Evidence-based Preclinical Medicine

Open Access

Evidence-based Preclinical Medicine ISSN 2054-703X

METHODS ARTICLE

A protocol format for the preparation, registration and publication of systematic reviews of animal intervention studies

Rob B. M. de Vries,[1†] Carlijn R. Hooijmans,[1†] Miranda W. Langendam,[2] Judith van Luijk,[1] Marlies Leenaars,[1] Merel Ritskes-Hoitinga[1] and Kimberley E. Wever[1*]

[1]*SYRCLE, Central Animal Laboratory, Radboud University Medical Center, Nijmegen, The Netherlands and* [2]*Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center (AMC), Amsterdam, The Netherlands*

(2015) Evidence-based Preclinical Medicine 1, 1, 1–9, e00007, DOI: 10.1002/ebm2.7

ABSTRACT

Systematic reviews are an important method to support evidence-based decisions in healthcare (research). Although not yet as common as clinical systematic reviews, the number of systematic reviews of animal studies has been increasing steadily in recent years. An important method to promote high-quality systematic reviews is to pre-specify the review methodology in a protocol, before the conduct of the systematic review itself. In contrast to clinical systematic reviews, a standard protocol format for systematic reviews of animal studies is not yet available. Here, we present a protocol format tailored to the preparation, registration and publication of systematic reviews of animal intervention studies (i.e. systematic reviews of animal experiments studying the efficacy and/or safety of interventions intended for use in human patients). In analogy to the Cochrane review protocol, the format helps authors predefine the methodological approach of their systematic review, from research question to data synthesis. We recommend that authors prospectively complete and agree on the protocol, and register and/or publish it to allow feedback on the proposed methodology and to avoid the introduction of bias during the review process. Opportunities for obtaining feedback, and for registration and publication of review protocols are also discussed.

Keywords: systematic review, protocol, animal, meta-analysis, pre-clinical studies, registration

FUNDING INFORMATION

This work was partially funded by The Netherlands Ministry of Health, Welfare and Sport (VWS), grant number 321200.

Introduction

Systematic reviews support evidence-based healthcare decisions by identifying, appraising, synthesizing and interpreting research-based evidence in a systematic and transparent way. Conducting systematic reviews is already common practice when summarizing clinical research evidence on the effectiveness of interventions. Although animal experiments are part of the evidence base for decisions in clinical research and practice, systematic reviews that summarize the research evidence from animal experiments are scarce. For several interrelated reasons, however, systematic reviews of animal studies may be very useful, particularly for the translation of animal data to patients.[1] Through an assessment of the risk of bias in the included studies, systematic reviews give insight into the quality of the available evidence.[2–5] Secondly, systematic reviews can assess the risk of publication bias[6,7] and identify whether there are fundamental differences in design between the animal studies and the subsequent clinical trials.[8] Furthermore, if they include a meta-analysis, systematic reviews provide pooled estimates of the direction and magnitude of the effect of an intervention. Such overall effect estimates may lead to alternative conclusions regarding the safety or efficacy of an

Address for correspondence: KE Wever, SYRCLE, Central Animal Laboratory, Radboud University Medical Center, PO Box 9101, 6500 HB, Nijmegen, the Netherlands. email: kim.wever@radboudumc.nl

[†]These authors contributed equally to this work.

intervention than those based on a qualitative assessment of the individual primary studies.[9,10]

For animal and clinical reviews alike, conducting a systematic review involves many methodological decisions, for example regarding the choice of inclusion criteria and subgroup analyses. In order to minimize bias in the review process, these choices should not be influenced by the results of the included studies. For that reason, the planned methods should be established and documented in advance.[11] The *a priori* preparation of a protocol describing the proposed methodological approach for the review is therefore highly recommended. From a practical point of view, preparing a protocol helps to structure the review process. In addition, registration/publication of review protocols reduces the potential for duplication of systematic reviews, prevents selective reporting of, for example the results of subgroup analyses, allows peer review of the planned methods and enables other review authors to learn from the methods chosen/developed.[12]

The Cochrane Collaboration provides a standard format for preparing a protocol for clinical systematic reviews of interventions or diagnostic tests[11] and publishes them *a priori* in the Cochrane Database of Systematic Reviews (see www.thecochranelibrary.com). For animal studies, such a standard protocol format is still lacking. Some of the more recent systematic reviews of animal studies have used protocols (as found e.g. on www.camarades.info), but these vary in the type and amount of information included. A standardized protocol format would promote complete and high-quality protocols, because it can give review authors guidance on all the elements and details they should include in their protocol. A standard format would facilitate peer review: it would be easy for reviewers to find the aspects of the protocol they are particularly interested in. Moreover, a standard format could help future central registration if the elements of the protocol format were matched to the fields expected to be required by a registration site, such as PROSPERO. Lastly, if review authors decided to publish their protocol as a journal article, the filled out format could be used as the basis for the article text.

Although systematic reviews of animal studies and of clinical trials consist of similar steps, the details of these steps can differ substantially. In animal systematic reviews, for instance, additional databases and search filters may need to be used to identify all relevant studies (e.g. Web of Science) and the risk of bias assessment often contains animal specific items (e.g. randomisation of the housing conditions[4]). Furthermore, the goal of a meta-analysis in an animal systematic review is different, since it is not to obtain a precise point estimate, but rather to assess the general direction and magnitude of the effect of an intervention and to explore potential sources of heterogeneity.[13] For the latter reason, animal systematic reviews often include a more elaborate evaluation of the study characteristics. For these reasons, we have developed a protocol format tailored to systematic reviews of animal studies.

Development of the animal systematic review protocol format

The presented protocol format (Appendix 1 and Appendix S1 for fillable MS Word format) is particularly suitable for systematic reviews of animal intervention studies, that is systematic reviews of animal experiments studying the efficacy and/or safety of interventions intended for use in human patients. This type of systematic review is most similar in design to systematic reviews of clinical trials in humans and the methodology has therefore been developed the furthest, allowing for detailed guidance to review protocol authors (e.g. the adapted population, intervention, comparison, outcomes (PICO) mnemonic for formulating the research question and the pre-specified options for literature databases, study quality criteria and meta-analysis). The protocol format may also be used as a starting point for other types of systematic reviews of animal studies (e.g. secondary analysis reviews or reviews providing a descriptive overview of disease models used or mechanisms studied), but some elements might not be applicable or need adaptation.

The protocol format was developed in four phases. In the first phase, a draft format was designed based on the Cochrane review protocol,[11] the preferred reporting items for systematic reviews and meta-analyses (PRISMA) checklist[14] and our experience in the conduct of systematic reviews of animal studies. In Appendix S2, the differences between our format and the Cochrane format as well as PRISMA are described.

Secondly, the protocol format was discussed with three scientific researchers, two methodologists, one veterinarian and two clinicians within the Radboud University Medical Center. As a result of these discussions, item 21 about defining the screening phases was added, in order to allow adaptation of the selection criteria to the data available per selection phase. For example, the outcome measures used are often not reported in the abstracts of animal studies and can therefore often only be applied as selection criteria in the full-text phase. In addition, we added an item (item 30) about sorting and prioritizing the exclusion criteria per selection phase, especially useful for preparing a structured flow chart of the results of the study selection.

In the third phase, the protocol format was tested by 7 researchers planning or already conducting a systematic review of animal studies (2 clinicians, 2 Phd students, 2 scientific researchers and 1 student). They were asked to fill out the protocol format and give feedback on (1) usability, (2) missing items, (3) possibilities for improvement and (4) whether or not the review authors understood what the items asked for. As a result of their feedback, items related to the collection of outcome data (items 39–41) were separated from those related to the extraction of study characteristics (items 31–36). This was partly done because in this way the items correspond more closely with the main steps of a systematic review, but also because experience taught us that extracting these two types of

data separately works better in practice. The test panel also suggested to provide checkboxes for standard options to improve interpretation of specific items. In response, we added checkboxes for items 17, 19 and 38.

As a fourth step, we discussed the resulting protocol format with members of CAMARADES (a supporting framework for groups involved in the systematic review and meta-analysis of data from experimental animal studies). Moreover, our protocol format was compared with five protocols already published on the CAMARADES website. At the time, the website contained 22 protocols, which were all supervised by CAMARADES. To increase possible variation between the protocols and increase the possibility of finding items which we missed so far, only the most recent review protocol per first author was selected. In addition, protocols that already used a previous version of our protocol format were excluded. From the resulting list, five random protocols were compared to our protocol. As a result of this step, we added "other study quality measures" and the CAMARADES' study quality checklist to item 38 and included item 49 about other details of the meta-analysis (e.g. correction for multiple testing, correction for multiple use of control group).

Structure of the protocol format

The protocol format (Appendix 1 and S1) consists of three sections, eight subsections and 50 items. The first section (A) pertains to general information about the review, the second (B) to the objectives of the review and the third (C) to the methods proposed to attain those objectives. The subsections of the format correspond to the different steps of a systematic review:

- formulating the research question (including background): items 10–16
- study identification: items 17–20
- study selection: items 21–30
- collection of study characteristics: items 31–36
- assessment of study quality: items 37–38
- extraction of outcome data: items 39–41
- data synthesis/meta-analysis: items 42–50

Importantly, for each step, both the content (the "what") and more procedural aspects (the "how") are registered. The items on study selection, for instance, pertain to the inclusion criteria to be used (content) as well as to the number of reviewers per selection phase (procedure).

SECTION A: GENERAL

The first section is comprised of general, more administrative data regarding the review, such as its title and both author and registration details. All authors and contributors of the review and their proposed role in the review process should be specified here. This encourages reflection on the composition of the review team at an early stage, to ensure that all fields of expertise necessary for the review are represented in the team. In addition, funding sources/sponsors as well as potential conflicts of interest, which might bias the conclusions of the review, should be mentioned here. Moreover, several registration details are asked for, particularly the stage of the systematic review at the time of registration, in order to determine whether (or the extent to which) the protocol was completed before the actual start of the systematic review itself.

SECTION B: OBJECTIVES

Items 10–16 concern the objectives of the review. They are intended to determine whether a review and particularly a systematic review will actually be useful for the field/topic in question (item 10) and, if so, which research question the review aims to answer. A specific research question—that is a question focussed on clearly defined interventions, animal models and outcome measures—is a defining feature of systematic reviews and it is a prerequisite for a high-quality review.[15] The protocol format therefore contains all elements of the PICO mnemonic: Population, Intervention, Comparison or Control group and Outcome measures (items 11–15). Of note, in the context of animal studies, the "P" not only refers to the species or strain of animal used but also to the disease model, that is the method or methods by which the disease is induced. The answers to the PICO items form the building blocks of the research question (item 16).

SECTION C: METHODS

Subsection search and study identification

In order to identify as many relevant primary animal studies as possible, searching in at least two bibliographic databases is recommended. Moreover, using comprehensive search strategies including thesaurus terms (such as MeSH terms) greatly increases the accuracy and completeness of the search results.[15] Items 17–20 in the methods section of the protocol promote the design of comprehensive strategies by explicitly mentioning databases/sources that are likely to contain references relevant to the field of animal intervention studies and by linking to relevant guides and tools. In addition, the protocol format points out different options for "hand searching".

Subsection study selection

Pre-specification of inclusion and exclusion criteria is crucial for preventing bias in the inclusion of studies in the review (e.g. inclusion based on the study results). Formulating these criteria is also a useful way to evaluate whether the research question is sufficiently specific and whether the search strategy is complete. The risk of subjectivity in study

selection is also reduced by independent assessment of each primary study by at least two reviewers. Accordingly, under item 22, the number of reviewers per study selection phase is registered as well as the way in which disagreements will be resolved. To guide the process of formulating inclusion and exclusion criteria, item 21 asks to define the screening phases and items 23–29 highlight several important study aspects, such as study design, disease model and outcome measure. The protocol format intentionally requires reviewers to document both the exclusion criteria and the corresponding inclusion criteria, to prevent gaps between the two. The exclusion criteria should then be specified per selection phase and prioritized (item 30). The selection criteria used in both selection phases may be identical, or additional criteria may be added for the full-text screening phase, for example because they pertain to details which are rarely stated in a study's abstract (see item 30 in Appendices S3 and S4). Prioritizing the exclusion criteria is especially useful for preparing a structured flow chart of the study selection phases. In addition, prioritizing these criteria is required when using Early Review Organizing Software (EROS; Institute of Clinical Effectiveness and Health Policy, Buenos Aires, Argentina; www.eros-systematic-review.org), a web-based application which specifically facilitates independent study selection.

Subsections study characteristics, risk of bias/study quality and collection outcome data

In order to prevent selective use or reporting of data from the studies included in the systematic review, details of the data extraction phase should be specified in the protocol. These details pertain not only to the study characteristics (items 31–36), but also to the study quality and risk of bias assessment (items 37–38) and outcome data (items 39–41).

In an animal systematic review, the characteristics of primary studies (items 31–36) are generally used to assess their external validity, and they function as the basis for explaining potential heterogeneity encountered in a meta-analysis (pre-specified subgroup variables).

The quality assessment is an important aspect of systematic reviews and the chosen methodological approach is specified in items 37–38 of the protocol. The assessment generally includes an assessment of the risk of bias in the primary studies. There are clear indications that in many animal studies the risk of bias is high,[3] leading to underestimation or overestimation of the safety and/or efficacy of interventions. SYRCLE has recently developed a standardized tool to assess the risk of bias in primary animal intervention studies.[4] However, other characteristics or study details related to quality/bias may be added or omitted as applicable (e.g. presence of a power calculation, adequate control of body temperature, presence of a conflict of interest statement etc.).

For the results section of the systematic review, outcome data from the primary studies will have to be collected to combine them either in a meta-analysis or in a more descriptive overview. Item 39 asks to define the type of data that need to be collected. If a distinction between primary and secondary outcome measures is considered to be appropriate, this can be indicated here. Not all data will be available in the format needed for the data synthesis (e.g. some data are only presented in graphs). For that reason, items 40–41 asks review authors to describe the procedure they will follow in order to acquire all relevant outcome data.

Subsection data analysis/synthesis

An important added value of systematic reviews, as compared to narrative reviews, is the synthesis of outcome data from individual studies. Preferably, this synthesis has the form of a meta-analysis, but such a statistical combination of outcome data is not always feasible or sensible.[13] The included studies may be too heterogeneous (e.g. due to large differences in design or outcome measures between the studies), or no quantitative data may be available. Items 42–43 of the protocol format therefore address how the authors aim to present their data and under which conditions a meta-analysis is deemed to be feasible and sensible. If a meta-analysis seems feasible, items 44–50 require authors to pre-specify important details of the meta-analysis, for example the effect measure to be used, the statistical model of analysis, the method to assess statistical heterogeneity and the subgroups to be explored. Pre-specifying the methodological details of the meta-analysis reduces the risk of inappropriate post-hoc analyses and selective outcome reporting (e.g. reporting only the results of subgroup analyses that show significant effects). This risk is higher in meta-analyses of animal data than in clinical meta-analyses, because the primary aim of the former is to explore which factors influence the overall effect, rather than determining a precise point estimate.[13]

Use of the animal systematic review protocol

In this article, we have presented a standardized protocol format tailored to systematic reviews of animal intervention studies, which is supported by the two largest networks/centres in the field, CAMARADES and SYRCLE. Because our format covers all steps of such a systematic review and asks for detailed information per step, protocols based on our format are unlikely to miss important information. By explicitly including the option "other" in all methodological subsections and by often asking open questions, we believe our format is sufficiently flexible to prevent review authors from refraining to describe certain information because the format does not seem to have room for it.

Two examples of completed protocols are presented in Appendices S3 and S4. These protocols were originally prepared using earlier versions of our protocol format, but were, for the purpose of this article, transferred to the latest version to function as illustrations of the information the various items in the protocol format call for.

We recommend researchers who intend to write a systematic review of animal intervention studies to complete the protocol before the start of the systematic review itself and have it approved by all intended authors. As a minimum, the completed protocol should be added as a supplementary file to the published systematic review. Any retrospective additions or alterations to the protocol must be made transparent in the materials and methods section of the systematic review itself, allowing readers to judge whether these may have introduced bias in the review.

The preferred option, however, is to prospectively register and/or publish the completed review protocol. Formal registration (or publication) of a systematic review protocol provides a permanent independent and dated public record of the protocol against which the final review can be compared. Registration indicates that the systematic review in question is in progress and therefore prevents duplication of systematic reviews.[16] Moreover, it helps to minimize selection bias (for example, the retrospective adaptation of inclusion criteria) and selective outcome reporting (for example, the reporting of only those subgroups that displayed statistically significant differences) in the systematic review.[17] Lastly, it helps avoiding publication bias on the level of systematic reviews, that is the publication of only those reviews that show positive results of an intervention. At the time of writing, the Advisory group of PROSPERO, the international prospective register of systematic review protocols for clinical studies (www.crd.york.ac.uk/PROSPERO[18]), has approved extending the database to include protocols of pre-clinical systematic reviews. Moreover, plans have been made to use a Delphi method to obtain consensus on the fields to be included in the pre-clinical template for registration on PROSPERO (for a comparison between the items in our format and the fields in PROSPERO see Appendix S2). Until this database is operational, protocols can be made publicly available through the websites of CAMARADES (www.dcn.ed.ac.uk/camarades/research.html#protocols) or SYRCLE (www.radboudumc.nl/Research/Organisationofresearch/Departments/cdl/SYRCLE/Pages/Protocols.aspx).

In addition to registration, the protocol can also be published. Published protocols have a more narrative structure, which makes them more readable. The major advantage of publication over registration in the pre-clinical field is that it allows for peer review of the protocol, which ensures that major flaws or biases can be discovered and corrected before the conduct of the review. This is desirable from the perspective of the both review authors and the scientific community, which profits most from high-quality reviews. Groups such as SYRCLE (www.SYRCLE.nl) and CAMARADES offer guidance and feedback to authors on protocols and systematic reviews of animal studies. Publication of protocols in open access journals such as Systematic Reviews (www.systematicreviewsjournal.com/) and particularly this journal, Evidence-Based Pre-clinical Medicine (www.onlinelibrary.wiley.com/journal/10.1002/(ISSN)2054-703X), is highly recommended. The latter journal has an editorial board of experts in the field of systematic reviews of animal studies, who can provide specialist feedback on the proposed methodology.

In addition to review authors, our format can be used by reviewers to assess protocols not presented in a standardized format or to detect potential biases in a systematic review that was based on a protocol using our format. Moreover, journal editors can encourage (potential) review authors to conduct systematic reviews and use our format as a starting point.

In order to promote implementation of our format, we presented an earlier version of the format at the Third International Symposium on Systematic Reviews in Laboratory Animal Science in Washington DC (November 13, 2014). In addition to publication in this journal—so far the only journal dedicated to evidence-based pre-clinical medicine—we intend to have a reprint of this article published in the journal Systematic Reviews. Moreover, both CAMARADES and SYRCLE will urge authors of the systematic reviews they supervise to use this format. Finally, the Delphi round planned for the inclusion of animal protocols in PROSPERO will ensure that all researchers working in the field of evidence-based pre-clinical medicine will become familiar with our format.

Conclusion

We present a tailored protocol format suitable for the preparation, registration and publication of systematic reviews of animal intervention studies. Preparing a protocol promotes researchers to prospectively reflect on the proposed methodology of their systematic review, which helps to prevent methodological flaws and minimize bias in the systematic review. In this way, protocols contribute to the conduct of high-quality animal systematic reviews. High-quality animal systematic reviews, in turn, stimulate researchers to reflect more extensively on the design of new animal experiments and/or clinical trials, which aids in preventing the "waste" of laboratory animals and trial participants through unnecessary, invalid or less informative studies.[19]

Acknowledgements

We would like to thank all researchers who have provided feedback on earlier versions of our protocol.

Conflict of Interest

All authors declare not to have any conflicts of interest.

Appendix 1

SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

FORMAT BY SYRCLE (WWW.SYRCLE.NL)
VERSION 2.0 (DECEMBER 2014)

Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review		
2.	Authors (names, affiliations, contributions)		
3.	Other contributors (names, affiliations, contributions)		
4.	Contact person + e-mail address		
5.	Funding sources/sponsors		
6.	Conflicts of interest		
7.	Date and location of protocol registration		
8.	Registration number (if applicable)		
9.	Stage of review at time of registration		
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?		
	Research question		
11.	Specify the disease/health problem of interest		
12.	Specify the population/species studied		
13.	Specify the intervention/exposure		
14.	Specify the control population		
15.	Specify the outcome measures		
16.	State your research question (based on items 11–15)		
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (e.g. Pubmed, Embase, Web of science)	☐MEDLINE via PubMed ☐Web of Science ☐SCOPUS ☐EMBASE ☐Other, namely: ☐Specific journal(s), namely:	
18.	Define electronic search strategies (e.g. use the step by step search guide[15] and animal search filters[20,21])	When available, please add a supplementary file containing your search strategy: [insert file name]	

19.	Identify other sources for study identification	☐Reference lists of included studies ☐Books ☐Reference lists of relevant reviews ☐Conference proceedings, namely: ☐Contacting authors/ organisations, namely: ☐Other, namely:	
20.	Define search strategy for these other sources		
	Study selection		
21.	Define screening phases (e.g. pre-screening based on title/abstract, full text screening, both)		
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved		
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: Exclusion criteria:	
24.	Type of animals/population (e.g. age, gender, disease model)	Inclusion criteria: Exclusion criteria:	
25.	Type of intervention (e.g. dosage, timing, frequency)	Inclusion criteria: Exclusion criteria:	
26.	Outcome measures	Inclusion criteria: Exclusion criteria:	
27.	Language restrictions	Inclusion criteria: Exclusion criteria:	
28.	Publication date restrictions	Inclusion criteria: Exclusion criteria:	
29.	Other	Inclusion criteria: Exclusion criteria:	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase: 1. 2. etc. Selection phase: 1. 2. etc.	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (e.g. authors, year)		
32.	Study design characteristics (e.g. experimental groups, number of animals)		
33.	Animal model characteristics (e.g. species, gender, disease induction)		
34.	Intervention characteristics (e.g. intervention, timing, duration)		
35.	Outcome measures		
36.	Other (e.g. drop-outs)		

	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved		
38.	Define criteria to assess (a) the internal validity of included studies (e.g. selection, performance, detection and attrition bias) and/or (b) other study quality measures (e.g. reporting quality, power)	☐By use of SYRCLE's Risk of Bias tool[4] ☐By use of SYRCLE's Risk of Bias tool, adapted as follows: ☐By use of CAMARADES' study quality checklist, e.g.[22] ☐By use of CAMARADES' study quality checklist, adapted as follows: ☐Other criteria, namely:	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (e.g. continuous/dichotomous, unit of measurement)		
40.	Methods for data extraction/retrieval (e.g. first extraction from graphs using a digital screen ruler, then contacting authors)		
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved		
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (e.g. descriptive summary, meta-analysis)		
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed		
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (e.g. mean difference, standardized mean difference, risk ratio, odds ratio)		
45.	The statistical model of analysis (e.g. random or fixed effects model)		
46.	The statistical methods to assess heterogeneity (e.g. I^2, Q)		
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)		
48.	Any sensitivity analyses you propose to perform		
49.	Other details meta-analysis (e.g. correction for multiple testing, correction for multiple use of control group)		
50.	The method for assessment of publication bias		
Final approval by (names, affiliations):		Date:	

REFERENCES

1. Hooijmans CR, Ritskes-Hoitinga M. Progress in using systematic reviews of animal studies to improve translational research. *PLoS Med.* 2013; **10**: e1001482.
2. Sena E, van der Worp HB, Howells D, Macleod M. How can we improve the pre-clinical development of drugs for stroke? *Trends Neurosci.* 2007; **30**: 433–9.
3. Kilkenny C, Parsons N, Kadyszewski E *et al.* Survey of the quality of experimental design, statistical analysis and reporting of research using animals. *PLoS One* 2009; **4**: e7824.
4. Hooijmans CR, Rovers MM, De Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med. Res. Methodol.* 2014; **14**: 43.
5. Van Luijk J, Bakker B, Rovers MM, Ritskes-Hoitinga M, De Vries RB, Leenaars M. Systematic reviews of animal studies; missing link in translational research? *PLoS One* 2014; **9**: e89981.
6. Sena ES, van der Worp HB, Bath PM, Howells DW, Macleod MR. Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. *PLoS Biol.* 2010; **8**: e1000344.
7. Korevaar DA, Hooft L, Ter Riet G. Systematic reviews and meta-analyses of preclinical studies: publication bias in laboratory animal experiments. *Lab. Anim* 2011; **45**: 225–30.
8. Hooijmans CR, De Vries RB, Rovers MM, Gooszen HG, Ritskes-Hoitinga M. The effects of probiotic supplementation on experimental acute pancreatitis: a systematic review and meta-analysis. *PLoS One* 2012; **7**: e48811.
9. Pound P, Ebrahim S, Sandercock P, Bracken MB, Roberts I, Reviewing Animal Trials Systematically Group. Where is the evidence that animal research benefits humans? *BMJ* 2004; **328**: 514–7.
10. Van der Worp HB, Howells DW, Sena ES *et al.* Can animal models of disease reliably inform human studies? *PLoS Med.* 2010; **7**: e1000245.
11. Green S, Higgins JPT. Preparing a Cochrane review. In Higgins JPT, Green S eds. *Cochrane handbook for systematic reviews of interventions. Version 5;1;0 [updated March 2011]*. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org
12. Light RJ, Pillemer DB. *Summing up: the science of reviewing research*. Cambridge, MA: Harvard University Press, 1984.
13. Vesterinen HM, Sena ES, Egan KJ *et al.* Meta-analysis of data from animal studies: a practical guide. *J. Neurosci. Methods* 2014; **221**: 92–102.
14. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* 2009; **6**: e1000097.
15. Leenaars M, Hooijmans CR, Van Veggel N *et al.* A step-by-step guide to systematically identify all relevant animal studies. *Lab. Anim* 2012; **46**: 24–31.
16. Moher D. The problem of duplicate systematic reviews. *BMJ* 2013; **347**: f5040.
17. Kirkham JJ, Altman DG, Williamson PR. Bias due to changes in specified outcomes during the systematic review process. *PLoS One* 2010; **5**: e9810.
18. Booth A, Clarke M, Dooley G *et al.* The nuts and bolts of PROSPERO: an international prospective register of systematic reviews. *Syst. Rev.* 2012; **1**: 2.
19. Macleod MR, Michie S, Roberts I *et al.* Biomedical research: increasing value, reducing waste. *Lancet* 2014; **383**: 101–4.
20. de Vries RB, Hooijmans CR, Tillema A, Leenaars M, Ritskes-Hoitinga M. Updated version of the Embase search filter for animal studies. *Lab. Anim.* 2014; **48**: 88. PMID: 23836850.
21. Hooijmans CR, Tillema A, Leenaars M, Ritskes-Hoitinga M. Enhancing search efficiency by means of a search filter for finding all studies on animal experimentation in PubMed. *Lab. Anim.* 2010; **44**: 170–175. PMID: 20551243.
22. Macleod MR, O'Collins T, Howells DW, Donnan GA. Pooling of animal experimental data reveals influence of study design and publication bias. *Stroke* 2004; **35**: 1203–1208. PMID: 15060322.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Fillable (MS Word) format of the systematic review protocol for animal intervention studies.

Appendix S2. The results of a comparison between the SYRCLE protocol format for systematic reviews of animal studies and 3 other formats.

Appendix S3. Protocol SR Pneumoperitoneum.

Appendix S4. Protocol SR Probiotics.