SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	Development of immunity in animals following Anti-rabies vaccination: a systematic review and meta-analysis	
2.	Authors (names, affiliations, contributions)	Hasanthi Rathnadiwakra[1], Chandrindu Abeykoon[2], Ranil Jayawardena[1], Mangala Gunatilake[1] [1]Department of Physiology, Faculty of Medicine, University of Colombo, Sri Lanka [2]Department of Veterinary Clinical Science, University of Peradeniya	
3.	Other contributors (names, affiliations, contributions)	Merel Ritskes- Hoitinga (Professor, SYRCLE team) Judith van Luijk (Postdoc, SYRCLE team)	
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5.	Funding sources/sponsors	None	
6.	Conflicts of interest	None	
7.	Date and location of protocol registration	June 2020 www.syrcle.nl	
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?	Vaccination of animal species responsible for transmission of rabies virus has become the most effective method to control rabies. Studies have shown that anti-rabies vaccination induce humoral and cellular immune response. However, the efficacy of the vaccine and subsequent immune response can be influenced by various factors. Assessment of this efficacy is largely measured in animals by determination of virus neutralising antibody titres which indicates humoral immunity. Initially rabies-virus neutralising antibodies had been measured in vivo using the mouse neutralization test. Currently there are several methods available for the determination of humoral immunity such as rapid fluorescent focus inhibition test (RFFIT), Fluorescent antibody virus neutralisation (FAVN) test and semi quantitative ELISA methods. Regarding cellular immunity detection, the techniques such as ELIspot assay, Luminex technique and MTT colorimetric assays are being used.	

		Since there are different immunity detection methods used in different countries, we are planning to compare all these methods with respect to the country, type/brand of vaccine used, type of animal model tested. We are planning to assess the reliability of each method and the presence of drawbacks. Also, we are planning to find whether there are any factors that affect the efficacy and immunity development following vaccination (such as animal dependant factors).	
	Research question		
11.	Specify the disease/health problem of interest	Rabies	
12.	Specify the population/species studied	Mammals	
13.	Specify the intervention/exposure	Anti-rabies vaccination	
14.	Specify the control population	Control populations as indicated in the studies e.g. un-vaccinated control groups and ect	
15.	Specify the outcome measures	Humoral and cellular immunity development	
16.	State your research question (based on items 11-15)	What is the status of humoral and cellular immunity development in animals against rabies following anti-rabies vaccination?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	✕ PubMed ✕ Web of Science ✕ SCOPUS ☐ EMBASE ☐ Other, namely: Non bibliographical database (Google) ☐ Specific journal(s), namely:	
18.	Define electronic search strategies	Please see below the protocol	
19.	Identify other sources for study identification	✕ Reference lists of included studies ☐Books ☐Reference lists of relevant reviews ✕ Conference proceedings, namely: Sri Lanka Veterinary Association ☐Contacting authors/ organisations, namely: ☐Other, namely:	
20.	Define search strategy for these other sources	Screening the reference list for relevant titles and screening the abstract of those relevant titles	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	1. Pre-screening based on title and abstract 2. Full text screening of relevant articles selected from first phase	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	(a) Two reviewers will independently screen title and full text for the inclusion criteria (b) Differences in opinion that cannot be resolved by discussion will be resolved by involving the third investigator	
	Define all inclusion and exclusion criteria based on:		

23.	Type of study (design)	Inclusion criteria: Animal intervention (studies will be included regardless of the methodology and quality) Exclusion criteria: Non-intervention studies	
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: All animal models for rabies (Regardless of age, gender, species, housing/setting) Exclusion criteria: Human, Animals with pathological conditions	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: Anti-rabies vaccination (All routes of administration, frequency, types, doses and combine vaccines) Exclusion criteria: Natural infection and other type of medical intervention (i.e: Other treatments/ drug administrations)	
26.	Outcome measures	Inclusion criteria: Humoral immunity; Antibody titres (IU/ml) in FAVN, RFFIT. Presence or absence of antibodies in ELISA, detection of antibodies/ humoral immune response by any other accepted method Cellular immunity; IFNɣ secreting cell count, Plasma cells and memory B cell count, Cytokine profiles in peripheral blood mononuclear cells (pg/ml or any other relevant unit) Exclusion criteria: Cellular immunity detection alone or other outcomes such as clinical signs	
27.	Language restrictions	Inclusion criteria: English. If studies found in other languages, those will be translated to English using Google translator and the necessary information will be gathered Exclusion criteria:	
28.	Publication date restrictions	Inclusion criteria: All publication dates Exclusion criteria: None	
29.	Other	Inclusion criteria: Primary studies, Studies that present original work Exclusion criteria: Non-primary studies, duplicate studies	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase tiab screening: 1. Not an animal study (Human study) 2. Not a primary study (review articles) 3. Not an intervention study (not given the rabies vaccine) Selection phase: 1. Other type of intervention, apart from vaccination 2. Different outcome measure (other than immunity) 3. Presence of pathological conditions in the study group 4. Duplicate study/ repeated studies in the same conditions, same country	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	Authors, year, country, journal	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Animal model tested, Number of animals used	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	Species, age, gender, random stray population/ domesticated population/ laboratory model, health status	

34.	Intervention characteristics (*e.g.* intervention, timing, duration)	Type of vaccine given (combined vaccines/ different brands), number of booster doses given, age of vaccination, route of administration,	
35.	Outcome measures	Frequency of sample collection, time separation between vaccination and 1st measurement, immunity level at each time points, method used to detect immunity, sample specifications (any criteria that was used to select samples for testing; eg; If haemolysed samples were tested or not)	
36.	Other (*e.g.* drop-outs)	Any confounding factors to immunogenicity, important finding/ conclusion, future directions	
	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	(a) Two reviewers will independently assess the bias (b) Differences that cannot be resolved by discussion will be resolved by involving the third investigator	
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 ☐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)	• Frequency of sample collection; number of samples collected after vaccination in each time interval-continuous data • Time separation between vaccination and 1st measurement; number of days – continuous data • Immunity level at each time points: Humoral immunity by antibody titre (IU/ml) – continuous data Presence or absence of antibodies in ELISA (dichotomous) and their respective percentages (continuous) Cellular immunity by different mononuclear cell counts and cytokine profiles (continuous data in pg/ml or any other relevant unit)	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	Extract data from tables, graphs (simple screen ruler) Contacting authors in case of missing data (only if the author contact details are available)	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	(a) Two authors will extract data independently (b) Differences will be resolved by discussion	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Meta-analysis with subgroup analysis for both outcome measures	

43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	If the studies are sufficiently comparable (based on the design etc), outcome data will be pooled. Minimum 3 studies will be considered If not comparable, subgroup analysis will be formed	
	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)	Humoral immunity measurement: Standardized mean difference of antibody titres. All data converted to (IU/ml) Cellular immunity: Standardized mean difference of number of cells and different cytokine concentrations (depending on the reported units whether they can be converted into a single unit)	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effects model	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I^2	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	Species variation Gender variation Type of vaccine (e.g. inactivated, killed etc)/ route of administration Duration of immunity development Different brands of anti-rabies vaccines used (The analysis will be done with respect to different manufacturers, and details will be collected about the sponsors and collaborates of those studies as well)	
48.	Any sensitivity analyses you propose to perform	During the review process, if any individual variations/ specialities of the studies which can influence the finding of the review are identified, a suitable sensitivity analysis will be performed (eg: differences in the number of booster vaccinations used, use of a different kind of animal model/ species which do not used commonly etc)	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	If multiple testing available, we will make subgroups with similar testing and analysis will be done. Similarly, subgroup analysis will be done based on specific control groups used in the studies.	
50.	The method for assessment of publication bias	Funnel plot will be drawn	
Final approval by (names, affiliations):		Date:	

Search Strings

Database	Sech equation	Number of hits (14/06/2020)
PubMed	((rabies[Title/Abstract]) AND (immunity[Title/Abstract])) AND ((vaccine[Title/Abstract]) OR (vaccination[Title/Abstract]))	416

Web of Science	#1. [vaccine AND vaccination] #2. [rabies AND immunity] #3. #2 AND #1	313
Scopus	(((TITLE-ABS-KEY (rabies) AND TITLE-ABS-KEY (immunity)) AND ((TITLE-ABS-KEY (vaccine) OR TITLE-ABS-KEY (vaccination)))	1124