Revista ARACÊ
ISSN: 2358-2472

REDUCING THE USE OF ANIMALS IN RESEARCH: TRENDS AND CHALLENGES IN ANIMAL EXPERIMENTATION BETWEEN 2016 AND 2021

https://doi.org/10.56238/arev6n3-318

Submission date: 25/10/2024 **Publication date:** 25/11/2024

Camila Botelho Miguel[1], Gabriela Severino da Silva[2], Glicélia Pereira Silva[3], Ferdinando Agostinho[4], Melissa Carvalho Martins de Abreu[5], Jamil Miguel Neto[6], Javier Emilio Lazo-Chica[7] and Wellington Francisco Rodrigues[8]

ABSTRACT

Introduction: In recent years, significant efforts have been directed towards reducing, refining, and replacing the use of nonhuman animals in research and teaching. Understanding the distribution and frequency of use of these animals is essential to develop indicators that guide these practices. Objective: The present study aimed to describe the frequency of use of animal experimentation models between 2016 and 2021, in addition to investigating potential temporal correlations. Methods: A retrospective cross-sectional study was conducted, accessing data from the public database of the National Center for Biotechnology Information. The data refer to mice, rats, rabbits, guinea pigs, monkeys, fish and dogs. Statistical analysis included Spearman's test to assess correlations, with a significance level of 5%. Results: Mice stood out as the most used models, representing 57.73% of the associations, followed by rats (26.27%) and fish (8.40%). Significant negative correlations were identified for rats (rho = -0.92), rabbits (rho = -0.97) and guinea pigs (rho = -0.96), while a positive correlation was observed for monkeys (rho = 0.91). Conclusion: The results indicate a reduction in the use of some non-primate species associated with basic research, along with an increase in the use of primates. This change highlights the

[1] Multidisciplinary Laboratory of Scientific Evidence, Centro Universitário de Mineiros, Unifimes, Mineiros, GO, Brazil.
Postgraduate Course in Tropical Medicine and Infectious Diseases, Federal University of Triângulo Mineiro, UFTM, Uberaba, MG, Brazil.
[2] Multidisciplinary Laboratory of Scientific Evidence, Centro Universitário de Mineiros, Unifimes, Mineiros, GO, Brazil.
[3] Multidisciplinary Laboratory of Scientific Evidence, Centro Universitário de Mineiros, Unifimes, Mineiros, GO, Brazil.
[4] Universidade de Rio Verde, UniRv, Rio Verde, GO, Brazil.
[5] Multidisciplinary Laboratory of Scientific Evidence, Centro Universitário de Mineiros, Unifimes, Mineiros, GO, Brazil.
[6] Multidisciplinary Laboratory of Scientific Evidence, Centro Universitário de Mineiros, Unifimes, Mineiros, GO, Brazil.
[7] Laboratory of Cell Biology of the Department of Structural Biology of the Institute of Biological and Natural Sciences, Federal University of Triângulo Mineiro, UFTM, Uberaba, MG, Brazil.
[8] Multidisciplinary Laboratory of Scientific Evidence, Centro Universitário de Mineiros, Unifimes, Mineiros, GO, Brazil.
Postgraduate Course in Tropical Medicine and Infectious Diseases, Federal University of Triângulo Mineiro, UFTM, Uberaba, MG, Brazil.
Laboratory of Cell Biology of the Department of Structural Biology of the Institute of Biological and Natural Sciences, Federal University of Triângulo Mineiro, UFTM, Uberaba, MG, Brazil.
E-mail: wellington.frodrigues@unifimes.edu.br

need for policies and practices that promote substitution and refinement in animal experimentation, aiming at animal welfare and scientific efficiency.

Keywords: Research. Animal Experimentation Models. Reduction. Refinement. Replacement.

Revista ARACÊ
ISSN: 2358-2472

INTRODUCTION

The use of non-human animals in research and applied to teaching practices has notoriously allowed contributions to the scientific and technological advancement observed today, on the other hand the practice collaborates to severe social, scientific and philosophical discussions over several years (FRANCO, 2013; LAFOLLETTE; SHANKS, 2020).

Animal experimentation is already recognized as being of paramount importance and contribution to scientific and educational advances (LAFOLLETTE; SHANKS, 2020), it is not yet possible to accurately mimic all the complex biological systems expected of living beings (GURA, 1997) However, in recent decades, several efforts have been made to ensure not only the welfare of experimental animals, but also management that allows the reduction, refinement and replacement of experimental animals by systems increasingly aligned with the characteristics of complex biological systems (MOVIA; BRUNI-FAVIER; PRINA-MELLO, 2020).

There is a consensus on ethical, civic and sincere behavior for the entire process in animal experimentation, including the approval of all protocols to an ethics committee prepared to evaluate it. Comprehensive guidelines for designing animal experiments have recently been developed to address this need (LEWIS, 2019). A commitment to improving animal welfare, scientific quality, staff care, and transparency for all stakeholders will also foster a culture of animal research care that benefits all parties. All of Russell and Burch's 3 R's (replace, reduce, and refine) play a role in planning and the reproducibility of studies and tests that may involve animals.

However, approaches that allow measuring the effects of policies aimed at reducing the use of experimental animals, as well as generating indicators for future practices, are important. Thus, the present study aimed to describe the frequency and potential temporal correlations for the use of animal experimentation models.

MATERIAL AND METHODS

ETHICAL ASPECTS

The present study is a secondary evaluation in a public digital collection and widely disseminated in the scientific and academic environment. Although the study does not require prior evaluation and approval by the Ethics Committee for the Use of Animals, as it is an approach to the use of animals, the study sought to consider the premises of the

Revista ARACÊ
ISSN: 2358-2472

National Council for the Control of Animal Experimentation as a guiding instrument for the construction of the study.

EXPERIMENTAL DESIGN

A secondary observational, analytical, cross-sectional, retrospective and prospective study was conducted. The retrospective period that configured the transversality addressed for this survey was between the years 2016 and 2021.

The prospective evaluation was defined by the time frame for a reduction in frequencies in negative correlations and a 2-fold increase in positive correlations. The following terms were used for the search: "models, animal", "mice", "rats", "rabbit", "guinea pig", "monkey" and "fish".

No calculation was necessary to determine the sampling, since the data collected were population-based between the study period.

ELIGIBILITY CRITERIA

Approaches to animal experimentation related to experimental models established with strains of mice, rats, rabbits, guinea pigs, monkeys or fish were included.

The documents included and analyzed in the study belonged to the digital collection of the National Center for Biotechnology Information, established in the Medline/Pubmed database. The search was carried out between the months of March (beginning: 28th) and April (end: 18th) of 2022, for the study period between the years 2016 and 2021.

Studies published in duplicates, such as "errata" or that did not contain mentions of experimental models in the title and/or abstracts of the documents were not included for the evaluations.

DATA ANALYSIS

The collected data were tabulated in Microsoft software (Windows 10), Excel®. The frequencies were used to evaluate the distributions for the use of the different experimental models, as well as the variations between the effect sizes given by the respective means and standard deviations, which were used for comparisons.

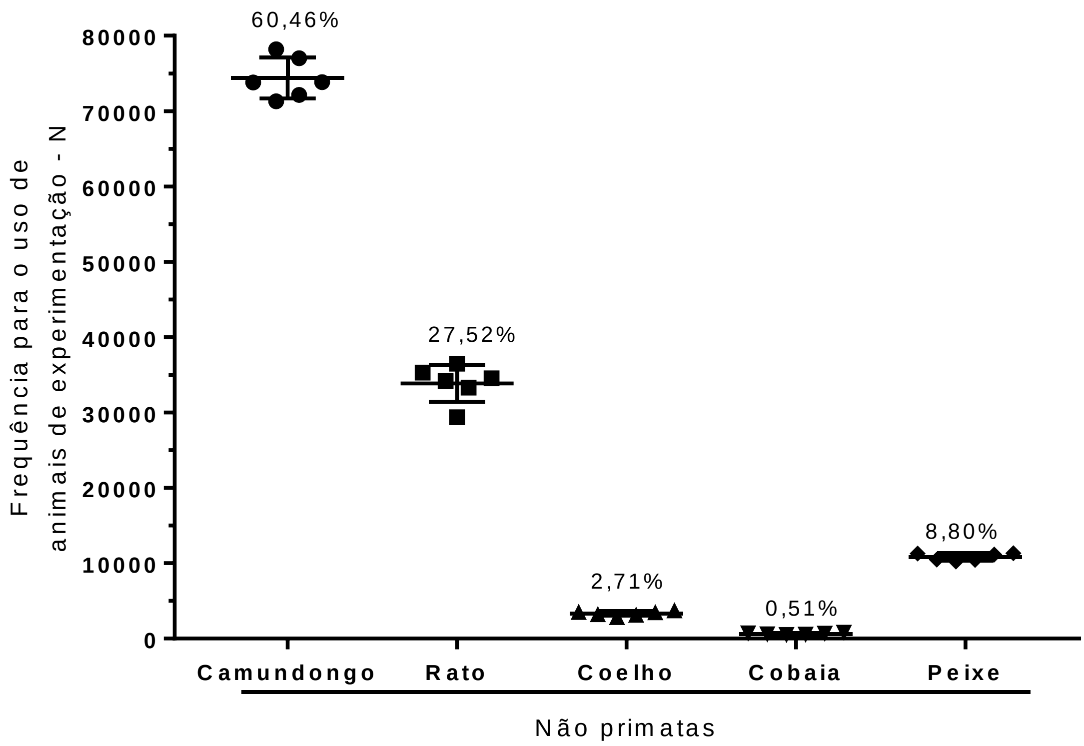
The Shapiro-Wilk test was used to evaluate distributions and/or normality. Correlations were determined using the Spearman test and predictions were evaluated using linear regression curves. The confidence level considered for all analyses was 5%.

Revista ARACÊ
ISSN: 2358-2472

RESULTS

The absolute and relative frequencies were initially evaluated for non-primate animal species, and it was possible to classify them in descending order for the citations in the different studies, where mice (74387± 2711 – 60.46%) appeared first, followed by rats (33859 ± 2445), fish (10823 ± 488.70), rabbits (3338 ± 312.90) and guinea pigs (626.30±110) (Figure 1). The distributions for the frequencies of citations to species differences in the evaluated period were Gaussian (w>0.84), demonstrating a standard of normality for the evaluations of the use of the animals.

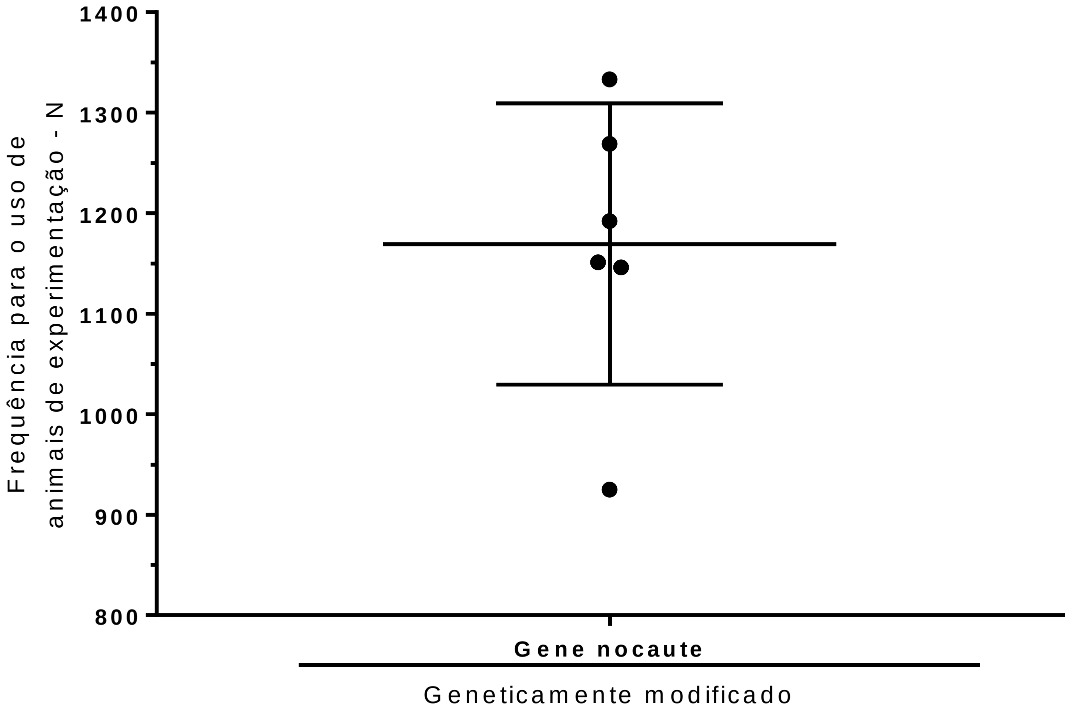
Figure 1. Profile for the distribution of the use of non-primate experimental animals for the period 2016 to 2021. Frequencies were obtained after consulting the Medline/Pubmed database for mouse, rat, rabbit, guinea pig and fish species. The data were tabulated into relative and absolute values, where the distributions are being demonstrated by the mean and standard deviation.



As the use of genetically modified animals has been the subject of several studies, its frequency has also been evaluated. It was possible to observe a normal distribution for the period analyzed (W = 0.92) and an average of 1169 studies per year with a standard deviation of 139.90 (95% CI = 1023 to 1316) (Figure 2).

Revista ARACÊ
ISSN: 2358-2472

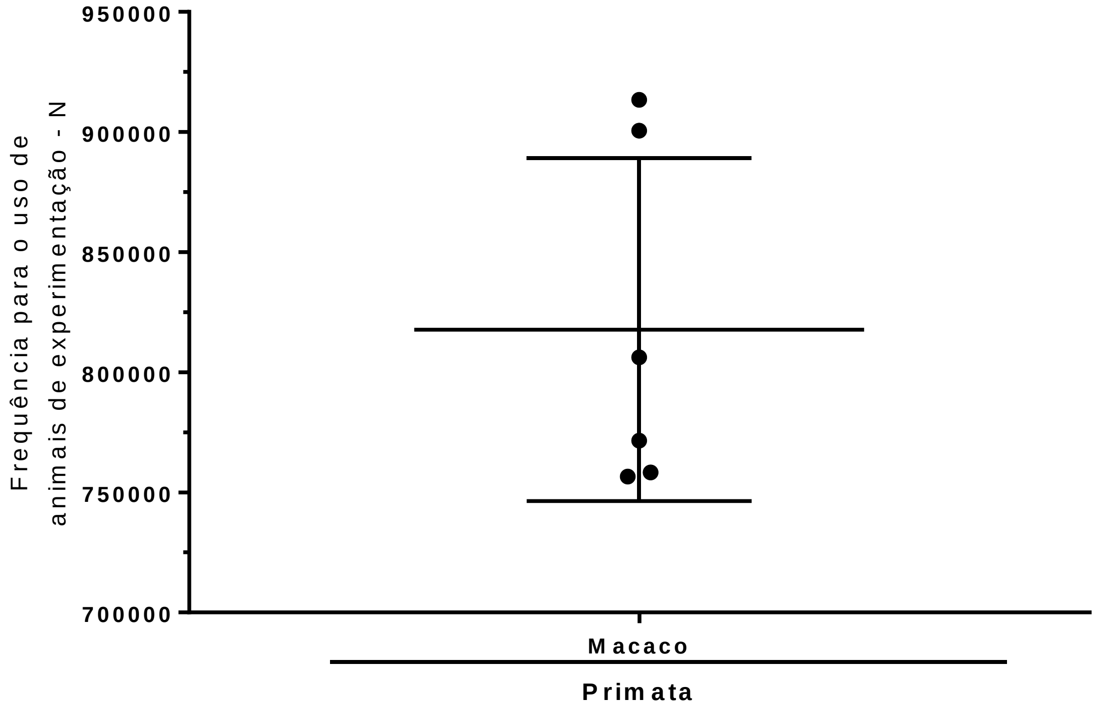
Figure 2. Frequency for the use of genetically modified experimental animals between the period 2016 and 2021. Frequencies were obtained after consulting the Medline/Pubmed database for genetically modified strains. The data were tabulated in absolute values, where the distributions are being demonstrated by the mean and standard deviation.



Following the descriptions for the frequencies of use of experimental animals, the frequencies for non-human primate animals in general were evaluated (Figure 3). A normal distribution was identified for the years of investigation (W = 0.81) with a mean of 817783 per year and a standard deviation of 71454 (95% CI = 722797 to 892769) (Figure 3).

Revista ARACÊ
ISSN: 2358-2472

Figure 3. Frequency for the use of primate experimental animals between the period 2016 and 2021. The frequencies were obtained after consulting the Medline/Pubmed database for the different species of primate experimental animals. The data were tabulated in absolute values, where the distributions are being demonstrated by the mean and standard deviation.

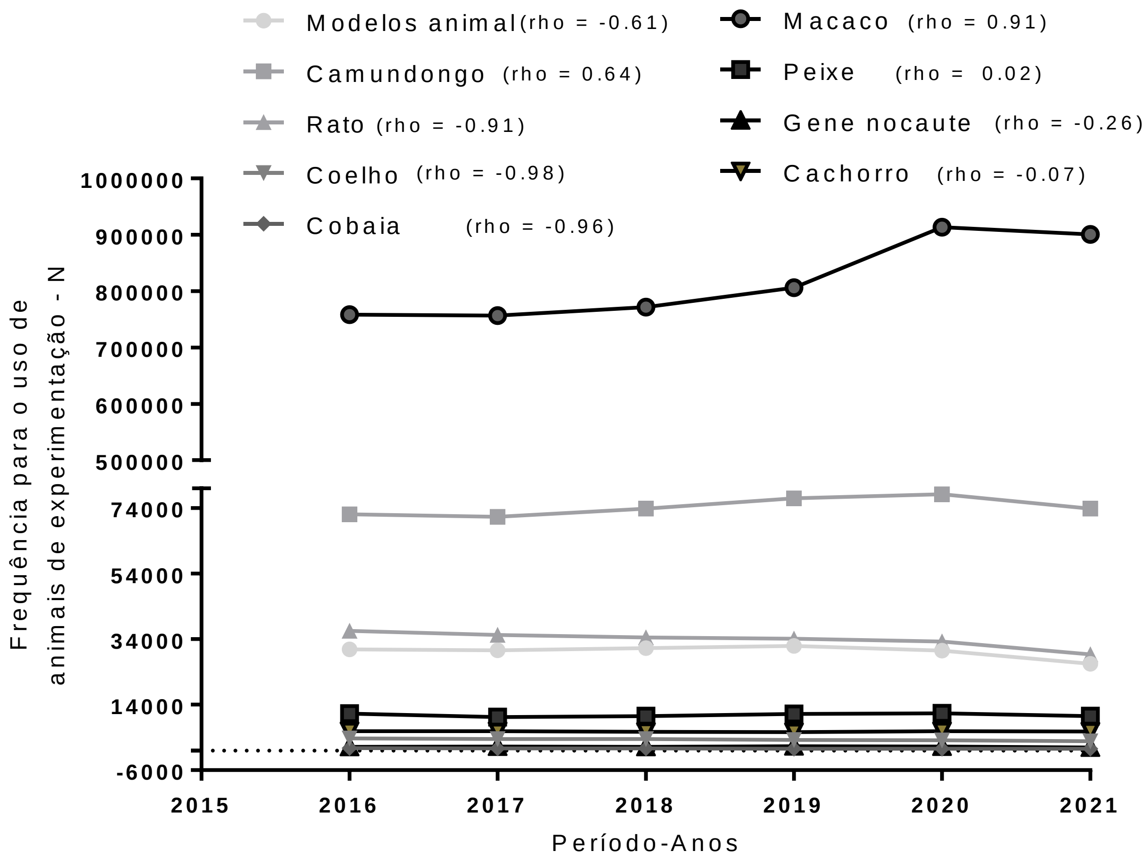


After surveying and knowing the mean distributions for the frequencies of different species of animals used in experimentation or teaching, potential correlations between the absolute frequencies and the study period were evaluated (Figure 4). Negative and significant correlations were observed for the animal models in general (rho = -0.61; $p<0.05$) and in a punctual way for the species of rats (rho = -0.91; $p<0.05$), rabbits (-0.98; $p<0.05$) and guinea pigs (rho = -0.96; $p<0.05$).

On the other hand, positive and significant correlations were observed for the species of mice (rho = 0.64; $p<0.05$) and monkeys (rho = 0.91; $p<0.05$). For dogs, fish and knockout animals, the fluctuations did not allow significant correlations ($p>0.05$) (Figure 4).

Revista ARACÊ
ISSN: 2358-2472

Figure 4. Temporal correlation for the frequencies of use of experimental animals and the six-year period (2016 to 2021). The frequencies were obtained after consulting the Medline/Pubmed database for the different species of primate experimental animals. The data were tabulated in absolute values and the series were determined between the values of F(X). To evaluate the correlations, the rho values were determined by the "Spearman" test. The level of significance used was 5%.



Finally, a prospective evaluation for all species with rho >|0.6| was carried out to estimate frequencies with a 50% difference from the frequencies of the year 2021, following the vector path (Table 1). It was possible to estimate that the first species to reduce its frequency by 50% was for guinea pigs estimated for the year 2025, followed later by rabbits (2030) and rats (2035).

On the other hand, the estimate for the increase by 2x the current frequency for monkeys was estimated for the year 2047 and for mice the year 2098. In general, the average reduction of experimental animal models by 50% was for the year 2046 (table 1).

Revista ARACÊ
ISSN: 2358-2472

Table 1. Prediction for the effects on the frequencies of use of different species of animals in experimentation.

Data	Animal Model	Mouse	Mouse	Rabbit	Guinea pig
2022 - Year	28093,47	77626,00	29666,60	2766,73	428,33
2023 - Year	27468,12	78551,43	28468,77	2603,42	371,76
2024 - Year	26842,78	79476,86	27270,94	2440,10	315,19
2025 - Year	26217,44	80402,29	26073,11	2276,79	258,62
≠ by 50% for 2021	13247.50 ↓	147678.00 ↑	14692.50 ↓	1424.00 ↓	253.50 ↓
Diff. by 50% - Year	2046	2098	2035	2030	2025
R value	-0,61	0,64	-0,92	-0,98	-0,96

≠ = difference. % = percentage. ↓↑ = shows the position for the differences for the year 2021 (divided or multiplied by 2). Diff. = difference. The years were estimated by means of interpolation and equation given in linear regression (criterion = r>0.6 in modulus). Note: For the monkeys, the data were heavily influenced by numbers of theoretical or observational descriptive studies (population descriptions only).

DISCUSSION

Here, in the present study it was possible to evaluate and discuss the frequencies for the use of different species used in animal experimentation, as well as to evaluate the time frame for modifications in 50% of the frequencies currently described.

Models associated with mice are still the most widely used models among non-primate animals, followed by rats, fish, rabbits, and guinea pigs. It is possible to observe a versatility for the induction of different experimental models with the use of mice for both infectious inflammatory diseases (ASSINGER; SCHROTTMAIER; SALZMANN; RAYES, 2019; CLEARY; PITCHFORD; AMISON; CARRINGTON *et al.*, 2020; RODRIGUES; MIGUEL; MARQUES; FROM COAST *et al.*, 2022), non-infectious (SHOCHET; HOLDSWORTH; KITCHING, 2020; TSCHÖPE; AMMIRATI; BOZKURT; CABIN *et al.*, 2021) and degenerative models (CHEN; LU; PENG; MAK *et al.*, 2022; DAWSON; GOLDE; LAGIER-TOURENNE, 2018) and also for different chronic diseases, including hypertension, obesity and diabetes mellitus (GIRALT-LÓPEZ; MOLINA-VAN DEN BOSCH; VERGARA; CAR-SHOP *et al.*, 2020; WOODS; SATOU; MIYATA; KATSURADA *et al.*, 2019) What may be contributing to the profile found regarding the high frequency of use of the species, but not only, other factors seem to be important, such as the optimization for the spaces occupied in vivariums, the availability for commercial 'kits' that evaluate different molecules of the species and of course its similarity with humans (BELONGIE; BRANSON; DOLLAR; RABAUD, 2005; SWEIS; OPEN; SCHMIDT; SEELAND *et al.*, 2018). Probably the factors discussed above will influence the order of frequencies for the use of other animal species for experimentation.

In recent decades there has been an increase in studies that have released promising experimental models for the use of fish, especially for the Zebrafish species

(ADAMSON; SHERIDAN; GRIERSON, 2018). Among contributions to the biological model, toxicological, neurobiological, immunological, cancer, metabolic disorders, among others, stand out (KEY; DEVINE, 2003; KUMAR; SINGH; SINGH, 2021; NOVAK; ŽEGURA; MODIC; HEATH *et al.*, 2017; VASYUTINA; ALIEVA; REUTOVA; BAKALEIKO *et al.*, 2022), certainly seems to be one of the complex biological models promising for different uses in research and teaching.

However, in the present evaluation no positive correlation was found for the use of models with Zebrafish, we believe that the versatility of the biological model is not yet a factor that allows it to be widely used, especially by research centers in developing countries, given that the implementation of a vivarium for the species in question, as well as its maintenance is still expensive and requires several specific care (AVDESH; CHEN; MARTIN-IVERSON; MONDAL *et al.*, 2012; PONPORNPISIT; JONGJAROENJAI; SUTHAMNATPONG; BURUT-ARCHANAI, 2022).

For some of the models evaluated, it was possible to verify a trend towards a decrease in the use of animals, as observed for rats, rabbits, guinea pigs and experimental animals in general. This was an important positive indicator of the study that corroborates the efforts linked to the refinement, reduction and substitution for the use of experimental animals widely disseminated (LILLEY; STANFORD; KENDALL; ALEXANDER *et al.*, 2020; TANNENBAUM; BENNETT, 2015). The inclusion of new computational and realistic resources has certainly contributed to the implementation and use of new strategies for research, reducing the number of experimental animals (NIEDERER; LUMENS; TRAYANOVA, 2019; YAMAMOTO; NAKAMURA; LIU; STEIN *et al.*, 2019), another important factor is the refinement for the research, with methods that guarantee replicability as well as the construction of assertive designs regarding the evaluation of statistical samples (BURR, 2018; STIGLIC; KOCBEK; FIJACKO; ZITNIK *et al.*, 2020).

On the other hand, there has been a notable increase in primate research in recent years. It seems that the frequencies for the use of non-human primates in research may vary according to the region evaluated, in the European Union for example there are reports that there is a decrease in the use of primates and for several other regions of the world the frequency has increased (CHATFIELD; MORTON, 2018). We believe that the phylogenetic proximity of primates contributed to the interest in their uses in different researches, as highlighted for a study with a multiple sclerosis model (BROK; BAUER; JONKER; BLEZER *et al.*, 2001), in addition, the safe practices and the speed of

Revista ARACÊ
ISSN: 2358-2472

investigations in recent years for Covid-19, quickly arriving at preventive measures (vaccines) and potential interventions for the disease have heated up the need for more assertive preclinical models for biomedical advances and applications (CAO; DENG; DAI, 2020; COHEN, 2020). In addition, epidemiological surveys, evaluations for the different biological interactions of primates with the environment, their hosts and relationships with humans are part of current investigations. In a recent report, disseminated practices restricted to some communities were demonstrated regarding the use of primate spices to seek the cure of different diseases, where in the same evaluation the authors highlighted the practice as a negative factor and the need for the digression of knowledge and practices aimed at strengthening health systems aiming to discourage the use of primates in these communities (DAOLAGUPU; TALUKDAR; CHOUDHURY, 2021). In another study, hematological, physiological, morphometric and parasitic parameters were evaluated in 26 monkeys (*Sapajus libidinosus*) after being rescued and allocated to 2 centers in northeastern Brazil (HERNÁNDEZ-CRUZ; FERREIRA; ROONEY; GUIDI *et al.*, 2022). On the other hand, it lacks attention and ethical strengthening for all practices aimed at testing in primates (MARTIN, 2008). In this sense, there is an eminent concern with the ethical issues of research applied to the use of primates for research, since laws may vary and be more flexible between different countries (CHATFIELD; MORTON, 2018).

Certainly, with the motivating practices for the implementation of alternative models for teaching and research, as well as the refinement of methods and the dissemination of studies that can make it possible to reduce efforts to search for answers to questions that are already tangible to be known through systematic reviews, there is a forecast for a reduction in the use of experimental animals (LEE; KANG; JEONG; KIM *et al.*, 2022), corroborating the expectations observed over the last decades, the study presented allowed us to prospectively evaluate the use of different species in the research and what seems to be that more accentuated modifications, for at least 50% of what we have today, will depend on another 3 decades. However, the promotion for the generation of alternative means to research and teaching must be part of and strengthened for the coming years and, in addition, the advancement of ethics committees for the use of animals will be fundamental for future practices (OLSSON; NIELSEN; CAMERLINK; PONGRÁCZ *et al.*, 2022), 2022), given that practices with experimental animals are still indispensable and fundamental alternatives to ensure the improvement and advancement of science, as well as to enable perspectives for new means of prevention, interventions, and management for overwhelming diseases (LAFOLLETTE; SHANKS, 2020).

Also the stratifications for other species, by countries or sociodemographic characteristics or even by scientific research rates and for the differences between the types of research should be raised to improve the indicators for the use of experimental animals and these points are the main considerations for the fragility of the study presented.

FINAL CONSIDERATIONS

In general, in recent years there has been a decline in the use of experimental animals, which was possible to verify for most of the species evaluated in the study, on the other hand there are no prospects for a reduction in the use of mice, the most frequent non-primate species currently used for animal experimentation and added to this there is a perspective for an increase in the use of non-human primate animals.

Alternative models for investigations, refinement of research design and data summarization are factors that have contributed to the reduction of the use of animals, but certainly policies inclined to good practices to the use of animals for experimentation as well as to the strengthening of ethics committees will be fundamental to enable better results in practice for the use of experimental animals.

THANKS

The authors thank the National Council for Scientific and Technological Development (CNPq) for supporting the research through the granting of a senior postdoctoral fellowship to the author Camila Botelho Miguel, according to Public Notice 32/2023 and Process 102630/2024-0, under the supervision of Carlo José Freire Oliveira.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

Revista ARACÊ
ISSN: 2358-2472

REFERENCES

1. Adamson, K. I., Sheridan, E., & Grierson, A. J. (2018). Use of zebrafish models to investigate rare human disease. Journal of Medical Genetics, 55(10), 641-649.

2. Assinger, A., Schrottmaier, W. C., Salzmann, M., & Rayes, J. (2019). Platelets in sepsis: an update on experimental models and clinical data. Frontiers in Immunology, 10, 1687.

3. Avdesh, A., Chen, M., Martin-Iverson, M. T., Mondal, A., et al. (2012). Regular care and maintenance of a zebrafish (Danio rerio) laboratory: An introduction. JoVE (Journal of Visualized Experiments), 69, e4196.

4. Belongie, S., Branson, K., Dollar, P., & Rabaud, V. (2005). Monitoring animal behavior in the smart vivarium. Wageningen, The Netherlands, 70-72.

5. Brok, H. P., Bauer, J., Jonker, M., Blezer, E., et al. (2001). Non-human primate models of multiple sclerosis. Immunological Reviews, 183(1), 173-185.

6. Burr, I. W. (2018). Statistical quality control methods. Routledge.

7. Cao, Y.-C., Deng, Q.-X., & Dai, S.-X. (2020). Remdesivir for severe acute respiratory síndrome coronavirus 2 causing COVID-19: An evaluation of the evidence. Travel Medicine and Infectious Disease, 35, 101647.

8. Chatfield, K., & Morton, D. (2018). The use of non-human primates in research. In Ethics Dumping (pp. 81-90). Springer, Cham.

9. Chen, C., Lu, J., Peng, W., Mak, M. S., et al. (2022). Acrolein, an endogenous aldehyde induces Alzheimer's disease-like pathologies in mice: A new sporadic AD animal model. Pharmacological Research, 175, 106003.

10. Cleary, S. J., Pitchford, S. C., Amison, R. T., Carrington, R., et al. (2020). Animal models of mechanisms of SARS-CoV-2 infection and COVID-19 pathology. British Journal of Pharmacology, 177(21), 4851-4865.

11. Cohen, J. (2020). COVID-19 shot protects monkeys. American Association for the Advancement of Science.

12. Daolagupu, D., Talukdar, N. R., & Choudhury, P. (2021). Ethnozoological use of primates in northeastern India. Journal of Threatened Taxa, 13(11), 19492-19499.

13. Dawson, T. M., Golde, T. E., & Lagier-Tourenne, C. (2018). Animal models of neurodegenerative diseases. Nature Neuroscience, 21(10), 1370-1379.

14. Franco, N. H. (2013). Animal experiments in biomedical research: A historical perspective. Animals, 3(1), 238-273.

Revista ARACÊ
ISSN: 2358-2472

15. Giralt-López, A., Molina-Van den Bosch, M., Vergara, A., García-Carro, C., et al. (2020). Revisiting experimental models of diabetic nephropathy. International Journal of Molecular Sciences, 21(10), 3587.

16. Gura, T. (1997). Systems for identifying new drugs are often faulty. American Association for the Advancement of Science.

17. Hernández-Cruz, G., Ferreira, R. G., Rooney, N. J., Guidi, R. D. S., et al. (2022). Haematology, physiological parameters, morphometry and parasitological status of rescued bearded capuchin monkeys (Sapajus libidinosus). Journal of Medical Primatology.

18. Key, B., & Devine, C. A. (2003). Zebrafish as an experimental model: Strategies for developmental and molecular neurobiology studies. Methods in Cell Science, 25(1), 1-6.

19. Kumar, V., Singh, C., & Singh, A. (2021). Zebrafish an experimental model of Huntington's disease: Molecular aspects, therapeutic targets and current challenges. Molecular Biology Reports, 48(12), 8181-8194.

20. Lafollette, H., & Shanks, N. (2020). Brute science: Dilemmas of animal experimentation. Routledge.

21. Lee, S. Y., Kang, J. H., Jeong, J. W., Kim, J. H., et al. (2022). Alternative experimental approaches to reduce animal use in biomedical studies. Journal of Drug Delivery Science and Technology, 103131.

22. Lewis, D. I. (2019). Animal experimentation: Implementation and application of the 3Rs. Emerging Topics in Life Sciences, 3(6), 675-679.

23.Lilley, E., Stanford, S. C., Kendall, D. E., Alexander, S. P., et al. (2020). ARRIVE 2.0 and the British Journal of Pharmacology: Updated guidance for 2020. British Journal of Pharmacology, 177(16), 3611.

24. Martin, C. (2008). Experimental use of nonhuman primates is not a simple problem. Nature Medicine, 14(10), 1011.

25. Movia, D., Bruni-Favier, S., & Prina-Mello, A. (2020). In vitro alternatives to acute inhalation toxicity studies in animal models—a perspective. Frontiers in Bioengineering and Biotechnology, 8, 549.

26. Niederer, S. A., Lumens, J., & Trayanova, N. A. (2019). Computational models in cardiology. Nature Reviews Cardiology, 16(2), 100-111.

27. Novak, M., Žegura, B., Modic, B., Heath, E., et al. (2017). Cytotoxicity and genotoxicity of anticancer drug residues and their mixtures in experimental model with zebrafish liver cells. Science of The Total Environment, 601, 293-300.

28. Olsson, I. A. S., Nielsen, B. L., Camerlink, I., Pongrácz, P., et al. (2022). An international perspective on ethics approval in animal behaviour and welfare research. Applied Animal Behaviour Science, 105658.

29.Ponpornpisit, A., Jongjaroenjai, M., Suthamnathpong, N., & Burut-Archanai, S. (2022). Application of a single-tank sequencing batch reactor for long-term zebrafish care. Thai Journal of Veterinary Medicine, 52(3), 451-463.

30. Rodrigues, W. F., Miguel, C. B., Marques, L. C., Da Costa, T. A., et al. (2022). Predicting blood parasite load and influence of expression of iNOS on the effect size of clinical laboratory parameters in acute Trypanosoma cruzi infection with different inoculum concentrations in C57BL/6 mice. Frontiers in Immunology, 1113.

31. Shochet, L., Holdsworth, S., & Kitching, A. R. (2020). Animal models of ANCA-associated vasculitis. Frontiers in Immunology, 11, 525.

32. Stiglic, G., Kocbek, P., Fijacko, N., Zitnik, M., et al. (2020). Interpretability of machine learning-based prediction models in healthcare. Wiley Interdisciplinary Reviews: Data Mining and Knowledge Discovery, 10(5), e1379.

33. Sweis, B. M., Abram, S. V., Schmidt, B. J., Seeland, K. D., et al. (2018). Sensitivity to “sunk costs” in mice, rats, and humans. Science, 361(6398), 178-181.

34. Tannenbaum, J., & Bennett, B. T. (2015). Russell and Burch's 3Rs then and now: The need for clarity in definition and purpose. Journal of the American Association for Laboratory Animal Science, 54(2), 120-132.

35. Tschöpe, C., Ammirati, E., Bozkurt, B., Caforio, A. L., et al. (2021). Myocarditis and inflammatory cardiomyopathy: Current evidence and future directions. Nature Reviews Cardiology, 18(3), 169-193.

36. Vasyutina, M., Alieva, A., Reutova, O., Bakaleiko, V., et al. (2022). The zebrafish model system for dyslipidemia and atherosclerosis research: Focus on environmental/exposome factors and genetic mechanisms. Metabolism, 155138.

37. Woods, T. C., Satou, R., Miyata, K., Katsurada, A., et al. (2019). Canagliflozin prevents intrarenal angiotensinogen augmentation and mitigates kidney injury and hypertension in mouse model of type 2 diabetes mellitus. American Journal of Nephrology, 49(4), 331-342.

38. Yamamoto, K. N., Nakamura, A., Liu, L. L., Stein, S., et al. (2019). Computational modeling of pancreatic cancer patients receiving FOLFIRINOX and gemcitabine-based therapies identifies optimum intervention strategies. PloS One, 14(4), e0215409.Adamson, K. I., Sheridan, E., & Grierson, A. J. (2018). Use of zebrafish models to investigate rare human disease. Journal of Medical Genetics, 55(10), 641-649.

39. Assinger, A., Schrottmaier, W. C., Salzmann, M., & Rayes, J. (2019). Platelets in sepsis: an update on experimental models and clinical data. Frontiers in Immunology, 10, 1687.

Revista ARACÊ
ISSN: 2358-2472

40. Avdesh, A., Chen, M., Martin-Iverson, M. T., Mondal, A., et al. (2012). Regular care and maintenance of a zebrafish (Danio rerio) laboratory: An introduction. JoVE (Journal of Visualized Experiments), 69, e4196.

41. Belongie, S., Branson, K., Dollar, P., & Rabaud, V. (2005). Monitoring animal behavior in the smart vivarium. Wageningen, The Netherlands, 70-72.

42. Brok, H. P., Bauer, J., Jonker, M., Blezer, E., et al. (2001). Non-human primate models of multiple sclerosis. Immunological Reviews, 183(1), 173-185.

43. Burr, I. W. (2018). Statistical quality control methods. Routledge.

44. Cao, Y.-C., Deng, Q.-X., & Dai, S.-X. (2020). Remdesivir for severe acute respiratory syndrome coronavirus 2 causing COVID-19: An evaluation of the evidence. Travel Medicine and Infectious Disease, 35, 101647.

45. Chatfield, K., & Morton, D. (2018). The use of non-human primates in research. In Ethics Dumping (pp. 81-90). Springer, Cham.

46. Chen, C., Lu, J., Peng, W., Mak, M. S., et al. (2022). Acrolein, an endogenous aldehyde induces Alzheimer's disease-like pathologies in mice: A new sporadic AD animal model. Pharmacological Research, 175, 106003.

47. Cleary, S. J., Pitchford, S. C., Amison, R. T., Carrington, R., et al. (2020). Animal models of mechanisms of SARS-CoV-2 infection and COVID-19 pathology. British Journal of Pharmacology, 177(21), 4851-4865.

48. Cohen, J. (2020). COVID-19 shot protects monkeys. American Association for the Advancement of Science.

49. Daolagupu, D., Talukdar, N. R., & Choudhury, P. (2021). Ethnozoological use of primates in northeastern India. Journal of Threatened Taxa, 13(11), 19492-19499.

50. Dawson, T. M., Golde, T. E., & Lagier-Tourenne, C. (2018). Animal models of neurodegenerative diseases. Nature Neuroscience, 21(10), 1370-1379.

51. Franco, N. H. (2013). Animal experiments in biomedical research: A historical perspective. Animals, 3(1), 238-273.

52. Giralt-López, A., Molina-Van den Bosch, M., Vergara, A., García-Carro, C., et al. (2020). Revisiting experimental models of diabetic nephropathy. International Journal of Molecular Sciences, 21(10), 3587.

53. Gura, T. (1997). Systems for identifying new drugs are often faulty. American Association for the Advancement of Science.

54. Hernández-Cruz, G., Ferreira, R. G., Rooney, N. J., Guidi, R. D. S., et al. (2022). Haematology, physiological parameters, morphometry and parasitological status of

Revista ARACÊ
ISSN: 2358-2472

rescued bearded capuchin monkeys (Sapajus libidinosus). Journal of Medical Primatology.

55. Key, B., & Devine, C. A. (2003). Zebrafish as an experimental model: Strategies for developmental and molecular neurobiology studies. Methods in Cell Science, 25(1), 1-6.

56. Kumar, V., Singh, C., & Singh, A. (2021). Zebrafish an experimental model of Huntington's disease: Molecular aspects, therapeutic targets and current challenges. Molecular Biology Reports, 48(12), 8181-8194.

57. Lafollette, H., & Shanks, N. (2020). Brute science: Dilemmas of animal experimentation. Routledge.

58. Lee, S. Y., Kang, J. H., Jeong, J. W., Kim, J. H., et al. (2022). Alternative experimental approaches to reduce animal use in biomedical studies. Journal of Drug Delivery Science and Technology, 103131.

59. Lewis, D. I. (2019). Animal experimentation: Implementation and application of the 3Rs. Emerging Topics in Life Sciences, 3(6), 675-679.

60. Lilley, E., Stanford, S. C., Kendall, D. E., Alexander, S. P., et al. (2020). ARRIVE 2.0 and the British Journal of Pharmacology: Updated guidance for 2020. British Journal of Pharmacology, 177(16), 3611.

61. Martin, C. (2008). Experimental use of nonhuman primates is not a simple problem. Nature Medicine, 14(10), 1011.

62. Movia, D., Bruni-Favier, S., & Prina-Mello, A. (2020). In vitro alternatives to acute inhalation toxicity studies in animal models—a perspective. Frontiers in Bioengineering and Biotechnology, 8, 549.

63. Niederer, S. A., Lumens, J., & Trayanova, N. A. (2019). Computational models in cardiology. Nature Reviews Cardiology, 16(2), 100-111.

64. Novak, M., Žegura, B., Modic, B., Heath, E., et al. (2017). Cytotoxicity and genotoxicity of anticancer drug residues and their mixtures in experimental model with zebrafish liver cells. Science of The Total Environment, 601, 293-300.

65. Olsson, I. A. S., Nielsen, B. L., Camerlink, I., Pongrácz, P., et al. (2022). An international perspective on ethics approval in animal behaviour and welfare research. Applied Animal Behaviour Science, 105658.

66. Ponpornpisit, A., Jongjaroenjai, M., Suthamnathpong, N., & Burut-Archanai, S. (2022). Application of a single-tank sequencing batch reactor for long-term zebrafish care. Thai Journal of Veterinary Medicine, 52(3), 451-463.

67. Rodrigues, W. F., Miguel, C. B., Marques, L. C., Da Costa, T. A., et al. (2022). Predicting blood parasite load and influence of expression of iNOS on the effect size of clinical

laboratory parameters in acute Trypanosoma cruzi infection with different inoculum concentrations in C57BL/6 mice. Frontiers in Immunology, 1113.

68. Shochet, L., Holdsworth, S., & Kitching, A. R. (2020). Animal models of ANCA-associated vasculitis. Frontiers in Immunology, 11, 525.

69. Stiglic, G., Kocbek, P., Fijacko, N., Zitnik, M., et al. (2020). Interpretability of machine learning-based prediction models in healthcare. Wiley Interdisciplinary Reviews: Data Mining and Knowledge Discovery, 10(5), e1379.

70. Sweis, B. M., Abram, S. V., Schmidt, B. J., Seeland, K. D., et al. (2018). Sensitivity to “sunk costs” in mice, rats, and humans. Science, 361(6398), 178-181.

71. Tannenbaum, J., & Bennett, B. T. (2015). Russell and Burch's 3Rs then and now: The need for clarity in definition and purpose. Journal of the American Association for Laboratory Animal Science, 54(2), 120-132.

72. Tschöpe, C., Ammirati, E., Bozkurt, B., Caforio, A. L., et al. (2021). Myocarditis and inflammatory cardiomyopathy: Current evidence and future directions. Nature Reviews Cardiology, 18(3), 169-193.

73. Vasyutina, M., Alieva, A., Reutova, O., Bakaleiko, V., et al. (2022). The zebrafish model system for dyslipidemia and atherosclerosis research: Focus on environmental/exposome factors and genetic mechanisms. Metabolism, 155138.

74. Woods, T. C., Satou, R., Miyata, K., Katsurada, A., et al. (2019). Canagliflozin prevents intrarenal angiotensinogen augmentation and mitigates kidney injury and hypertension in mouse model of type 2 diabetes mellitus. American Journal of Nephrology, 49(4), 331-342.

75. Yamamoto, K. N., Nakamura, A., Liu, L. L., Stein, S., et al. (2019). Computational modeling of pancreatic cancer patients receiving FOLFIRINOX and gemcitabine-based therapies identifies optimum intervention strategies. PloS One, 14(4), e0215409.