How to calculate sample size in animal studies?

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ABSTRACT

Calculation of sample size is one of the important component of design of any research including animal studies. If a researcher select less number of animals it may lead to missing of any significant difference even if it exist in population and if more number of animals selected then it may lead to unnecessary wastage of resources and may lead to ethical issues. In this article, on the basis of review of literature done by us we suggested few methods of sample size calculations for animal studies.

Key words: Alpha error, animal studies, power, sample size

How many animals I should use for my study? This is one of the most confusing questions faced by a researcher. Too small sample size can miss the real effect in experiment and too large sample size will lead to unnecessary wasting of the resources and animals.[1] Issue of sample size has been highlighted adequately for the clinical trials and clinical studies, but not explored much in the case of animal studies in published literature. It is very important to teach young researchers and post-graduate students regarding importance and methods of sample size calculation. To clarify this issue of sample size in animal studies, we decided to search various articles available regarding the sample size in animal studies. We did PubMed search by using various MeSH terms such as "sample size," "sample size calculations," "animal studies" etc., and their combinations. We have also searched various articles through Google and Google Scholar. We have also searched various websites related to animal research (http://www. 3rs-reduction. co.uk/html/6__power_and_sample_size.html, http://www. acuc.berkeley.edu/, http://www.bu.edu/orccommittees/iacuc/ policies- and-guidelines/sample-size-calculations/, http://www. ucd.ie/researchethics/etc.). First author read all available literature and an understanding about the concept is made in consultation with the second author. Here, we are explaining briefly about the method of sample size calculations in animal studies based on review of the literature carried out by us.

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Basically, there are two methods of sample size calculation in animal studies. The most favored and most scientific method is calculation of sample size by power analysis.[2] Every effort should be carried out to calculate sample size by this method. This method is similar to the method used for calculation of sample size for clinical trials and clinical studies. Simple calculation can be carried out manually with the help of some formula [Appendix 1], but for complex calculations statistical software can be used or help from a statistician can be sought. To calculate the sample size by power analysis a researcher must have knowledge and information about these concepts:

- Effect size: This is the difference between the mean of two groups (quantitative data) or proportions of events in two groups (qualitative data). A researcher should decide before the start of the study that how much minimum difference between two groups can be considered as clinically significant. The idea about clinically significant difference between the groups should be taken preferably from previously published studies[2-5]
- Standard deviation: Standard deviation measures variability within the sample. Information about standard

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deviation is needed only in the case of quantitative variables. Information about the standard deviation of a particular variable can be taken from previously published studies. If no such study is available then author should conduct a pilot study first and standard deviation can be calculated from the pilot study[2-5]

- Type 1 error: This is measured by significance level, which is usually fixed at the level of 5% ($P = 0.05$). This is an arbitrary value and can be decreased or increased according to the research question[2-5]
- Power: Power of a study is probability of finding an effect, which the study is aimed to find. This may be kept between 80% to even 99% depending on research question, but usually, it is kept at 80%[2-5]
- Direction of effect (one tailed or two tailed): When a researcher wants to explore the effect of some intervention, the actual effect observed in sample may be in same direction as researcher thought or it may be just opposite to that. If researcher feels that effect may be in both directions then he should use two tailed test and if he has strong reason to believe for the effect to lie in one direction then he can use one tailed test. In animal research, two tailed tests are usually used[2]
- Statistical tests: For sample size calculation, it is important to have an idea about statistical test, which is to be applied on data. For simple statistical tests such as Students *t*-test or Chi-square test, manual calculation based on formula can be carried out [Appendix], but for complex tests like ANOVA or non-parametric tests help of statistician or use of software is needed[2,4]
- Expected attrition or death of animals: Final sample size should be adjusted for expected attrition. Suppose a researcher is expecting 10% attrition then the sample size calculated by formula or software should be divided by 0.9 to get actual sample size. Suppose sample size calculated by software is 10 animals per group and researcher is expecting 10% attrition then his final sample size will be 11 animals per group (10/0.9 = 11.11). Similarly, for 20% attrition sample size should be divided by 0.8.[5] This can be explained in the form of structured formula i.e.,

Corrected sample size = Sample size/ (1− [% attrition/100])

We suggest use of freely downloadable software G Power (Faul, Erdfelder, Lang and Buchner, 2007) for sample size calculation. This software is equally good for sample size calculation for clinical trials also. This software can be used for simple as well as complex sample size calculations.[6] G Power can calculate sample size based on pre-designed effect size at small, medium, and large difference between the groups based on Cohen's principles.[7] Information about other freely available software and calculators for sample size calculation is given in Appendix 2. More complex sample size will need more sophisticated software such as "nQuery advisor" or "MINITAB."

Second method of calculation is a crude method based on law of diminishing return. This method is called "resource equation" method.[2,8,9] This method is used when it is not possible to assume about effect size, to get an idea about standard deviation as no previous findings are available or when multiple endpoints are measured or complex statistical procedure is used for analysis. This method can also be used in some exploratory studies where testing of hypothesis is not the primary aim, but researcher is interested only in finding any level of difference between groups.

According to this method a value "E" is measured, which is nothing but the degree of freedom of analysis of variance (ANOVA). The value of E should lie between 10 and 20. If E is less than 10 then adding more animals will increase the chance of getting more significant result, but if it is more than 20 then adding more animals will not increase the chance of getting significant results. Though, this method is based on ANOVA, it is applicable to all animal experiments. Any sample size, which keeps E between 10 and 20 should be considered as an adequate. E can be measured by following formula:

E = Total number of animals − Total number of groups

Suppose a researcher wants to see the effect of a drug and he made five groups (one group as control and four groups of different doses of that drug) with 10 rats each. In this case E will be

$E = (10 \times 5) - 5$

$E = 50 - 5 = 45$, which is more than 20 hence sample size in this experiment is more than necessary. However, if sample size is five per group then E will be 20, which is the acceptable limit and hence can be considered as adequate sample size.

This method is easy, but it cannot be considered as robust as power analysis method.

We want to suggest researchers to include a statement about method of calculation of sample size and justification of sample size in the manuscript they want to publish. Animals in research: Reporting *in vivo* experiments guideline recommends inclusion of a statement mentioning justification of the sample size used in research and detail of method of calculation of sample size.[10] All components of sample size calculation such as effect size, type 1 and type 2 error, one tailed/two tailed test, standard deviation etc., should be reported in manuscript sent for publication the way it is suggested for the clinical research.[11] Shortage of resources (budget, manpower), time constrain etc., cannot be considered as valid justification regarding decision of sample size. Many researchers consider six animals per group as adequate sample size, but after reviewing available literature on this issue we came to a conclusion that this notion of six animals per group has little scientific and statistical basis. This is a brief description and readers are requested to read more resources available for better understanding of various concepts related to the sample size calculation in animal studies.

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Appendix 1

- Formula for sample size calculation for comparison between two groups when endpoint is quantitative data.

When the endpoint is in quantitative data (ratio and interval scale) such as weight, height, concentration of drug etc., then this formula can be used for calculation of sample size for comparison between two groups.[5]

Sample size = $2\ SD^2\ (Z^{\alpha/2} + Z^{\beta})^2/d^2$

Where
Standard deviation = from previous studies or pilot study
$Z^{\alpha/2} = Z_{0.05/2} = Z_{0.025} = 1.96$ (From Z table) at type 1 error of 5%
$Z^{\beta} = Z_{0.20} = 0.842$ (From Z table) at 80% power
d = effect size = difference between mean values

Hence now formula will be

Sample size = $2\ SD^2\ (1.96 + 0.842)^2/d^2$

For example, if a researcher wants to see the effect of a potential antihypertensive herbal drug in rats. He wants to compare the new drug with placebo. Mean blood pressure according to previous study in the spontaneous hypertensive rats was 170 mmHg. Researcher thinks that if a new drug reduced this blood pressure to 130 mmHg then it should be considered as significant. Suppose standard deviation found in previously carried out studies was 25 mmHg and researcher selects the level of significance at 5% and power of study at 80%. He thinks suitable statistical test in this condition will be two tailed unpaired *t*-test. The effect size in this situation will be 170 − 130 = 40. Hence, the sample size will be

Sample size = $2\ (25)^2\ (1.96 + 0.842)^2/(40)^2$ (see formula) = 6.09

Hence in this case the researcher needs 6 animals per group. If researcher thinks that 10% of animal may die during an experiment and that may decrease the power of the study then, he can adjust this attrition in calculated sample size. For 10% attrition he has to choose 7 rats per group (6/0.9 = 6.7).

- Formula for sample size calculation for comparison between two groups when endpoint is qualitative.

When endpoint is in qualitative scale such as alive/death, diseased/non-diseased, male/female etc., then, this formula can be used for sample size calculation for comparison between two groups.[5]

Sample size = $2\ (Z^{\alpha}_{/2} + Z^{\beta})^2 \times P\ (1 - P)/(p_1 - p_2)^2$

$Z^{\alpha}_{/2} = Z_{0.05/2} = Z_{0.025} = 1.96$ (From Z table) at type 1 error of 5%
$Z^{\beta} = Z_{0.20} = 0.842$ (From Z table) at 80% power
$p_1 - p_2$ = Difference in proportion of events in two groups
P = Pooled prevalence = (prevalence in case group [p_1] + prevalence in the control group [p_2])/2

For example, a researcher is interested in exploring the effect of an herbal combination against methotrexate induced liver injury. Suppose survival is an important endpoint in this study. Previous study says that if methotrexate is given for 4 weeks intraperitonealy to rats 50% of them will die within this period hence survival is 40% (0.4 proportions). Researcher feels that if new herbal preparation increases survival to 90% (0.9 proportions) then, these finding can be considered as significant. Effect size will be difference between percentages. 0.4 − 0.9=−0.5.

At 5% of significance level and 80% power, sample size will be

Pooled prevalence = 0.4 + 0.9/2 = 0.65
Sample Size = $2\ (1.96 + 0.842)^2 \times 0.65\ (1 - 0.65)/(-0.5)^2 = 14.26$

Hence the researcher needs 14 rats per group. This value, if adjusted for 10% attrition will be around 16 rats per group.

Hence simple calculation for two unpaired groups can be done manually by given formula, but for more than two groups and for other complex calculations software should be used. Analysis of variance is one of the most frequently used the statistical test in animal research as multiple groups are taken in animal research. To calculate the sample size for ANOVA, it is better to use software like G Power. If author wants to do manual calculation then, he can think of effect size as difference between means or proportions of two groups out of all groups where there will be maximum difference and then he can calculate sample size by above formulae given for two groups.

Appendix 2

Few free software and calculators available online for sample size calculation

These software's can be used for sample size calculations through power analysis method. Some of them can also be used for other statistical methods.

Open Epi
http://www.openepi.com/OE2.3/Menu/OpenEpiMenu.htm
Biomath
http://www.biomath.info/power/index.htm
EpiTools epidemiological calculators.
http://epitools.ausvet.com.au/content.php?page = SampleSize
Java applets for power and sample size
http://homepage.stat.uiowa.edu/~rlenth/Power/#Download_to_run_locally
StatPages
http://statpages.org
Department of Biostatistics, Vanderbilt University
http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize
G Power
http://www.psycho.uni-duesseldorf.de/aap/projects/gpower
Power analysis for ANOVA
http://www.math.yorku.ca/SCS/Online/power
Statistics calculators
http://danielsoper.com/statcalc3/default.aspx

REFERENCES

1. Fitts DA. Ethics and animal numbers: Informal analyses, uncertain sample sizes, inefficient replications, and type I errors. J Am Assoc Lab Anim Sci 2011;50:445-53.
2. Festing MF, Altman DG. Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR J 2002;43:244-58.
3. Lenth RV. Some practical guidelines for effective sample size determination. Am Stat 2001;55:187-93.
4. Jones SR, Carley S, Harrison M. An introduction to power and sample size estimation. Emerg Med J 2003;20:453-8.
5. Naduvilath TJ, John RK, Dandona L. Sample size for ophthalmology studies. Indian J Ophthalmol 2000;48:245-50.
6. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39:175-91.
7. Cohen J. Statistical Power Analysis for the Behavioral Sciences. 2nd ed. Hillsdale, NJ: Lawrence Erlbaum; 1988.
8. National centre for replacement, refinement, and reduction animals in research. Experimental design/statistics. [about 2 screen] Available from: http://www.nc3rs.org.uk/category.asp?catID=7 [Last cited on 2012 Nov 02].
9. Festing MF. Design and statistical methods in studies using animal models of development. ILAR J 2006;47:5-14.
10. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. J Pharmacol Pharmacother 2010;1:94-9.
11. Jaykaran P, Yadav N, Chavda, Kantharia ND. Some issues related to the reporting of statistics in clinical trials published in Indian Medical Journals: A survey. Int J Pharmacol 2010;6:354-9.

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