

REVIEW

Preferred Reporting Items for Animal Studies in Endodontology: a development protocol

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Abstract

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The regulated use of animals in endodontic research is often necessary to investigate the biological mechanisms of endodontic diseases and to measure the pre-clinical efficacy, biocompatibility, toxicology and safety of new treatments, biomaterials, sealers, drugs, disinfectants, irrigants, devices and instruments. Animal testing is most crucial in situations when research on humans is not ethical, practical or has unknown health risks. Currently, there is a wide variability in the quality of manuscripts that report the results of animal studies. Towards the goal of improving the quality of publications, guidelines for preventing disability, pain, and suffering to animals, and enhanced reporting requirements for animal research have been developed. These guidelines are referred to as Animals in Research: Reporting *In Vivo* Experiments (ARRIVE). Henceforth, causing any form of animal suffering for research purposes is not acceptable and cannot be justified under any circumstances.

The present report describes a protocol for the development of welfare and reporting guidelines for animal studies conducted in the specialty of Endodontology: the Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) guidelines. The PRIASE guidelines will be developed by adapting and modifying the ARRIVE guidelines and the Clinical and Laboratory Images in Publication (CLIP) principles. The development of the new PRIASE guidelines will include a five-step consensus process. An initial draft of the PRIASE guidelines will be developed by a steering committee. Each item in the draft guidelines will then be evaluated by members of a PRIASE Delphi Group (PDG) for its clarity using a dichotomous scale (yes or no) and suitability for its inclusion using a 9-point Likert scale. The online surveys will continue until each item achieves this standard, and a set of items are agreed for further analysis by a PRIASE Face-to-face Consensus Meeting Group (PFCMG). Following the consensus meeting, the steering committee will finalize and confirm the PRIASE guidelines taking into account the responses and comments of the PFCMG. The PRIASE guidelines will be published and disseminated internationally and updated periodically based on feedback from stakeholders.

Keywords: animal, consensus, delphi technique, endodontics, health research reporting guidelines, protocol.

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Introduction

Animal testing is crucial in situations when research on humans is not permitted due to ethical concerns, or when a new material, device or drug has unknown human health risks (Henderson *et al.* 2013). The use of appropriate animal model(s) prior to clinical trials in humans is thus an essential stage in research within the broad field of Endodontology. In Endodontics, animal testing is often necessary following laboratory experiments and prior to clinical trials of new treatments, stem cell therapies, drugs, materials, sealers, irrigants, disinfectants, instruments and devices. This is also necessary for investigating the biological mechanisms of endodontic diseases and tissue healing and regeneration potentials. Animal testing can play a pivotal role for validating the safety, biocompatibility and toxicology of new clinical techniques or biomaterials and regenerative therapies for treating conditions such as apical periodontitis. Apical periodontitis is a host immune response in the periapical region due to the presence of a microbial infection within the root canal system (Ricucci & Siqueira 2010). Endodontic disinfection is necessary to debride the microbes and infected tissue from the root canal system. Subsequently, the root canal space is filled with a sealer and suitable materials and the tooth restored to function. The objective of most surgical and non-surgical endodontic treatments is to remove infected, diseased and necrotic tissues to achieve healing and regeneration (Saoud *et al.* 2016). Researchers must often use animal experimentation to collect research data, because these experiments often cannot be replicated effectively in a laboratory using extracted teeth.

Rodents, including rats and mice appear to be the most common types of animals used in Endodontic research. Larger animals including dogs, cats, ferrets, guinea pigs, rabbits, sheep, mini-pigs and even non-human primates have also been used in past animal tests. However, studies using large animal have become rare, due to public opposition to animal testing on pet species, and some prohibitions were introduced on non-human primate research. Tests with animals are expensive, and they require extensive time and effort to comply with national animal welfare guidelines. The physiopathology and metabolism of rodents and small animals is dissimilar to humans and so, experimental animal models are likely to lack clinical relevance and will thus yield results that are not reproducible in humans. In addition, many

animals used in research do not have a fully functional immune system and thus lack a clinically relevant immune response to endodontic treatment. In these studies, the authors should clarify that the use of immune suppressed animals (e.g. SCID) does not replicate functioning immune responses. Another common problem is that dental and endodontic instruments are often too large for use in the minuscule root canals of small animal teeth. Measures need to be taken to adapt endodontic instruments and material volume usage to the correct scale for the anatomy and size of the animal teeth and oral tissue.

Modern non-invasive imaging technologies, such as micro-computed tomography should be employed whenever possible to allow data to be collected at several time-points, thereby reducing the numbers of animals needed to obtain data. The animal care regulations are similar for all vertebrate mammals, and it is not clear if there is a need to distinguish the reporting requirements for rodents, household pets (dogs, cats, rabbits, ferrets) and farm animals (pigs, sheep). Most animal studies are performed over a short time span because of the high costs involved and are therefore not long enough to detect chronic inflammatory reactions, systemic diseases or tumours that have developed in response to test materials. Some recommendations are needed for animal testing in terms of duration (e.g. 7 and 28 days) to increase the reliability of the safety, biocompatibility, inflammatory, systemic, cancer or allergy data. At present, there appear to be few or no animal studies which report unexpected animal deaths, and adverse events such as lethargy and behavioural signs of suffering. Yet this restricted information can be useful to readers who can make their own assumptions about the safety of experimental treatments. In spite of these problems, challenges and advantages, animal research today is considered a contentious area of science, especially since some animal studies can fail to report the results adequately, often lacking sufficient information to replicate the experiments (Kilkenny *et al.* 2010). A review of some animal studies revealed that most had poorly designed experiments, raising both ethical and scientific concerns. A review commissioned by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), further highlighted serious flaws in the way research using animals has been conducted and reported (Kilkenny *et al.* 2009). Poor quality reporting in publications using animal models will translate into difficulties in clinical correlation and or decision-

making. This in turn would compromise the subsequent development of treatment policies or guidelines.

To address the issues of inadequate reporting of animal studies, the NC3Rs published the 'Animals in Research: Reporting *In Vivo* Experiments' (ARRIVE) guidelines (Kilkenny *et al.* 2010). The ARRIVE guidelines have been developed primarily from the 'Consolidated Standards of Reporting Trials' (CONSORT) statement (Schulz *et al.* 2010). They are made up of 20 items focusing on reporting of title, abstract, introduction, methods, results and discussion of an animal study. In addition to the general items applicable to any scientific study, additional items such as details of the type of experimental animal, housing, husbandry and the allocation of animals to experimental groups are included to better reflect the focus of study designs involving animals (Kilkenny *et al.* 2010). The ARRIVE guidelines are readily available, and several journals and research institutes have endorsed them; however, the quality of reporting in animal research can sometimes be sub-optimal, making them impossible to reproduce (Flórez-Vargas *et al.* 2016, Nam *et al.* 2018). Indeed, a working group has been formed recently to revise the existing ARRIVE guidelines (Percie du Sert *et al.* 2018).

Authors need to be mindful whilst describing the data and images obtained from animals in a manuscript, to ensure that the interpretations and conclusions of the study are unbiased, accurate and that they do not overgeneralize the animal results to humans. In some scientific publications, the reliability of radiographs, micro-computed tomography and histologic data collected can easily be distorted by interpretation difficulties, and inter- or intra-rater disagreements due to the substantial variability that can be observed in biological specimens. Furthermore, due to limits on word count, number of pages and figures, the analysis and description of images in manuscripts can often be superficial and incomplete. Sometimes, there were no controls to help validate the data and artefacts can occur due to a poor study design that are not discovered until after the study has ended. Additionally, application of software-based image analysis and machine-learning data collection algorithms should be described clearly with relevant interpretation calibrations. Due to the high risk of errors created by these potential problems, complete and detailed information about the images on which the findings of the study rely upon becomes vital. In response to this, Lang *et al.* (2012) proposed six principles for reporting images in publications within their

document, 'Clinical and Laboratory Images in Publications (CLIP) principles'. The CLIP principles will be modified and adapted within the new PRIASE guidelines to help authors to improve the quality of images used in animal studies as well as how they are reported.

The existing ARRIVE guidelines can be applied to any field of biomedical sciences as they provide a general overview of the required items in a manuscript. However, animal studies in Endodontology often require specialized endodontic information that are not included within any existing guidelines or other policy documents. Therefore, it has become necessary to develop and validate guidelines for animal studies in Endodontology. The aim of this project is to formulate a protocol to develop and disseminate the Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) guidelines. PRIASE guidelines will help to improve the quality, accuracy, reproducibility, completeness and transparency in reporting all types of animal studies within the Endodontology specialty. Additionally, supplementary information on the lack of availability of alternatives to animal testing, and the minimization of the numbers of animals used in testing will be requested. In addition to details of the steps taken to minimize animal injury and disability, to prevent animal oral suffering and to monitor animal oral suffering will be requested along with housing conditions, feeding animals with painful teeth and the amount of veterinary care. Information will also be requested concerning unexpected deaths and the emergency euthanization of animals. It should also be necessary to employ painless euthanization procedures on the animals at the conclusion of the study. Euthanasia by neck breaking, drowning, asphyxia, gassing or choking animals to death is no longer acceptable. It is also not acceptable to withhold analgesics or pain relief to alleviate any potential suffering, whilst intentionally inflicting severe prolonged pain and/or disability, such as through (i) exposed pulp and open root canals to create infections, (ii) the creation of large infected lesions by plaque infection, (iii) restricting blood flow to create gangrenous/necrotic tissues, (iv) severing nerves and muscles, (v) exposing animals to severe heat or cold, burn, chemical or radiation injuries, (vi) mimicking trauma, wounding, severing limbs, harvesting tissues and organs, (vii) starving animals and/or feeding them toxic substances and (viii) re-using animals in further painful studies. Therefore, causing any form of animal suffering involving prolonged pain and disability for

research purposes is not acceptable and cannot be justified under any circumstances. This is because animal suffering during research goes against the majority wishes of the general public, dentists, dental patients, dental suppliers and researchers. To ensure the prevention of animal suffering becomes the highest priority for researchers and their assistants, studies suspected of inflicting unnecessary animal suffering should not be considered for publication in Endodontology. Finally, the guidelines will help editors and peer reviewers of scientific articles to critically assess the quality of animal welfare and to ensure that all the essential details about the animal studies will be reported during the manuscript submission process.

Methodology

The development of the PRIASE guidelines will adhere to the recommendations from the Guidance for Developers of Health Research Reporting Guidelines (Moher *et al.* 2010) and follow similar methodology to that used to develop the guidelines for Preferred Reporting Items for Case reports in Endodontics (PRICE) (Nagendrababu *et al.* 2019a), the Preferred Reporting Items for RANdomized Trials in Endodontics (PRIRATE) (Nagendrababu *et al.* 2019b) and the Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) (Nagendrababu *et al.* 2019c). The process will involve five phases with a steering committee being responsible for facilitating the development of the guidelines. The process will involve the creation of a PRIASE Delphi Group (PDG) and PRIASE Face-to-face Consensus Meeting Group (PFCMG) comprising a diverse range of experts who will participate in the consensus process for the guideline development. Figure 1 shows the five-step consensus process in the form of a flow chart.

Phase I: initial steps

The project leaders (VN, PD) conducted a thorough literature search including the EQUATOR Network database of reporting guidelines to identify the need for the development of guidelines on reporting animal studies in Endodontology. The project leaders decided to combine, adapt and augment the ARRIVE guidelines (Kilkenny *et al.* 2010) and the CLIP principles (Lang *et al.* 2012) to create the new PRIASE guidelines specifically tailored to the field of Endodontology. A steering committee comprising nine members (PD, VN, AK, PM, MHN, JF, EP, JJ, SP) was formed to

develop the initial draft PRIASE guidelines and to refine the process of achieving consensus with the assistance of world-leading experts in the field of Endodontology as well as general dental practitioners and members of the general public.

Phase II: pre-meeting activities

The steering committee will form the PRIASE Delphi Group (PDG), comprising of 30 experts including 22 academicians or researchers and four Endodontists, who must satisfy at least one of the following criteria to be eligible to participate in the Delphi process: (i) published at least one animal study in Endodontics; (ii) published any reporting guidelines for *in vitro/in vivo* research; (iii) a minimum of 15 years clinical experience in Endodontics. Additionally, two general dentists and two public representatives will be included in the Delphi process. The PDG group will be invited to participate in an explicit consensus development process. Following the confirmation of the PDG members, a document explaining the Delphi process and their role will be shared with them. The Delphi process will involve sequential surveys to achieve consensus on the inclusion or exclusion of the proposed items in the PRIASE guidelines (checklist and flow chart) drafted by the steering committee. Each item of the draft PRIASE guidelines will be assessed by the PDG members independently and confidentially to confirm: (1) the clarity of the item using a dichotomous scale (yes or no) and (2) the suitability of the item for its inclusion on a 9-point Likert scale (1 = 'definitely not include' to 9 = 'definitely include'). Additionally, the PDG members can express their comments for each item. This will allow the steering committee to better analyse the response of the PDG members (Maher *et al.* 2015). Items being scored as 7–9 by at least 70% and 1–3 by <30% of PDG members will be included in the PRIASE checklist. Items getting a score of 1–3 by more than 70% and 7–9 by at most 30% of members will be excluded. Results of each Delphi round will inform the subsequent round by proposing the necessary modifications of the items. This process will continue until this standard is achieved and a final set of items are agreed for the PRIASE guidelines (Agha *et al.* 2017). At the end of each Delphi round, the PDG members will be provided with a summary of the results and the set of revised items.

Following the initial consensus on the items within the draft PRIASE guidelines, a face-to-face consensus meeting will be conducted. This meeting will comprise



Figure 1 Five-step consensus process. PDG, PRIASE Delphi Group; PFCMG, PRIASE Face-to-face Consensus Meeting Group; PRIASE, the Preferred Reporting Items for Animal Studies in Endodontology; PRIDE, Preferred Reporting Items for study Designs in Endodontology.

two chair persons and 18 members selected by the steering committee. The eligibility criteria for the PFCMG will be the same as the PDG; PDG members will be eligible to be part of the PFCMG. Additionally, two Endodontic postgraduate students will be invited to participate in the meeting and provide their views on the guidelines. Following the confirmation of members, information on the venue, date and time of the meeting will be provided to the PFCMG. At least

10 days prior to the meeting, the PFCMG members will be provided with the draft PRIASE checklist, flow chart, results of Delphi process, members' details and the agenda for the face-to-face meeting.

Phase III: face-to-face consensus meeting

The meeting will start by reviewing the objectives of the meeting and presenting the results of the

Delphi process by the project leads (VN, PD). Following this, the rationale for including the items in the PRIASE checklist will be discussed along with the content of the flow chart. The PFCMG will also discuss and clarify any outstanding issues during the meeting. Subsequently, the PFCMG will discuss the elaboration and explanation of each included item in the PRIASE checklist and the flow chart to finalize the reporting guidelines. Furthermore, plans for disseminating PRIASE guideline, journal endorsement and strategies to ensure adherence to the reporting guideline will be discussed. Notes of discussions during the meeting will be kept for future reference.

Phase IV: post-meeting activities

The steering committee will amalgamate the results of the Delphi process and the discussions that occurred during the face-to-face meeting to finalize the PRIASE guidelines. The guidelines will be supplemented with an explanation of the rationale and evidence for each included item and elaborate the details of the item. Each item in the PRIASE checklist will be accompanied by at least one illustrative example of good reporting to guide the reporting of animal studies and their critical appraisal by researchers, journal editors, peer reviewers and readers. The examples of good reporting for each item will be prepared by the steering committee and will be sent to six members (three from the PDG and three from the PFCMG) for their approval. This document will serve as a 'user manual'. The steering committee will be responsible for the publication of the PRIASE guidelines and any supporting documents in peer-reviewed journals. Additionally, the reporting guidelines will be presented at various international endodontic and dentistry conferences and meetings.

Phase V: post-publication activities

The steering committee will be responsible for ensuring editors of relevant journals will endorse the PRIASE guidelines. To ensure effective implementation of the PRIASE guidelines and their ancillary documents a dedicated website, the Preferred Reporting Items for study Designs in Endodontology (PRIDE) will be established and made freely available. The steering committee will welcome and address feedback and criticism from stakeholders. The PRIASE guideline will also be translated into various languages. Finally, the

steering committee will ensure that the PRIASE guidelines are updated periodically, to reflect potential changes to good practice.

Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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