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PRIASE 2021 guidelines for reporting animal studies in Endodontology: a consensus-based development

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Abstract

Animal testing is crucial in situations when research on humans is not allowed because of unknown health risks and ethical concerns. The current project aims to develop reporting guidelines exclusively for animal studies in Endodontology, using an established consensus-based methodology. The guidelines have been named: Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) 2021. Nine individuals (PD, VN, AK, PM, MN, JF, EP, JJ, SJ), including the project leaders (PD, VN) formed a steering committee. The steering committee developed a novel checklist by adapting and integrating their animal testing and peer-review experience with the Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines and also the Clinical and Laboratory Images in Publications (CLIP) principles. A PRIASE Delphi Group (PDG) and PRIASE Online Meeting Group (POMG) were also formed. Thirty-one PDG members participated in the online Delphi process and achieved consensus on the checklist items and flowchart that were used to formulate the PRIASE guidelines. The novel PRIASE 2021 guidelines were discussed with the POMG on 9th September 2020 via a Zoom online video call attended by 21 individuals from across the globe, and seven steering committee members. Following the discussions, the guidelines were modified and then piloted by several authors whilst writing a manuscript involving research on animals. The PRIASE 2021 guidelines are a checklist consisting of 11 domains and 43 individual items together with a flowchart. The PRIASE 2021 guidelines are focused on improving the methodological principles, reproducibility and quality of animal studies in order to enhance their reliability as well as repeatability to estimate the effects of endodontic treatments and usefulness for guiding future clinical studies on humans.

Introduction

Animal studies fulfil specific roles in dental research, particularly within Endodontology where there is a need to exclude confounding human variables to aid in the understanding of an array of biological and molecular mechanisms of infection, disinfection, inflammation, necrosis, healing, regeneration and disease progression. Animal studies are also essential for testing the safety and effectiveness of new dental materials, medicaments, drugs, devices and instruments which have unknown health risks for human participants. Despite the extensive use of animals in experiments, the clinical translation of research outcomes from these animals can be challenging due to the differences in anatomical dimensions, where human-sized instruments are difficult to use and/or be assessed in small animal root canals (Yoneda *et al.* 2017). In addition, there are potential physiological differences whereby human teeth completely stop growing at maturity, but mature rodent teeth can keep increasing in length by up to 1 mm per day (Law *et al.* 2003). There are also metabolic differences between humans and animals, where healing and regeneration responses can be observed in the exposed pulp of rodent teeth (Kakehashi *et al.* 1965) that will not occur within the inflamed pulps of human teeth (Mjör 2002). Despite these controversial differences, some published studies have argued that the healing of rat molar pulp tissue after direct pulp capping is histologically comparable with human teeth (Stanley 1992, Dammaschke 2010). The improper clinical translation of findings from animal studies can explain the unrealistic expectations for the success of some biomaterials for vital pulp therapies in humans, due to the correlative absence of a dentine bridge and pulpitis after the direct capping of the exposed dental pulp (Accorinte *et al.* 2005). Non-human primate dental pulps may heal following acid etching and direct pulp capping with composite resins (Cox *et al.* 1987), which is contraindicated for human teeth, because it is a disastrous treatment (Hörsted-Bindslev *et al.* 2003).

In addition to the problems of translation, small differences, artifacts or flaws in the experimental design can lead to reproducibility problems, even between similar animal studies, where the success of pulp capping treatments (Cox *et al.* 1987) cannot be replicated in similar animal models, where they may result in disastrous treatment failures (Pameijer & Stanley 1998). Some of the reasons why animal studies cannot adequately replicate human variables are because of the irreconcilable differences in anatomy, ages, health status, histo-pathophysiology, disease progression, infections, medications and treatment history that are more suitably addressed within a clinical trial (van Lujik *et al*. 2014). A review of the methodological quality of systematic reviews involving animal studies within dentistry reported the need for improvements in their methodological principles (Faggion Jr *et al*. 2012). In addition, improvements are required to increase their reproducibility, validity and quality of reported methods and results so as to improve their reliability to estimate the effects of treatment as well as to guide future clinical studies on humans.

In an initial attempt to improve the reporting quality of animal studies, the ARRIVE (Animals in Research: Reporting *In Vivo* Experiments) guidelines were developed (Kilkenny *et al*. 2010) as an extension of the Consolidated Statement for Reporting Trials (CONSORT) (Schulz *et al.* 2010). The ARRIVE guidelines consist of 20 items that are essential when reporting experiments on animals. They include the need to report the number and specific characteristics of the animals used, e.g. species, strain, sex, age and genetic background, details of housing and breeding, as well as the components of the study design including experimental, statistical, and analytical methods. Clearly, these detailed descriptions are intended to promote high-quality, comprehensive reporting in animal research (Kilkenny *et al*. 2010). More recently, the ARRIVE 2.0 guidelines have been published (Percie du Sert *et al.* 2020); they contain a checklist of 20 items divided into two sets: those that are deemed "Essential" constitutes the minimum requirement to be included in reports of animal studies, and those that are "Recommended" describes the research content. The classification of the items into two categories has facilitated reporting of animal research by allowing an initial focus on the

most critical issues (Percie du Sert *et al*. 2020). However, the existence of multiple guidelines for reporting the methods and results of animal studies can be confusing for authors and peer-reviewers. Thus, there is a clear requirement for a consensus checklist of essential pre-peer-review reporting items, which can provide specific guidance to authors, while facilitating the peer review process.

Ideally, animal studies should report every useful detail to the peer-reviewers and readers, but publication word limits, make that goal virtually impossible. A more realistic goal is for animal studies to report the most significant information. That will include the accuracy, validity, comprehensiveness, interpretation and implications of images in journal articles, such as has been addressed by the development of the Clinical and Laboratory Images in Publications (CLIP) principles (Lang *et al.* 2012). Therefore, these CLIP principles can be included into the reporting of animal studies in the field of Endodontology, to ensure that the images, figures, data and results are reported uniformly to aid in improving the reproducibility and comparability of different animal studies.

Endodontology is a highly specialized field within dentistry that has its own language, definitions and terminologies, which requires customized information specific to the location, severity, type and cause of the disease, trauma and infection, pulp chamber access preparation, canal instrumentation, root canal centring, root canal debridement, periapical lesions, pulp sensibility testing, tissues, medicaments, disinfection solutions, ultrasonic activation, chelating agents, sealers, root canal filling techniques, handling and placement of biomaterials, use of restorative materials, imaging and assessing treatment outcomes. Hence, by soliciting and integrating the input of peer-reviewers from across the world on the information needed to improve the quality of animal studies, together with the ARRIVE guidelines (Kilkenny *et al*. 2010), ARRIVE 2.2 guidelines (Percie du Sert *et al*. 2020), and CLIP guidelines (Lang *et al*. 2012), the unique Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) 2021 guidelines have been created. The PRIASE 2021 guidelines have been designed to improve the quality, accuracy, reproducibility, completeness and transparency of reports describing all types of animal studies in Endodontology (Nagendrababu *et*

al. 2019). This article aims to provide insight into the development of the PRIASE guidelines for reporting animal studies in Endodontology through a consensus-based approach.

Methods

An ethical approval memo for the project leaders (PD, VN) to pursue this research was issued by the Institutional Review Board on Research and Ethics of the International Medical University (IMU), Kuala Lumpur, Malaysia (No: IMU 450/2019) and University of Sharjah, Sharjah, UAE (REC-20-11-06-01). The PRIASE 2021 guidelines were developed in accordance with the recommendations given in the Guidance for Developers of Health Research Reporting Guidelines (Moher *et al*. 2010) and a more detailed protocol has been published (Nagendrababu *et al*. 2019)

Initial steps

The project leaders (VN and PD) identified the knowledge gap for guidelines for authors when reporting animal studies in Endodontology. A steering committee (SC) consisting of eight members, including the project leaders (PD, VN, AK, PM, MN, JF, EP, JJ, SJ) drafted a preliminary version of the PRIASE guidelines. This draft checklist was developed by soliciting and integrating the input of peer-reviewers from across the world about the information needed to improve the quality of animal studies, together with the ARRIVE statements (Kilkenny *et al*. 2010, Percie du Sert *et al.* 2020) and CLIP principles (Lang *et al*. 2012) to fit the specialty of Endodontology. Subsequently, the draft checklist and a preliminary flowchart were used during an online Delphi survey to build a consensus on the contents of the checklist and the suitability of the flowchart.

Online Delphi process

The next phase of the study involved a PRIASE Delphi Group (PDG) that comprised 31 experts including 23 academics / researchers, four Endodontists, two general dentists and two

representatives of the public. The professional PDG members fulfilled at least one of the following criteria to be eligible to participate in the Delphi process: (i) published at least one animal study in Endodontology; (ii) published any reporting guidelines for *in vitro* /*in vivo* research; (iii) had a minimum of 15 years of academic or clinical experience in Endodontics. All 31 eligible members were invited by a letter to participate in an online Delphi survey. The letter introduced the aims and rationale of the study, described the Delphi process and the role of the PDG members.

Those individuals who had volunteered to join the PDG were sent a document that provided further information on the online Delphi process and contained the draft PRIASE 2021 checklist and a flowchart. The criteria and scoring method for including or excluding items from the draft checklist were also described in detail. Using the online Delphi questionnaire, each PDG member assessed the items of the draft PRIASE 2021 checklist on their suitability and clarity. The clarity of each item was assessed using a dichotomous scale of 'yes' or 'no' whilst the suitability of the item on a 9-point Likert scale (1 = 'definitely not include' to 9 = 'definitely include'). Additionally, the PDG members were given an opportunity to add comments on any of the items that could potentially strengthen the quality of the text.

The steering committee analysed the scores awarded to each item based on a previously agreed set of inclusion and exclusion criteria. Items scored as 7–9 by at least 70% and 1–3 by less than 30% of PDG members were included in the PRIASE 2021 checklist for the second round of the Delphi process. Items scored as 1–3 by more than 70% and 7–9 by at most 30% of members were excluded. The scores were shared with the PDG members and those items that needed modification after the first round were revised and included in the PRIASE 2021 checklist and were re-scored by the PDG members during a second round of the Delphi survey (Agha *et al*. 2017). Finally, the revised

PRIASE 2021 checklist and flowchart created by the consensus-building process were discussed during a subsequent PRIASE online meeting.

Online meeting

The eligibility criteria for the members of the PRIASE Online Meeting Group (POMG) were the same as those for the PDG with several individuals being members of both groups. On confirmation of their POMG membership, the results of the online Delphi rounds, the revised PRIASE 2021 checklist and flowchart, agenda of the meeting as well as the details of the date and time of the meeting were shared. The online meeting was conducted on 9th September 2019 via Zoom.

Post-meeting activities

Based on the discussions and outcomes of the meeting, a final list of the PRIASE items was created, and the final design of the flowchart prepared. The PRIASE 2021 guidelines were then piloted by several experts who each drafted a manuscript using the PRIASE 2021 checklist and flowchart. Finally, the steering group reviewed the guidelines and made minor changes to improve the understanding and readability of the items and flowchart.

Results

Online Delphi process

In total, 31 individuals agreed to participate in the Delphi process. Rounds 1 and 2 received a response rate of 100 % and 94% respectively. Round 1 consisted of a PRIASE checklist with 45 items and a flowchart. Among the 45 items, 44 received a score between 7 and 9 by ≥70% of members and were included in the PRIASE checklist; there was disagreement over only one item. Based on the comments provided by PDG members, the steering committee revised that one item, added one new item, removed one item because it was duplicated in another item and improved the resolution of

the flowchart. Thus, round 2 consisted of a PRIASE checklist with these 2 items and the revised flowchart. In round 2, both items were awarded a score between 7 and 9 by ≥70% of members and were included in the checklist.

Online meeting

Due to the COVID-19 pandemic, the PRIASE steering committee replaced the planned face-to-face consensus meeting with an online virtual meeting using Zoom.. In total, seven steering committee members (PD, VN, AK, PM, MN, JF, JJ) and 19 academics/clinicians and two postgraduate students attended the meeting across the world, which was chaired by two steering committee members (PD, VN). The PRIASE 2021 checklist and flowchart resulting from the online Delphi process were discussed to determine the views of members on whether the items should be included or excluded and whether the specific text for each item was clear and understandable, or needed modification.

Post-meeting activities

The steering committee revised the PRIASE 2021 checklist and flowchart based on the comments received during the POMG. The final checklist and flowchart were then piloted to ensure they could be used during the development of actual manuscripts reporting animal studies. The final PRIASE 2021 checklist comprised of 11 domains (Title, Keywords, Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusion(s), Funding and support, Conflict of interest and Quality of images) with 43 individual items. The PRIASE 2021 checklist is presented in Table 1. Figure 1 is the PRIASE 2021 flowchart consisting of 12 domains that summarizes the key steps involved in reporting animal studies.

Discussion

The outcome of this study was the creation of the practical and useful PRIASE 2021 guidelines in the form of a checklist to be used to guide authors to improve the quality of their animal testing studies besides manuscript preparation prior to peer-review. The widespread adoption of the PRIASE 2021 guidelines is intended to guide authors to become more successful, by avoiding the pitfalls of working on poorly designed and badly executed animal studies that are unsuitable for publication. In addition, the fulfilment of the PRIASE 2021 guidelines and checklist over the longer term are intended to make the publications of animal studies more accurate, reliable and reproducible. It is estimated that at best, only 50% of all the preclinical biomedical research is reproducible (Hunter 2017). It is essential that PRIASE 2021 address these problems within publications because they can have a profound negative impact on innovations within Endodontology, by delaying the translation of novel research advances that are needed to benefit patients.

Animal testing can be a highly controversial and divisive area even within Endodontology as shown by the hundreds of comments provided by the 31 individuals who formed this group. The members of the PRIASE group only supported animal testing when it was conducted with restrictions to prevent animal suffering. Animal testing can invoke strong ethical concerns among endodontic professionals and the general public. The living conditions of animals kept for research purposes can affect people's attitudes towards animal research, and if animals are well-housed and cared for, people's support for animal research will perhaps increase (Ormandy & Schuppli 2014). The PRIASE 2021 guidelines request very precise details of the animal care and welfare, even beyond those regulated by Institutional Animal Care and Use Committees (IACUC) and ethical review committees, to help authors identify and prevent problems. For these reasons the PRIASE 2021 guidelines require that researchers adhere to very high standards of animal welfare and care, which includes pain monitoring and pain alleviation to prevent any suffering or disability. Authors will recognize from the PRIASE 2021 guidelines an unwillingness of peer-reviewers to overlook any animal care or

welfare problems. The current opinions of peer-reviewers are reflected in the PRIASE 2021 checklist. Each checklist item is identified for a specific reason and violations of the checklist will likely make a manuscript unacceptable for publication.

At the beginning of this undertaking, there was a risk that the group may never be able to arrive at a consensus for the PRIASE 2021 guidelines, because it became clear that almost everyone had a fixed mindset of ideas about animal testing that could not be changed by mere factual arguments. The fixed mindset can be explained by the emotional attachment that many individuals have developed by caring for their pets, such as cats and dogs (Ormandy & Schuppli 2014), making them unwilling to view their pets as potential test subjects for experimentation. Thus, although an ethics approval committee or Institutional Animal Care and Use Committee (IACUC) may approve an animal testing protocol and decide that it is entirely legal and completely ethical, researchers proposing to conduct experiments on cats, dogs and non-human primates will need to recognize that peer-reviewers, editors, and publishers have an absolute right to deny a publication. Even if an animal study did not use pets, but caused severe prolonged pain, suffering, or disability, an author will be unlikely to convince peer-reviewers and editors that even the most technically brilliant manuscript is worthy of publication. Thus, researchers are advised to carefully consider their choice of animals and to employ the most humane test methods required by the PRIASE 2021 guidelines, because anything that could upset readers is likely to be unacceptable for publication. Researchers can check the policies of journals with the editor regarding animal testing, and also search within the past issues of journals for animal testing publications to see any limitations of animal studies that are acceptable for publication.

Peer-reviewers recognize that animal testing cannot be entirely abolished at this time because it is required by international testing standards; ISO 10993 and ISO 7405 (Dammaschke

2010) to evaluate the biological safety of novel biomaterials, devices and medicaments prior to clinical trials (Stanley 1992). Without animal testing no new research to develop safe and effective treatments that benefit patients would be possible. Therefore, it is ethically and morally unacceptable to cease the animal testing of treatments and medicaments that are needed to alleviate human pain, suffering and disabilities.

The opinions of the individuals that contributed to the guidelines emphasised that highly-regulated animal testing described by the PRIASE 2021 guidelines was necessary to protect the health and safety of humans. Replacement alternatives to live animal testing involving the *in vitro* organ culture of teeth (Murray *et al.* 2008), and *ex vivo* models of periodontal tissues and inflammatory bone destruction (Sloan *et al.* 2013) could help address the concerns that opponents to animal testing may have. Unfortunately, many aspects of animal testing have barely changed over the past 60 years, since the publication of Russell and Burch's seminal book phrasing the 3Rs to refine, replace, reduce the use of animals used for testing (Russell & Burch 1959). Despite these widespread problems, the implementation of the PRIASE 2021 guidelines within Endodontology will help to reduce the numbers of animals used for animal testing by guiding researchers to avoid wasteful investigations that lack accuracy, reproducibility and reliability.

The PRAISE guidelines request high-quality images and figures be used to effectively communicate the most significant information to readers. High quality illustrations are an important avenue to support findings, report discoveries and have the potential to generate new research questions (Kotz & Cals 2013, Polepalli Ramesh *et al*. 2015). Due to the relative importance of images in conveying information from animal studies, nine items related to images were included in the PRIASE 2021 checklist, e.g. radiographs, scans, histology slides, and clinical photographs. The domain covering the quality of images will guide authors to provide detailed information to explain

the precise nature of what the images convey. The individuals who contributed to the guidelines recognized the reluctance of some authors to show surgical photographs of animal experiments, but these are often necessary for the education of readers. As a general rule, if authors are reluctant to present photographs of the animal experiments to avoid the risk of upsetting some readers, then it automatically suggests that the experiments should not have been performed in the first place. Editors and peer-reviewers can never accept painful experiments on animals are justified merely for the sake of a publication.

A flowchart provides a diagrammatic sequence for the readers to appreciate the main components of a study and also provide authors with a template when writing their manuscripts. It has been reported that the CONSORT flowcharts enhanced the reporting of clinical trials (Egger *et al*. 2001). Hence, the inclusion of a flowchart in the PRIASE guidelines should benefit both authors and readers.

Future plans

1. *Explanation and elaboration document*: The rationale and importance of items in the checklist and flowchart will be explained and clarified in an additional report. Suitable examples from the literature or hypothetical scenarios will be provided to support the explanations.

2. *Translation*: For the benefit of global readers and authors, the guidelines will be translated and published in several languages.

3. *Preferred Reporting Items for study Designs in Endodontology (PRIDE) website*: The PRIASE 2021 guidelines and flowchart are freely accessible and downloadable from the PRIDE website (www.pride-endodonticguidelines.org). Feedback from readers, authors, academics,

students, researchers and journal editors can be provided through the website and will help in the revision of the guidelines over time.

4. *Endorsement*: The editors of journals who publish animal studies in Endodontology will be approached to adopt the guidelines within their Author Guidelines to inform authors during the preparation of manuscripts.

Conclusion

A well-documented and validated consensus process was adopted to develop the PRIASE 2021 guidelines and flowchart. The guidelines consist of a checklist of 11 sections with a total of 43 items. The PRIASE 2021 guidelines are focused on improving the methodological principles, reproducibility and quality of animal studies to ensure their reliability to estimate the effects of endodontic treatments, while guiding future clinical studies on humans.

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Conflict of Interest statement

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

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Figure Legend

Figure 1 PRIASE 2021 flowchart

Table 1 PRIASE 2021 checklist of items to be included when reporting animal studies in Endodontology

Section/ Topic	Item Number	Checklist Items	Reported on page number
Title	1a	The specific animal species and its health or disease status (sometimes called "animal model") must be provided.	
	1b	The specific test, field, subject and treatment of interest within the animal model must be provided.	
Keywords	2a	Keywords such as "animal model" or "*in vivo* model" and the specific area(s) of interest must be provided.	
Abstract	3a	The Introduction of the Abstract must explain the significance of the study.	
	3b	The unambiguous aim(s) and objective(s) of the study must be provided.	
	3c	The most important details of the animal and the experimental model must be provided.	
	3d	Key details of the methodology must be provided.	
	3e	The most relevant and important results must be presented succinctly including differences among the means, medians or modes of the dependent variables (treatment outcome and test results) and any significant P-values.	
	3f	Succinct conclusions supported by the results must be provided.	
Introduction	4a	The relevant background information must be provided using terminologies consistent with professional standards and previous publications.	
	4b	The appropriateness of the selected animal model to address the aims and objectives of the study must be explained.	
	4c	A justification of the reasons why the investigation was necessary using an animal model must be provided.	
	4d	The unambiguous aim(s) and objectives(s) of the animal study must be provided.	
Materials and Methods	5a	The reference number of the approval granted by the ethics board, such as an Institutional Review Board or Institutional Animal Care committee, must be provided along with a reference to the applicable institutional and/or national regulations that were enforced. Any identifying details about the authors institution should not be disclosed during the blind peer review.	

	5b	The sample size must be justified by citing prior similar studies and/or be estimated by using statistical power calculations to ensure an adequate sample size is used to detect any significant differences and answer the research questions. This is to avoid making any type I and type II errors.	
	5c	Details of how animal pain and disability was monitored and how animal suffering was prevented during all aspects of experimentation must be provided.	
	5d	The job titles and qualifications of the animal caretakers must be provided.	
	5e	Specific details of the animals must be provided, including their species, strain, immune system, breeding programme, age, weight, health status, and any special characteristics.	
	5f	The experimental design must include details of the numbers of animals, numbers of experimental units (e.g. teeth), and timelines (e.g. 5, 30 and 60 days) used.	
	5g	The primary outcome data measures or categories as well as any other secondary outcome data measures or categories that will be assessed must be provided.	
	5h	Details must be provided on (1) steps in the interventions and treatments, (2) instruments, medicaments or device allocation, and (3) concealment and randomization prior to data collection.	
	5i	Details regarding post-disease and post-operative care of the animals must be provided.	
	5j	Details on the statistical analysis, statistical tests, type of software used, and steps taken to control, interpret success or failure, and to validate the accuracy of the data must be provided.	
Results	6a	Average baseline characteristics of the animals (e.g. age, weight, gender, microbiological status) at the beginning of the experiment must be provided.	
	6b	The results for each group of primary and secondary outcomes should describe the means, median or mode; as well as differences and their statistical significance.	
	6c	All adverse events during the animal experimentation and the method of euthanasia must be reported.	
	6d	Any changes made to the experimental protocols to prevent the occurrence of animal adverse health events, analgesic or other medication overdoses or underdoses, or unexpected deaths must be provided.	
Discussion	7a	A discussion on how the methods and results are relevant to the study aims, and how the results support or dispute prevailing theories advocated in prior publications must be provided.	

	7b	An objective presentation of the strengths and limitations of the animal model, study design, methods, materials, instruments, drugs and devices, and outcomes must be provided, including any biology/functional variability between the animal model and humans.	
	7c	The potential influence of the results on future research plans must be discussed.	
	7d	If appropriate, the impact the findings have on human health, treatments or healthcare must be explained.	
Conclusion(s)	8a	A rational basis for the conclusion(s) must be provided, that is, they must be directly supported by the results of the study.	
	8b	Explicit conclusion(s) from the study, including appropriate follow-up research ideas, must be provided.	
Funding and support	9a	All funding, donations, assistance and support provided for the study must be reported.	
Conflicts of interest	10a	An explicit statement on conflicts of interest must be provided.	
Quality of images	11a	Details of the equipment (model, supplier, city, country), software (version, supplier city, country) and settings used to acquire image(s) must be described in the Methods and/or figure legend.	
	11b	The reason why the image(s) was acquired and rationale for its inclusion in the manuscript must be provided in the text.	
	11c	The circumstances (conditions) under which the image(s) was viewed and evaluated must be provided in the text.	
	11d	The resolution, magnification and any important manipulation(s) on any image (e.g. brightness, image smoothing, staining etc.) must be described in the text or legend.	
	11e	An interpretation of the findings (meaning and implications) from the image (s) must be provided in the text.	
	11f	The legend associated with each image must clearly describe the subject matter specific feature(s) illustrated. Images of animals must describe their age and test duration, and other relevant features such as important anatomical landmarks and relevant features.	
	11g	Arrow markers and relevant labels must be provided in image(s), if relevant, in order to identify key information.	
	11h	The legend of each image must include an explanation whether it refers to pre-treatment, intra-treatment, post-treatment or post-sacrifice, and if relevant, how images were standardised over time.	

Figure 1: PRIASE 2021 flowchart