not always in accordance with our regulations. While our researchers' view that stray animals should not be used is in line with our regulations, their view that owned animals cannot be used is not. The inability to use owned animals in scientific studies could be a factor that severely restricts clinical research in veterinary medicine and could negatively affect their health by preventing access to experimental treatment options. On the other hand, while 75% of our researchers believe that animals that are carriers of infectious agents cannot be used in studies, this situation is quite common and even considered natural for conventional animals. Similarly, 20% or fewer of our researchers consider the use of infected or infected animals appropriate. This situation is neither realistic nor necessary. As we mentioned among our findings, two of our researchers have actually expressed the necessary scientific approach very correctly. While conjunctivitis does not constitute a valid reason for excluding an animal from a study as long as it is unrelated to the research subject and remains treatable, it is a correct approach to be aware that pathogens may be found in conventional animals. However, in the latter case, it should not be overlooked that certain pathogens capable of affecting all animals within the colony uniformly may introduce confounding factors into the study outcomes (Nicklas et al., 1999).

While a large majority of our researchers believe that pathogen-free animals should be used in studies, 66 participants reported obtaining information regarding

animal health status from suppliers. Considering the total number of researchers involved in experimental studies, this figure may initially appear satisfactory; however, upon detailed examination of the data these participants requested, it was observed that 64 of these researchers obtained only information such as age, species, and breed, while the majority did not inquire about detailed data recommended by research guidelines such as ARRIVE (Animal Research: Reporting of In-vivo Experiments) (Kilkenny et al., 2010a) which could influence the outcomes of their studies. The ARRIVE guidelines, first published in 2010, emphasize that information reflecting the health status of animals used in experiments is an essential part of experimental studies and underline the necessity of evaluating their impacts on experimental outcomes (Kilkenny et al., 2010b; Pritchett-Corning et al., 2014). Many journals that accept publications involving animal research now require information reporting to comply with the ARRIVE guidelines.

26 researchers reported incidentally detecting a health issue in experimental animals, aside from routine health monitoring. Despite encountering such frequent problems, the absence of microbiological screening may result in the complete oversight of hard-to-detect conditions that could influence study outcomes at a subclinical level in terms of physiological, behavioral, and biochemical parameters. The most frequently encountered incidental findings were infections and tumors, cysts, or organ anomalies. An interesting point is that, among the 26 researchers who incidentally identified a pathological condition unrelated to their study, only two had checked whether the animals used in their research carried pathogens that could potentially affect the study outcomes.

Our participants' views on the training received by those who will use animals in procedures during certification were, unfortunately, not entirely aligned with our legislation. A reassuring aspect of this issue was the significant difference observed between certified and non-certified individuals, indicating that the training was at least somewhat effective. The most striking impact of certification training was the change in participants' approaches to anesthesia, analgesia, and euthanasia methods. While 26% of non-certified individuals considered training in these areas unnecessary, 100% of certified individuals deemed it essential. Another notable point was that certification training made a significant difference in the use of humane endpoints. However, it is quite disappointing that 22% of our certified participants still considered this unnecessary.

Conclusion

In conclusion, at least 60% of experimental animal units in our country do not conduct any form of microbiological surveillance. This issue not only significantly impacts the reliability of research data but also increases variability in collected data, potentially leading to excessive use of animals in experiments. Although the current regulations mandate health monitoring and emphasize that units should have strategic plans and programs in this regard, the fact that only 17% of all facilities housing laboratory rodents perform microbiological surveillance suggests that the obligation to develop strategic plans does not serve as an effective directive. On the other hand, the ability to monitor SPF (Specific Pathogen-Free) animals indicates that developing national guidelines for microbiological surveillance of conventional animals could be an efficient approach.

Perhaps the most crucial point for researchers is the necessity of always keeping in mind that ensuring the reliability of data and strict adherence to ethical principles in our studies is our responsibility. We cannot absolve ourselves of this responsibility with a statement like "because I assume that this has already been checked".

Ethics Committee Approval: The necessary permission for this research was obtained from the Gazi University Clinical Research Ethics Committee on 27.01.2020-117.

Author Contributions: Concept - AMS; Design - AMS, BK, YT; Supervision - AMS; Resources - NONE; Materials - AMS, BK; Data Collection and/or Processing - AMS, BK, YT; Analysis and/or Interpretation - AMS, BK; Literature Search - AMS, BK, YT; Writing Manuscript - AMS, BK, YT; Critical Review - AMS, BK, YT

Peer-review: Externally peer-reviewed.

Funding: The authors did not receive any funding for this study.

Declaration of Interests: The authors have no conflict of interest to report.

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