Journal of Biostatistics and Epidemiology

J Biostat Epidemiol. 2023;9(4): 474-483

Original Article

Sample Size Calculation Based on Research Approaches in Animal Studies

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ARTICLE INFO

ABSTRACT

Received 03.05.2023
Revised 27.05.2023
Accepted 28.06.2023
Published 15.12.2023

Key words:
Sample size;
Animal studies;
Effect size;
Ethical considerations;
Statistical power.

Introduction: Sample sizes that are too small can produce inconclusive results, while sample sizes that are too large may raise ethical concerns and produce trivial outcomes. Ethical considerations of sample size calculation in animal studies are essential and researchers should consider the 3R approach. Therefore, accurately calculating the sample size is essential to ensure adequate statistical power and avoid wastage of resources.
Methods: The paper presents several innovative approaches for conducting small-scale sampling in animal studies. It includes a comprehensive review of relevant literature, discussing various proposed methods for determining sample size in animal studies.
Results: In this study, various formulas are available for preparing sample size calculations that are relevant to the research design. These include t-tests (for one sample and two independent/paired samples), ANOVA, ANCOVA, simple/multiple linear regression, as well as proportion studies and studies utilizing correlation coefficients.
Conclusion: Our aims to equip researchers with formulas for reliable findings and adherence to ethical principles for sample size calculating in animal studies.

Introduction

The determination of an appropriate sample size is crucial in both animal and human studies. In animal studies, the sample size can be determined based on factors such as the effect size, variability, and the specific outcome being examined. But what is important in animal studies is to focus on small sample size and avoid too small sample size. When the number of animals in a study is limited, there is a risk of losing any significant differences that may exist in the overall population. This can result in unreliable or inaccurate study results and

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important discoveries can be overlooked. On the other hand sample sizes that are too large can raise ethical issues over the unnecessary use of animals and may produce trivial results.[1]
Challenges in large animal sampling can briefly include the following: ethical considerations, cost-effectiveness, and regulatory limitations, such as strict regulations for a limited selection of the number of animals in the study.[2]

Previous studies show while a large sample size may enhance the chances of identifying a statistically significant result, it does not guarantee that the observed effect holds any meaningful implications in practical terms. Therefore relying solely on statistical significance without considering effect size can lead to misleading conclusions and we should pay attention to the effect size, which refers to the magnitude or strength of the relationship between the variables under study.[2] Therefore, even if a claim is statistically significant due to a large sample size, it is crucial to assess its effect size before drawing any definitive conclusions.

When conducting an animal study, the sample size involves considering various factors, such as type I (α) and type II (β) errors, one-tailed/two-tailed tests, effect size, and standard deviation. One commonly used method for determining sample size is power analysis, which takes into account these factors along with the desired power of the study.[3]

In this paper, we present several innovative approaches with considering various statistical factors such as type I (α) and type II (β) errors for conducting small-scale sampling, particularly in the context of animal studies. In Section 2, we will conduct a comprehensive review of relevant literature. In the Section 3, various proposed methods by researchers for determining sample size on animals are introduced. In Section 4 we have presented formulas for determining sample volume, which are suggested for testing the mean of a single sample, two independent samples, dependent samples, and studies that utilize correlation coefficient.

Literature Review

This literature review delves into the ethical and scientific intricacies of this concept, examining how improper sample size can undermine the very purpose of medical research.

Zhang and Hartmann[4] conducted a mini review on how to calculate sample size in animal and human studies. The review summarizes key statistical concepts and terms related to determining sample size in research. It discusses topics like mean, standard deviation, hypothesis testing, errors, power, effect size, and allocation ratio. The authors also provide practical examples of calculating sample size using information from pilot studies, previous studies, or estimated effect sizes.

Pakgohar and Khalili[5] delve into the crucial aspect of determining an appropriate sample size in qualitative research. By examining 27 frequently employed non-random sampling methods in qualitative research, they successfully establish the minimum sample size required for each method. This results proves invaluable for animal studies that employ non-random sampling methods.

Wang and Ji[6] provides an overview of study design and statistical considerations for sample size estimation in clinical research. The article discusses statistical considerations in the choice of a sample size for randomized controlled trials and observational studies, and provides strategies for reducing sample size when planning a study.

Chow et al,[7] studied on sample size calculation in microarray studies, which are a type of animal studies that use DNA chips to measure the expression levels of thousands of genes simultaneously. The chapter covers various statistical and biological issues that affect the sample size calculation in microarray studies, such as gene selection, permutation tests, resampling methods, prior information, Bayesian methods, experimental designs, and platforms.

Ricci et al.[8] review guidelines for determining sample size adequacy in animal model studies in nutrition research. They highlight that small sample sizes are common but can only be justified for large effect sizes. The authors stress the importance of considering other factors, such as precision analysis and nonparametric methods, when assumptions of normality are not met or when small samples are available.

Schmidt et al.[9] explain the importance of finding a balance between accurately observing the true impact of wild animals on crops and biodiversity, while also avoiding false results. They propose a framework that combines ecological knowledge and experimental data to create effective strategies for managing agroecosystems. They provide examples and stress the importance of considering statistical power, precision, ecological variability, and complexity in studies. They suggest tailoring sample size, experimental design, and data analysis to the specific objectives. They also recommend using adaptive management to make improvements based on the obtained results.

Sample Size Calculations in resource equation methods

In this section, the suggested methods of sample size calculation for animal studies are introduced. These methods are different in terms of techniques, but they have in common the resource equation method. Every equation has been referenced to the presenters of the method.

The "resource equation method" is an alternative way to determine sample size for small and complex biological experiments with multiple treatment groups. This method is useful when analyzing the results using analysis of variance, as power analysis is difficult in these situations. The experiment should have an appropriate sample size when the error degrees of freedom in the analysis of variance range between 10 and 20.[10]

Therefore, we can utilize the following study methods to determine the sample size, which will be presented subsequently.

Sample Size calculation in ANOVA design based on Number of groups

The resource equation method measures a value called "E," which represents the degree of freedom in analyzing variance. The constant E should ideally fall between 10 and 20. If E is less than 10, adding more animals increases the likelihood of obtaining significant results. However, if E is greater than 20, adding more animals does not increase the chances of significant results. This method, based on ANOVA, is applicable to all animal experiments. Any sample size that keeps E between 10 and 20 is considered adequate, and E can be calculated using a specific formula.[11]

$$E = N_t - N_g \quad (1)$$

Where, N_t=Total number of animals = and N_g=Total number of groups is used to calculate sample size in an experiment.

Sample Size calculation in Randomize Block ANOVA design

The resource equation method equation for determining the appropriate size is:[12]

$$E = N_t - Tr - B + 1, \quad (2)$$

Where, $N_t = n \times B$ is the number of observations in any treatment, 'Tr' is the number of treatments, B is the number of blocks, and E should be between approximately 10 and 20.

Consider a hypothetical scenario where we aim to design an experiment with 3 treatments and 5 blocks. Within each treatment, we plan to have 6 observations in each block. In this case, the total sample size would be calculated as: $E = N_t - Tr - B + 1 = (6 \times 5) - 3 - 5 + 1$, resulting in a sample size of 23. It is evident that selecting six observations in each cell within each treatment falls slightly below the maximum of 20 observations. This sample size is considered acceptable and does not exceed a burdensome level. Now, let us suppose we double this value. In that case, the new sample size would be calculated as: $E = (12 \times 5) - 3 - 5 + 1$, resulting in a sample size of 53. Research conducted by Fasting (2006) has demonstrated that despite doubling the sample size, the p-value has decreased from 0.007 to 0.001. Consequently, this increase in sample size has had minimal impact on enhancing the statistical power of the hypothesis test.[12]

Sample Size calculation in one-way ANOVA design

When conducting a one-way ANOVA, the within-subject error degrees of freedom (DF_w) can be calculated using the following formula:[13]

$$DF_w = N_t - k = k.n - k = k(n - 1). \quad (3)$$

Here, N_t represents the number of subjects, k represents the number of groups, and n represents the number of subjects per group.

To determine the value of n, the formula can be rearranged as follows:

$$n = \frac{DF_w}{k} + 1 \quad (4)$$

To find the minimum and maximum numbers of animals per group, the acceptable range of DF_w is taken into consideration. The formulas are modified by replacing DF_w with the minimum =10 and maximum =20 values:

$\mathrm{Min}(\mathrm{n}) = \frac{10}{\mathrm{k}} + 1 =$ rounded up to integer number animals/group

$\mathrm{Max}(\mathrm{n}) = \frac{20}{\mathrm{k}} + 1 =$ (rounded down to)integer number animals/group. (5)

Finally, to calculate the minimum and maximum numbers of animals required, the following formulas are used:

$$\mathrm{Min}(N_t) = \mathrm{Min}(\mathrm{n}) \times \mathrm{k} = \mathrm{k}(10/\mathrm{k} + 1)$$
$$\mathrm{Max}(N_t) = \mathrm{Max}(\mathrm{n}) \times \mathrm{k} = \mathrm{k}(20/\mathrm{k} + 1). \quad (6)$$

It is important to note that the minimum and maximum numbers of animals per group are rounded up and down, respectively, in order to maintain the degrees of freedom (DF) within the limit.

Sample Size calculation in repeated measure ANOVA design

In a repeated-measures ANOVA design, there is only one group of subjects involved. This means that the focus is solely on the within-subject factor. The within-subject error degrees of freedom (DF_w) can be calculated using the formula:[13]

$$DF_w = (n-1)(r-1). \qquad (7)$$

Here, n represents the total number of subjects, while r denotes the number of repeated measurements.

$$n = \frac{DF_w}{r-1} + 1 \qquad (8)$$

To find the minimum and maximum numbers of animals required, the DFs in the formulas are replaced with 10 and 20, respectively.
The formulas become:

$\mathrm{Min}(n) = \frac{10}{r-1} + 1 =$ rounded up to integer animals/group

$\mathrm{Max}(\mathrm{n}) = \frac{20}{r-1} + 1 =$ (rounded down to) integer.animals/group. (9)

Remark: If the animals need to be sacrificed at each measurement, the total sample sizes are calculated by multiplying the minimum and maximum sample sizes by the number of measurements (r).

Sample Size calculation in repeated-measures ANOVA with one between-subject factor

When conducting a repeated-measures ANOVA with one between-subject factor (the group), there are two error degrees of freedom (DFs) to consider: the between-subject error DF_b and the within-subject error DF_w (13-14): Then the between-subject error DF is calculated as follows:

$$DF_w = N - k = kn - k = k(n-1) \qquad (10)$$

n a repeated-measures ANOVA with one between-subject factor, DF_w is calculated as:

$$DF_w = (N-k)(r-1) = (kn-k)(r-1) = k(n-1)(r-1) \qquad (11)$$

Therefore, DF_t is the sum of Between-subject error (DF_b) and Within-subject error (DF_w):

$$DF_t = DF_b + DF_w$$
$$DF_t = k(n-1) + k(n-1)(r-1) = k \times r \times (n-1) \qquad (12)$$

Here, N represents the total number of subjects or observations in the study, k represents the number of groups or conditions in the study, n represents the number of subjects or observations in each group or condition and r represents the number of repeated measurements or observations taken on each subject or observation within each group or condition.
By rearranging the terms, we can obtain the value of n:

$$n = \frac{\mathrm{DF}_t}{k \times r} + 1 \qquad (13)$$

The formulas use specific values (10 and 20) for the minimum and maximum number of animals (n and N) by substituting them into the degrees of freedom (DF) calculations. This lets us figure out the least and most animals needed for the experiment.

$$\mathrm{Min}\left(n\right)=\frac{10}{k\times r}+1 \text{ animals / group}$$

$$\mathrm{Max}\left(n\right)=\frac{20}{k\times r}+1 \text{ animals / group.} \tag{14}$$

The minimum and maximum total numbers of animals required can be calculated as:

$$\mathrm{Min}\left(N\right)=\min\left(n\times k\right) \text{ animals / group}$$

$$\mathrm{Max}\left(N\right)=\max\left(n\times k\right) \text{ animals / group.} \tag{15}$$

It is important to note that whenever the experiment involves sacrificing the animals at each repetition, n and N must be multiplied by r.

Sample size based on Power and Infection Proportion

This formula can be used to determine the number of animals that need to be sampled to detect the presence of a pathogen. Constant β represents the chosen statistical power of hypothesis and $q = 1- p$ in which p is the proportion of animals infected. In conclusion, sample size needed to determine if an event has occurred depends on the chosen power, the proportion of infected animals, and the pathogen's prevalence. A larger sample size is required when the prevalence is low.

Sample size for the continuous variables can be determined by (15):

$$n=\frac{\log\beta}{\log q} \tag{16}$$

Results

In this section, proposed methods for determining sample size for sensitive experiments and animal studies are presented.

Sample Size calculation in two independent samples T test

It is worth mentioning that although the formula provided is for a one-way ANOVA, it can also be applied to an independent *t*-test involving two groups. In this case, the value of *k* is set to 2. The formula works because the error DF for a one-way ANOVA and the DF for an independent *t*-test are equal when comparing two groups. Conducting both a one-way ANOVA and an independent t-test (assuming equal variance) will yield the same P-value.

In order to ensure that DF_w stays within the desired range of 10 to 20 (refer to (10)), and '*k*' in two independent samples T test is equal to 2 we have:

$$\mathrm{Min}\left(\mathrm{n}\right)=\frac{10}{2}+1=6$$

$$\mathrm{Max}\left(\mathrm{n}\right)=\frac{20}{2}+1=11. \tag{17}$$

Finally, to calculate the minimum and maximum numbers of animals required, the following formulas are used:

$$\mathrm{Min}\left(N_t\right)=\mathrm{Min}\left(\mathrm{n}\right)\times 2=12$$

$$\mathrm{Max}\left(N_t\right)=\mathrm{Max}\left(\mathrm{n}\right)\times 2=22. \tag{18}$$

Sample Size calculation in paired samples T test

It is worth mentioning that although the provided formula is designed for a repeated measure ANOVA, it can also be applied to a paired samples t-test involving two steps. In this scenario, the value of k is set to 2. The formula is effective because the error degrees of freedom (DF) for a repeated measure ANOVA and the *DF* for a paired samples t-test are equal when comparing two groups.

The within-subject error degrees of freedom (DF_w) can be calculated using the formula:

$$DF_w = (N-1)(2-1). \tag{19}$$

Then

$$N = \frac{DF_w}{2-1} + 1 = DF_w + 1 \tag{20}$$

To find the minimum and maximum numbers of animals required, the DFs in the formulas are replaced with 10 and 20, respectively obtains:

$$11 \leq N_t \leq 21\ . \tag{21}$$

Sample Size calculation in ANCOVA test

When conducting ANCOVA test the adjusted within-group degree of freedom is calculated as follows:

$$DF_{w(adj)} = DF_{t(adj)} - DF_{b(adj)} - DF_{cov} = (N-1)-(k-1)-1 = N - k - 1 = k\,(n-1) - 1 \tag{22}$$

By rearranging the terms, we can obtain the value of n:

$$n = \frac{\mathrm{DFw}(\mathrm{adj})+1}{k} + 1 \tag{23}$$

The formulas use specific values (10 and 20) for the minimum and maximum number of animals (n and N) by substituting them into the degrees of freedom (DF) calculations. This lets us figure out the least and most animals needed for the experiment.

$$\mathrm{Min}(n) = \frac{10+1}{k} + 1 \text{ animals / group}$$
$$\mathrm{Max}(n) = \frac{20+1}{k} + 1 \text{ animals / group.} \tag{24}$$

It is important to note that whenever the experiment involves sacrificing the animals at each repetition, n and N must be multiplied by k.

Sample Size calculation in Multiple Regression model

When conducting a simple linear regression model, the degree of freedom for error is calculated using the following formula:

$$DF_e = N - 2 \tag{25}$$

Here, 'N' represents the total number of observations in the model. Additionally, 'K' denotes the number of independent variables present in the model.

To illustrate this further, let's consider the specific values of 10 and 20 for the minimum and maximum number of animals, respectively. By substituting these values into the degrees of freedom (DF) equation, we can determine the impact on the model. Then

$$\mathrm{Min}(N) = 10 + 2 \text{ animals / group}$$
$$\mathrm{Max}(N) = 20 + 2 \text{ animals / group.} \tag{26}$$

When it comes to multiple regression models, it is recommended to multiply the minimum and maximum number of samples obtained from the simple linear regression model by the number of predictor variables included in the regression model.

Sample Size in Hypothesis test of mean based on χ^2 and F statistics

Let us the observed values of μ_0 and σ_0^2 are obtained under the null hypothesis.
It is a well-established fact in statistical theory that:

$$\Pr\left(\frac{\bar{x}-\mu_0}{\frac{s}{\sqrt{n}}} \le t_{\alpha,n-1}\right) = 1-\alpha \qquad (27)$$

Moreover, when $X \sim T(n)$, then we would have: $Y=X^2 \sim F(1,n)$. Hence

$$\mathrm{pr}\left(\frac{\left(\bar{x}-\mu_0\right)^2}{\frac{s^2}{n}} \le f_{\alpha,1,n-1}\right) = 1-\alpha \xrightarrow{\text{yields}} \mathrm{pr}\left(s^2 \ge \frac{n\left(\bar{x}-\mu_0\right)^2}{f_{\alpha,1,n-1}}\right) = 1-\alpha \qquad (28)$$

Furthermore

$$\mathrm{pr}\left(\frac{(n-1)s^2}{\sigma_0^2} \ge \chi^2_{1-\alpha,\,n-1}\right) = 1-\alpha \xrightarrow{\text{yields}} \mathrm{pr}\left(s^2 \ge \frac{\sigma_0^2 \cdot \chi^2_{1-\alpha,\,n-1}}{n-1}\right) = 1-\alpha. \qquad (29)$$

So, we have

$$\frac{\sigma_0^2 \cdot \chi^2_{1-\alpha,n-1}}{n-1} = \frac{n(\bar{x}-\mu_0)^2}{f_{\alpha,1,n-1}} \qquad (30)$$

That implies

$$n(n-1) = \frac{\sigma_0^2 \cdot \chi^2_{1-\alpha,n-1} \cdot f_{\alpha,1,n-1}}{(\bar{x}-\mu_0)^2} \qquad (31)$$

In addition, according to $\frac{\left(\bar{x}-\mu_0\right)^2}{\sigma_0^2} \sim \chi^2_{0,1}$ we can represent the mentioned function as

$$n(n-1) \ge \frac{\chi^2_{1-\alpha,n-1} \cdot f_{\alpha,1,n-1}}{\chi^2_{\alpha,1}} \qquad (32)$$

Obtaining the solution for the quadratic equation in terms of n implies

$$n^* \ge \frac{\chi^2_{1-\alpha,n-1} \cdot f_{\alpha,1,n-1}}{\chi^2_{\beta,1}} + 1 \qquad (33)$$

The constant 'n' represents the sample size in the pretest while n^* denotes the sample size calculation for the study. By default, we can set the pretest sample size to 5, assuming a power of hypothesis of at least 0.8, which is equivalent to β=0.2. This yields a minimum required sample size of

$$n^* \ge \frac{3.32 \times 5.18}{1.64} + 1 \approx 12.$$

Sample Size calculation in correlational Study design

Based on the t-test statistics for the Pearson correlation significance test, we can derive the following equation:

$$n = T^2_{\alpha/2,n_0}\left(\frac{1}{r_{xy}^2} - 1\right) + 2 \qquad (34)$$

In this equation, 'T' represents the critical value of the t-test, which is used to determine the significance of the Pearson correlation as following:

$$T = \sqrt{\frac{n-2}{1-r_{xy}^2}} \times r_{xy}\,. \qquad (35)$$

Table 1 calculated n by substituting '$T_{\alpha/2,n_0}$'

Table 1. Sample Size Recommend at critical value α and correlation r.

α |r|	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5	0.55	0.6
0.05	31	21	15	12	10	8	7	6	6	5	5
0.04	34	22	16	13	11	9	8	7	6	5	5
0.03	38	25	18	14	12	10	8	7	6	6	5
0.02	43	28	21	16	13	11	9	8	7	6	6
0.01	55	36	26	20	16	13	11	10	8	7	6

at critical value α and pre trained sample size: $n_0=5$. Clearly increasing n_0 obtains decreasing 'n' in equation.[35] Additionally, 'r_{xy}' denotes the Pearson correlation value estimated by the researcher during the pre-study phase.

Conclusion

Sample size estimation is an important part of designing studies in clinical and preclinical research. Its purpose is to ensure that studies have enough statistical power to detect meaningful effects, while also minimizing the chances of false positive or false negative results.

In animal studies, researchers must provide a justification for the number of animals used based on ethical considerations.

Therefore, when determining the sample size for animal studies, it is advisable for researchers to take into account several factors. These factors include the 3R ethical approach, statistical principles, and the need for a robust sample size for accurate estimation. To ensure these considerations are met, it is recommended that researchers consult with statisticians. By following this approach, researchers can conduct studies that have enough power to detect potential effects, while also adhering to ethical principles and minimizing the use of animals. Ultimately, this approach enhances the overall quality and reproducibility of research.

This paper aims to explore research methodology and present various formulas for determining sample size. The formulas covered include t-tests (for one sample and two independent/paired samples), ANOVA, ANCOVA, simple/multiple linear regression, as well as correctional and proportion studies.

The objective of this study is to provide a comprehensive understanding of the research process and equip researchers with the necessary tools to determine appropriate sample sizes for their studies. By utilizing these formulas, researchers can ensure the reliability and validity of their findings.

Acknowledgements

The authors would like to thank all participants of the present study. We would like to thank the reviewers for their thoughtful comments and efforts towards improving our manuscript.

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