APPENDIX II: Evidence Profiles

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Evidence Profile: Question 1

Question 1: Does novel evidence support the use of PrEP in particular sub-populations, apart from persons bearing an occupational rabies exposure risk?

Population	Adults or children residing in remote areas with a continual or frequent risk of rabies exposure
Intervention	Large scale PreP for specific sub-populations (other than professionals at risk) to prevent human rabies
Comparator	No PrEP to children or adults, standard PEP (including RIG) in case of rabies exposure
Outcome	No or insufficient level of neutralizing Abs ($\geq$ 0.5 IU/ml) and inadequate immune response in case of a rabies exposure, rabies infection

Background:

Poor and rural populations are disproportionately affected by rabies, with the majority of deaths occurring in children under the age of 15 in Asia and Africa. Children living in or visiting rabies-affected areas are at particular risk. Persons who have received PrEP require fewer doses of PEP and can be treated without RIG, which is costly and difficult to procure. PrEP can play a valuable role in protecting persons at high risk, especially in areas where control of disease in the animal reservoir (domestic or sylvatic) is virtually impossible or difficult to implement, or where access to PEP and RIG is unreliable or non-existent. The majority of human rabies cases in the world are due to exposures to rabid dogs (WHO, 2013). However, in parts of Latin America, particularly in the Amazonian region, bat-mediated rabies exposures contribute up to a third of the human rabies cases.

Current position and practice:

The WHO Rabies Vaccine position paper (2010) recommends PrEP for anyone who will be at continual, frequent or increased risk of exposure to the rabies virus, because of either their residence or occupation. However, in practice, PrEP is hardly ever made available to children or entire populations living in areas of high rabies risk (WHO, 2013). The position paper calls for studies on the feasibility, cost-effectiveness and long-term impact of incorporating cell culture and embryonated egg-based vaccines (CCEEVs) into the immunization programmes of children in places where canine rabies is a public health problem. No separate reference is made to regional specificities, e.g. bat-mediated rabies virus exposures in high risk areas (e.g. Amazonian region).

New evidence:

PrEP in areas of high rabies risk, focus on children:

A systematic review on PrEP summarizes relevant new evidence (Kessels et al., 2017), including experiences and results from national programmes implementing PREP for high-risk populations in the Philippines (mainly canine-mediated rabies) and Peru (both, canine- and bat-mediated rabies). This review also addresses available evidence on cost-effectiveness of such interventions in specific sub-populations. Meeting reports of experts on rabies and paediatric health in Asia and the Middle East have recommended PrEP programmes for those in high risk populations, especially children (Dodet, 2008, 2010; Aikimbayev et al., 2014). In India, the Academy of Paediatrics has called for the inclusion of PrEP for high-risk children in the immunization schedule for children aged 0-18 years (Vashishtha et al., 2014).

In total, 7 studies investigated the safety and immunogenicity of intradermal PrEP in children from 2 months to 15 years of age using purified chick embryo cell vaccines (PCECV) (Kamoltham et al., 2007,

2011; Shanbag et al., 2008; Pengsaa et al., 2009; Strady et al., 2009; Malerczyk et al., 2013; Ravish et al., 2013). All describe it to be safe and immunogenic in both infants and children. The detailed results of the cited studies are displayed in the Table 1 below.

Combination of rabies vaccines with other childhood vaccines:

Three studies found PrEP safe and immunogenic for up to 5 years in combination with other childhood vaccines such as Japanese encephalitis, diphtheria, tetanus, pertussis and oral and inactivated poliomyelitis vaccines (Vien et al., 2008; Lang et al., 2009; Pengsaa et al., 2009). See also Table 1.

Additional information:
A more recent prospective cohort study in the Para State of Brazil evaluated the persistence of rabies virus-neutralizing antibodies annually over 4 years in people who received either PrEP or PEP (Medeiros et al., 2016). The cohort included 506 persons from 2 to 83 years of age living in an area with considerable rabies exposure risk (bats, dogs and other animals). 85-88% of the not re-boosted participants evaluated at yearly follow-up visits remained seroconverted (see Table 2). Similar rabies virus-neutralizing antibodies persistence profiles were observed in participants originally given PEP or PrEP, and the GMT of the study population remained >1 IU/mL 4 years after vaccination. There were no rabies human cases recorded in the study cohort during the entire follow up period. No cost-effectiveness analysis was conducted.

New evidence from cost-effectiveness modelling (see Annex 1):

The full background document "Consideration of rabies pre-exposure vaccination (PrEP) within the routine EPI schedule in rabies endemic countries" is available as Annex 1. The study aimed at quantifying the potential benefits and relative costs of inclusion of rabies PrEP within a routine EPI schedule in settings where rabies is endemic.

Conclusion:

The results highlight that PrEP as a large scale public health intervention, e.g. PrEP delivery as part of the EPI programme, is likely to be substantially more expensive than other measures to prevent human rabies deaths, such as PEP and dog mass vaccination campaigns. PrEP for entire populations is highly unlikely to be an efficient use of resources and should only be considered in extreme circumstances, where the incidence of rabies exposures is unusually high (incidence >6%). Modelling could be used to support decision making in specific high-exposure contexts of local settings.

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Table 1 Published data from clinical trials or observational studies in children receiving PrEP by IM and ID routes. (adapted from Kessels et al)

Author(s)	Year	Location	Study population	Study type	Cohort	Vaccine	Route	Regimen	Primary antibody response (IU/ml)	Recall antibody response (IU/ml)	Comments
Lang, J. Et al.; Vien, N.C. Et al.; Lang, J., et al.	2009 2008 1997	Vietnam	Infants (< 1)	prospective cohort studies RCT	84 228 84	PVRV	IM	2 doses at 2 and 4 mo of age, yr 5	20.1	>1	Priming combined with DTP-IPV [2009, 2008 studies]
Pengsaa, K., et al.	2009	Thailand	Toddlers (1-1.5)	RCT	200	PCECV	IM	D 0, 7, 28, yr 1;	IM: 15-41	IM: 103-299	Higher titres in IM group;
							ID	D 0, 28, yr 1	ID: 4.1-8.5	ID: 8.0-38	Priming combined with JE
Lumbiganon, P., et al.	1989	Thailand	Children (2-15)	clinical study	13 and 12	PCECV	IM/ID	D0, 7, 28	4.7-47	n.t.	Higher titres in IM group
Kamoltham, T., et al.; Kamoltham, T., et al.	2007 2011	Thailand	Children (5-8)	RCT follow up study	703	PCECV	ID	D 0, 7, 28, yr 1 or later; D 0, 28, yr 1 or later	>2	8.9-27.3	Higher recall responses upon priming with 3 doses
Shanbag, P., et al.	2008	India	Children (6-13)	observer-blind RTC	175	PVRV/ PCECV	IM	D 0, 7, 28	12.2-14.5	n.t.	All subjects developed RVNA

											concentrations > 0.5 IU/ml
Strady, C., et al	2009	France	Children/Adults (12-79)	clinical study	407	HDCV/	IM	D 0, 7, 28, yr 1;	0.1-48	51 IU (3-dose priming	Routine booster injection after 1 year could increase levels and duration of antibody titres.
						PVRV		D 0, 28, yr 1	(analysed 1 yr later)	13 IU 2-dose priming	

Table 2: Detailed results on the persistence of rabies neutralizing antibodies in an Amazonian population (Medeiros et al.)

Location	Study population	Study type	Cohort size	Vaccination Regime	Time Since PREP	Antibody titre	Conclusions
Para state, Brazil	Immunized population (aged 2 - 83 years) at risk of vampire bat rabies and who had titres >0.5IU/ml in 2005	Prospective cohort study	448 PEP in 2005 58 PrEP in 2005	IM PEP IM PREP	2 y (2007)	>0.5IU/ml: 84.6%	There was a total of 89 cases of re-exposure to rabies (mostly by dogs (52 participants) but also bats, cats, and monkeys). No cases of rabies occurred among the study participants.
					3 y (2008)	>0.5IU/ml: 88.0%	
					4 y (2009)	>0.5IU/ml: 85.7%	

ANNEX 1

Consideration of rabies pre-exposure vaccination (PrEP) within the routine EPI schedule in rabies endemic countries

Background

There are considerable efforts underway to reduce the global burden of human rabies, with the goal of reaching zero human rabies deaths by 2030. The two primary (and complementary) strategies to prevent human rabies deaths are (1) canine vaccination to eliminate rabies at its source and (2) offering post-exposure prophylaxis (PEP) in the form of rabies vaccination and, in some settings, immunoglobulin, to individuals who have been bitten by suspected rabid mammals. A third preventive strategy that may be considered is pre-exposure prophylaxis (PrEP) in which a series of injections of rabies vaccine is given to prime the immune system. Individuals who have received PrEP still require PEP, but they require fewer doses of vaccine than unprimed individuals and do not require rabies immunoglobulin.

A systematic review on the safety, immunogenicity, cost-effectiveness and recommendations for use of rabies PrEP has recently been published (Kessels *et al* 2017). The review concluded that "Pre-exposure rabies prophylaxis is safe and immunogenic and should be considered: (i) where access to postexposure prophylaxis is limited or delayed; (ii) where the risk of exposure is high and may go unrecognized; and (iii) where controlling rabies in the animal reservoir is difficult." National rabies PrEP programmes have been implemented in Peru (where there are high exposures via vampire bats) and the Philippines (where children at risk of dog-transmitted rabies were targeted).

Offering more widespread PrEP, for example within the routine EPI immunisation schedule in rabies endemic countries, raises considerable practical and operational difficulties, as delivering multiple doses of vaccine within a short time scale (such as a week) lies outside the standard EPI programme. However, if PrEP could be a cost-effective method to prevent human rabies, ways to overcome these challenges should be considered. In contrast, if PEP alone, without widespread PrEP, can bring additional benefits, efforts should focus on improving PEP access in marginalized communities. We developed models to quantitatively assess the potential costs and effectiveness of these strategies.

Aims

To quantify the potential benefits and relative costs of including rabies PrEP within a routine EPI schedule in settings where rabies is endemic.

Methods

We took two different approaches to address this question: (a) development a model of a hypothetical birth cohort of 100,000 children to investigate the trade-off between bite incidence and the relative cost of PrEP + PEP *versus* PEP alone; (b) adaptation of an existing model specific to N'Djamena, Chad to investigate the costs and benefits of PrEP compared to both PEP and dog vaccination.

<u>a. Hypothetical birth cohort</u>
We developed a simple simulation model to estimate the relative cost of PrEP +PEP *versus* PEP alone in a population in a setting endemic for rabies. This cost ratio is largely dependent on two

parameters: the incidence of dog bites (for which individuals will seek PEP) and the cost per course of PrEP +PEP vs PEP alone.

Bite incidence. In the latest global burden of rabies study (Hampson et al 2015) the incidence of dog bites in endemic settings varied from around 12 per 100,000 (in Chad) up to 2 orders of magnitude higher at around 1200 per 100,000 (India, Sri Lanka, Cambodia, Myanmar). A more recent systematic review covering the period 2013-2015 reported typical bite incidence in the range 10 to 130 per 100,000 per year (WHO, unpublished). The highest reported bite incidence we have identified in the literature is 4840 per 100,000 in rural Cambodia which is far higher than reported in any other setting (Ponsich et al 2016). Since the costs of PEP are only relevant for individuals who seek care, the crude dog bite incidence should be modified by the proportion of people seeking care. We chose to model a typical range of 10 to 500 per 100,000 per year.

Costs of PrEP and PEP. There are a range of different regimens being considered for both PEP and PrEP; we did not model these individually and assumed that any differences in health benefits would be marginal. Individuals who are primed by PrEP do not require expensive immunoglobulin. We assumed that costs of PEP for those who have been primed is the same as the cost of PrEP. Although it is the relative costs that are important, for the sake of the simulation we assumed the cost of PrEP varied between $5 and $20 and the cost of PEP in naïve individuals varied from $10 to $160.

Simulation. Assuming that both bite incidence and costs of PrEP and PEP followed a uniform distribution we ran 10,000 simulations in R to estimate the ratio of costs for a hypothetical cohort of 100,000 children. We assumed that EPI vaccine uptake was high. We assumed that protection from PrEP lasted for 20 years, on the basis of 3 studies showing immunogenicity out to 5 years (Kessels et al, 2017) and that dog bites are most common in children. Future costs were not discounted.

b. N'Djamena, Chad

Model: For the setting of N'Djaména, data from a rabies elimination project exists and was used to estimate the comparative cost-efficiency of PEP alone (scenario 1), PEP with dog vaccination (scenario 2) and a holistic rabies control approach with perfect communication between veterinary and human health sector accompanying PEP and dog vaccination (scenario 3). This analysis was published recently (Mindekem et al., 2017).

PrEP: To evaluate the effectiveness of PrEP for a real life example, additional scenarios were simulated that include PrEP vaccination of a yearly cohort of children and PEP for 100% prevention of human rabies deaths.

Parameters: For rabies exposure cases the real number of suspected rabies bite cases were taken from the data set collected in 2012 in health facilities in N'Djaména before the dog mass vaccination campaign. The yearly extrapolated numbers of exposures of the whole city was observed to be 374 of which 42% are children below 15 years of age. Currently in N'Djaména the Essen 5 dose regimen is used and costs of a whole PEP treatment are 198 USD including transport and personnel costs. The PEP cost does not include immunoglobulin, because it is virtually unavailable in Chad. PrEP costs were calculated to be 48'560 FCFA (83 USD) on the basis of the local cost for 3 doses of vaccine, transport cost, loss of work time and cost for technician. It was further assumed that a pre-vaccinated child needs 2 additional doses of vaccine if exposed. Cost of this shortened PEP schedule are 39'000 FCFA (66.5 USD) including cost for additional wound treatment. It was assumed PrEP coverage was 55% of all surviving infants based on the observed measles 1 vaccination coverage in Chad. This is probably optimistic as a full PreP schedule requires 3 visits rather than just 1 for measles. To achieve this coverage among surviving infants approximately 57270 children have to be

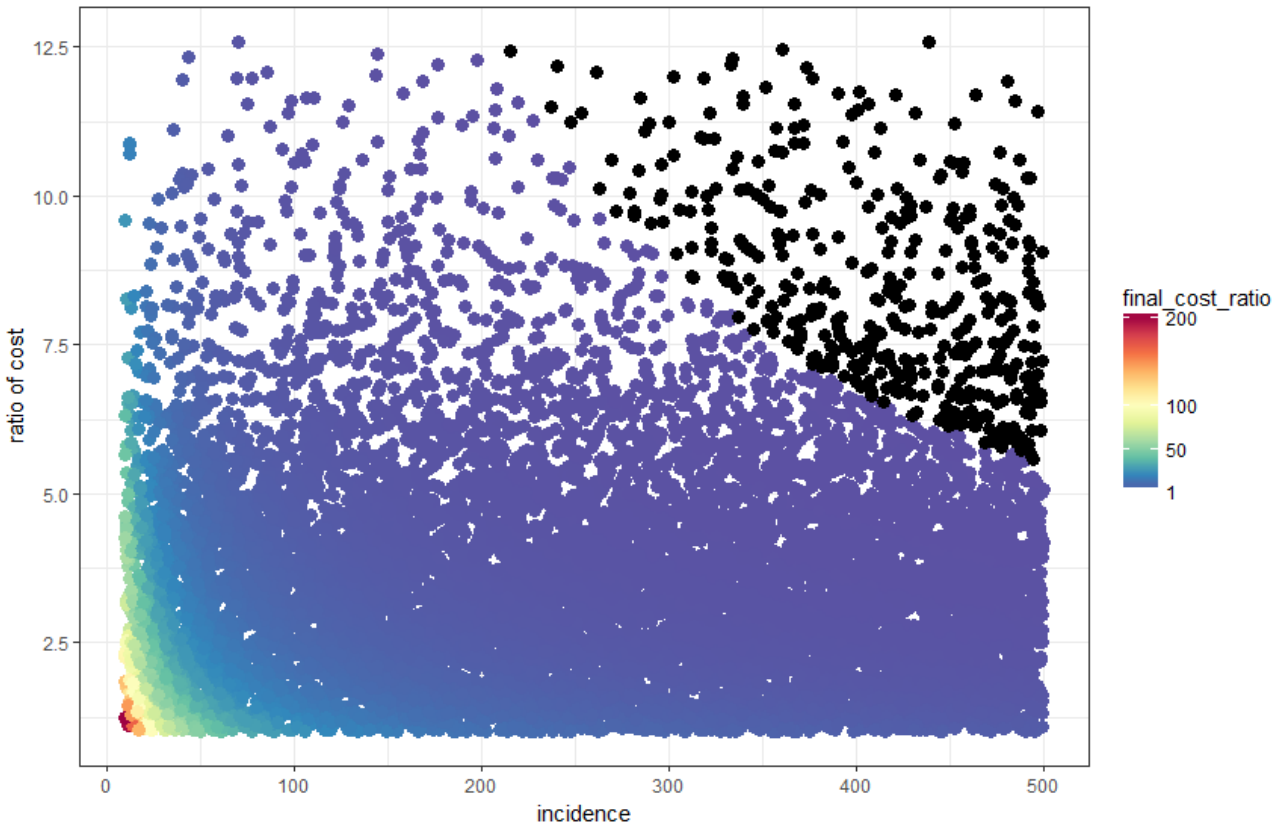
vaccinated against rabies each year in N'Djaména. Based on a demographic model without sex stratification we simulated the change of PrEP coverage in the population of children below 15 years of age over a period of 20 years. This coverage was multiplied with the percentage of children among all yearly exposure victims which resulted in an increasing number of children requiring two PEP doses instead of 5. The overall cost of scenario 4 is the sum of PreP cost, PEP cost for pre vaccinated children and PEP cost of unvaccinated children and adults. The cumulative costs were discounted at a rate of 0.04. The DALYs averted where calculated on the basis of a 19% risk of developing rabies after exposure and the age distribution observed among bite victims.

Results

a. Hypothetical birth cohort

The use of PrEP +PEP was at least twice as expensive compared to PEP alone in 75% of simulations. In some simulations where bite incidence was low and costs of PEP in naïve individuals were also relatively low, the ratio was in the range of 100-200. In 4% of simulations (indicated as black points in figure1) the ratio was ≤1, meaning that PrEP+PEP was less expensive than PEP alone; here both bite incidence and relative cost of PEP in naïve individuals were high.

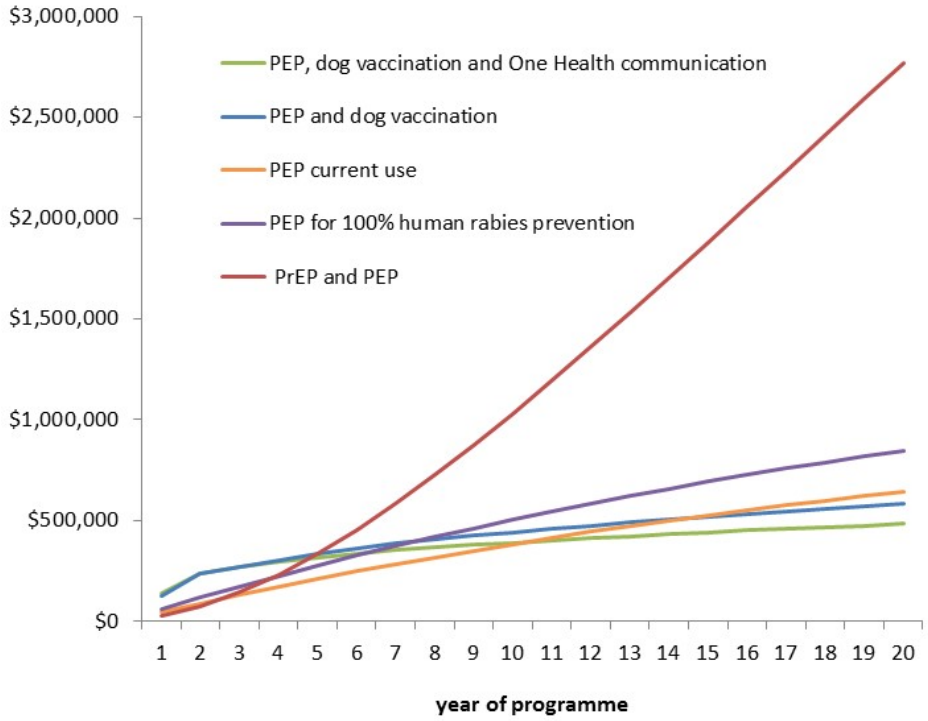
Figure 1: Ratio of the costs of PrEP +PEP versus PEP alone, by varying dog bite incidence and relative cost of PEP in naïve individuals compared to cost of PrEP + PEP in primed individuals. Note that simulations with a final cost ratio ≤1 are indicated in black.



b. Chad

The results of the estimations presented in figure 2 below show that the cost of PrEP with PEP is more than five times higher than the costs of all other included scenarios. The cost invested per DALY averted are $3242 USD as opposed to $43 USD invested per DALY averted with a sole PEP approach.

Figure 2: Total costs of different rabies control strategies in Ndjamena, Chad



Discussion

Through our model simulations we have shown that the use of pre-exposure vaccination for rabies is unlikely to be an efficient use of resources. We have assumed that PEP will always be necessary after exposure to a potentially rabid animal (accepting this as the ethical position), therefore there are limited health benefits and substantial costs associated with PrEP. Use of PrEP in the EPI schedule targets many more children than are likely to be exposed to rabies and unlike most other infectious diseases, this risk is identifiable (i.e. animal bite victims can be targeted for PEP). Of course, this assumes that PEP is available, which may not always be the case. In view of the current shortage of human rabies vaccine observed in some countries it will be fatal to rabies exposure victims to divert vaccine away from PEP to PrEP. Furthermore based on our experience from Chad we hypothesize that marginalized communities (nomadic groups, very remote villages) that already have low access to PEP will also be less likely to have access to PrEP and therefore the issue of health inequality will remain.

We have used two different modelling approaches, one generic and one context specific, to address the potential costs and cost-effectiveness of PrEP. For the hypothetical birth cohort we simulated from a range of dog bite incidence and relative costs. We did not however take into account differential protection as all regimens are expected to provide good protection, nor did we account for age-specific variation in the incidence of dog bites. In the specific example, model parameters were based upon extensive field studies in Chad. We did not model the use of PrEP in other specific settings but there are additional studies reported in the literature (summarised in Kessels et al,

2017). In one study from Thailand, they similarly found that bite incidence would need to be much higher than has been observed to make PrEP cost-comparable (Chulasugandha et al, 2006).

These analyses suggest that investing in PEP, or indeed dog vaccination, will be preferable to investing in PrEP. Our findings are in agreement with the recent systematic review of PrEP (Kessels et al 2017). Even if the price of rabies vaccine were to be considerably lower, the *marginal* cost-effectiveness of PrEP is still likely to be less favourable than PEP or dog vaccination, simply because many more individuals need to be targeted. The use of absolute thresholds for assessing cost-effectiveness are not recommended by WHO and "should never be used as a stand-alone criterion for decision-making" (Bertram et al 2016).

Conclusions

We find that PrEP as part of the EPI programme is likely to be substantially more expensive than other measures to prevent human rabies deaths, such as PEP and dog vaccination. PrEP is highly unlikely to be an efficient use of resources and should only be considered in extreme circumstances, where the incidence of rabies exposures is high. Modelling could be used to support decision making in specific high-exposure contexts.

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GRADE Tables: Question 1

Does novel evidence support the use of PrEP in particular sub-populations, apart from persons bearing an occupational rabies exposure risk?				
			Rating	Adjustment to rating
Quality Assessment	No. of studies/starting rating		4 RCT	4
	Factors decreasing confidence	Limitation in study design	Non-serious[1]	0
		Inconsistency	Non-serious	0
		Indirectness	Non-serious	0
		Imprecision	Non-serious	0
		Publication bias	Serious[2]	-1
	Factors increasing confidence	Strength of association	Applicable[3]	1
		Dose-response	Non-applicable	0
		Mitigated bias and confounding	Applicable[4]	1
	Final numerical rating of quality of evidence			5
Summary of Findings	**Statement on quality of evidence**			Evidence supports a moderate level of confidence that the true effect lies close to that of the estimate of effect on health outcome
	Conclusion PrEP is safe and immunogenic in children and other sub-populations living in at risk areas, including when administered with childhood vaccinations or Japanese encephalitis vaccines in children and adults.			

[1] Recall antibody response was not tested in Shanbag et al.
[2] While clinical publications on safety and immunogenicity of PrEP in children are available, data on large scale implementation, its cost-effectiveness and impact on health at population level are limited. The majority of trials were conducted in South and South East Asia.
[3] All studies prove that PrEP is safe and immunogenic in children and other sub-populations at risk and could be implemented at large scale pending cost-effectiveness assessment in more settings
[4] All the RCTs addressed control of potential confounding.

Does novel evidence support the use of PrEP in particular sub-populations, apart from persons bearing an occupational rabies exposure risk?				
			Rating	Adjustment to rating
Quality Assessment	No. of studies/starting rating		6 observational	2
	Factors decreasing confidence	Limitation in study design	Serious[1]	-1
		Inconsistency	Non-serious	0
		Indirectness	Non-serious	0
		Imprecision	Non-serious[2]	0
		Publication bias	Serious[3]	-1
	Factors increasing confidence	Strength of association	Applicable[4]	1
		Dose-response	Non-applicable[5]	0
		Mitigated bias and confounding	Non-applicable	0
	Final numerical rating of quality of evidence			1
Summary of Findings	**Statement on quality of evidence**			Evidence supports a low level of confidence that the true effect lies close to that of the estimate of effect on health outcome
	Conclusion PrEP is safe and immunogenic in children and other sub-populations living in at risk areas, including when administered with childhood vaccinations or Japanese encephalitis vaccines in children and adults			

[1] Recall response was not tested in Lumbignanon et al. the study has a limited sample size high variation in antibody titres and uses other antibody testing methods than the other studies. Limitations due to small sample size.

[2] Strady et al: Vaccine-related factors explained only 32% of variation in antibody titres following PrEP

[3] While clinical publications on safety and immunogenicity of PrEP in children are available, data on large scale implementation, its cost-effectiveness and impact on health at population level are limited.

[4] Most studies prove that PrEP is safe and immunogenic in children and other sub-populations at risk

[5] The cohort study of Medeiros et al additionally assessed the rabies exposures and mortalities in the cohort during the entire study period. No human rabies cases occurred.

Evidence Profile: Question 2

Question 2: Does novel evidence support the need for rabies booster doses in persons at continual or frequent risk of occupational rabies exposure?

Population	Healthy persons with a continual or frequent risk of occupational rabies exposure and who have received at least a complete primary series of PrEP or PEP
Intervention	No PrEP boosters
Comparator	PrEP boosters as a precautionary measure with or without determination of antibody titres
Outcome	Maintenance of a sufficiently protective level of neutralizing Abs and rapid immune response in case of an unnoticed rabies exposure, potential vaccine savings. (in case of an obvious exposure, standard WHO recommendations for PEP in already immunized patients would apply)

Background:

PrEP mitigates the risk of occupational exposure to rabies virus. The potential frequency and possible ways of exposure, as well as virus load or its virulence might be highly variable and dependent on the setting, the protective measures implemented and the professional activity. Employers may have legal obligations towards staff at risk.

Current position and practice:

Routine booster doses of rabies vaccines are not required for individuals living in or travelling to high-risk areas who have received a complete primary series of PrEP or PEP with a CCEEVs. Individuals who had received their primary series 5–21 years previously showed good anamnestic responses after booster vaccination. Long-term immunity is also achieved with intradermal immunization, and may persist even when antibodies are no longer detectable. The ability to develop an anamnestic response to a booster vaccination is related neither to the route of administration of the initial series (*i.e.* IM or ID), nor to whether the patient completed a PrEP or PEP series.

However, periodic routine booster injections are recommended as an additional precaution for persons whose occupation puts them at 'continual' or 'frequent' risk of exposure. The current risk categorization and corresponding recommendations on PrEP for specific subsets of professionals at risk is annexed as Table 1a (source Müller et al 2015). Routine pre-exposure booster immunization consists of one dose of modern cell culture vaccine, either ID or IM. If available, antibody monitoring of at-risk personnel is preferred to the administration of routine boosters. For professionals who are potentially at risk of laboratory exposure to high concentrations of live rabies virus, antibody testing should be done every 6 months. Professionals who are not at continual risk of exposure through their activities, such as certain categories of veterinarians and animal health officers, should have serological monitoring every 2 years. Because vaccine-induced immunity persists in most cases for years, a routine booster would be recommended only if rabies virus neutralizing antibody titres fall below 0.5 IU/ml.

New evidence:

A general compilation of new evidence on requirements for routine booster vaccination are available in Kessels et al. 2017. Another study of general interest is from Mansfield et al 2016 who assessed neutralising antibody responses in a cohort of rabies-vaccinated recipients over a period of twenty years. There are only 2 new studies available that investigate the potential frequency or need for routine boosters in occupationally exposed persons (see Table 2). The two studies (Lim & Barkham

and Cunha) were conducted with a limited study cohort size and one cover only a short post PrEP monitoring period. The nature of rabies exposure or risk category of the occupational rabies hazard of the study participants is not further specified in either study:

Lim *et al.* conducted a retrospective cohort study on 66 government-employed veterinarians and animal health workers (convenience sample) who received primary rabies vaccination with purified Vero cell vaccine. One year later, 26 (39%) demonstrated antibody titres below the recommended minimum of 0.5 IU/ml, and thus required a routine booster. Serological surveillance to determine the need for a booster appears justified in those with an ongoing need for protection and those who may face unrecognized exposures. This category would include both, veterinarians as well as travellers with extensive informal animal exposures, such as volunteers in animal sanctuaries or expatriates adopting stray animals in rabies-enzootic areas. In such high-risk persons who lack access to serologic testing, the authors recommend providing a routine booster 1 year after primary rabies vaccination to ensure adequate minimum protective titres.

Cunha *et al.* conducted a randomized controlled study involving veterinarians, biologists, students, researchers, municipal guards and zoonotic control workers. The authors compared measurable immune-response of subjects receiving either IM or ID administration of PrEP. The study concluded that ID PrEP was more cost-effective than IM administration in this context and that serology after the 3rd dose of PREP was unnecessary. However, the study authors admit potential bias in the results of subjects who received ID PrEP, due to administration techniques of vaccine and due to laboratory techniques used to measure the humoral response.

Conclusion

As novel, generic evidence on PrEP is available, combine it with expert opinion, this might be an opportunity for updating the Table 1 (Müller et al 2015). The table would additionally cover aspects of timely access to vaccines after exposure, broaden the specifications of the typical target population or professionals at risk and provide more flexible options for serologic testing (see revised proposal Table 1b).

References:

Müller, T et al. Chapter 21: Elimination of rabies - a missed opportunity. pp 527-571

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Table 1a: Current decision matrix for rabies immunization: Criteria for pre-exposure immunization, Müller et al. 2015:

Criteria for Pre-exposure Immunization			
Exposure category	Nature of risk	Typical populations	Preexposure regimen
'Continuous'	Virus present continuously, usually in high concentrations. Specific exposures may be unrecognized. Bite, non-bite, or aerosol exposures.	Rabies research workers.[c] Rabies biologics production workers.	Primary course. Serologic testing every ~6 months. Booster immunization if antibody titer falls below 'acceptable' level.[c,d]
Frequent	Exposure usually episodic, with source recognized, but exposure also may be unrecognized. Bite, nonbite, or aerosol exposures.	Rabies diagnostic workers,[c] cavers, veterinarians and staff, and animal control and wildlife workers in areas where rabies is enzootic. All persons who handle bats.	Primary course. Serologic testing every ~2 years. Booster vaccination if antibody titer is below 'acceptable' level.
Infrequent (greater then population-at-large)	Exposure nearly always episodic with source recognized. Bite or nonbite exposures.	Veterinarians and animal control staff working with terrestrial animals in areas where rabies is uncommon to rare. Veterinary students. Travelers visiting areas where rabies is enzootic and immediate access to appropriate medical care including biologics is limited.	Primary course. No serologic testing or booster vaccination
Rare (population-at-large)	Exposure always episodic. Bite or nonbite exposure.	Population-at-large, including individuals in rabies-epizootic areas.	No vaccination necessary, unless exposed.

[a]Adapted from recent WHO and U.S. Advisory Committee on Immunization Practices (ACIP) guidelines.

[b]Pre-exposure immunization. Preexposure immunization consists of three doses of cell culture vaccine, ID or IM (i.e., deltoid area), one each on days 0, 7, and 21 or 28. Administration of routine booster doses of vaccine depends on exposure risk category as noted above.

Post-exposure immunization. All PEP should begin with immediate thorough cleansing of all wounds with soap and water. Persons not previously immunized: RIG, 20 IU/kg body weight, as much as possible infiltrated at the bite site (if feasible), with the remainder administered IM; cell culture vaccine, ID or IM (i.e., deltoid area), one each on days 0, 3, 7, and 14. Persons previously immunized: Two doses of modern cell culture vaccine, ID or IM (i.e., deltoid area), one each on days 0 and 3. No RIG should be administered. Pre-exposure immunization with modern cell culture vaccine; prior PEP with modern cell culture vaccine; or persons previously immunized with any other type of rabies biologic and a documented history of an 'acceptable' rabies virus neutralizing antibody response to the prior vaccination.

[c]Assessment of relative risk and any extra monitoring of immunization status of laboratory workers is the responsibility of the laboratory supervisor (as an example, see guidelines in the current edition of the United States Department of Health and Human Services' Biosafety in Microbiological and Biomedical Laboratories).

[d]Routine Pre-exposure booster immunization consists of one dose of modern cell culture vaccine, ID or IM (i.e., deltoid area). An acceptable antibody level is a 1:5 titer (complete inhibition in the RFFIT at a 1:5 dilution, approximately equivalent to 0.1 IU/ml) or ~ 0.5 IU/ml. Boost if the virus neutralizing antibody titer falls below this level, as long as the person remains at risk of viral exposure.

Table 1b: Indications for pre-exposure rabies immunization, adapted from Müller et al. 2015

Examples of typical individuals and populations	Likelihood and nature of exposure to rabies virus	Timely access to rabies biologics	Recommendations on pre-exposure immunization [a] and serologic testing
Occupational exposure			
Individuals involved rabies research, rabies biologics production [b].	Virus may be present continuously, usually in high concentrations. Specific exposures may not be recognized. Bite, non-bite, or aerosol exposures.	Yes	PrEP recommended. Suggested timeframes for serologic testing: After primary immunization and the every ~6 months up to every 1-2 years. Routine booster vaccination [c], if antibody titer falls below 0.5 IU/ml [d].
Individuals working in rabies diagnostic laboratories [b], in hospitals with clinical rabies cases [e], animal disease control, wildlife management, bat handling or with professional activities in caves likely to lead to direct contact with bats.	Settings or areas where rabies is enzootic and where exposure may not be recognized. Presence of bats, particularly non-haematophagous bats. Bite, non-bite, or aerosol exposures.	Variable, mostly yes Variable	PrEP recommended. Serologic testing every ~2 years. Routine booster vaccination if antibody titer is below 0.5 IU/ml. PrEP recommended. No serologic testing or routine booster vaccination.
Individuals working or residing in remote areas for extended periods and involved in e.g. dog vaccination campaigns, animal disease control programmes, peace keeping, military or religious missions.	Remote settings where rabies is enzootic. Exposure typically episodic with source recognized. Bite or non-bite exposures. Partly remote settings where rabies is enzootic. Exposure typically episodic with source recognized. Bite or non-bite exposures.	Variable, mostly not Variable	PrEP recommended. Serologic testing unnecessary unless risk of exposure remains. Otherwise, test and boost if antibody titer falls below 0.5 IU/ml, or alternatively give a routine booster vaccination before departure.
Individuals involved in e.g. animal disease control with direct contact with terrestrial animals.	Settings where rabies is uncommon to rare. Exposure typically episodic with source recognized. Bite or non-bite exposures.	Variable, mostly yes	PrEP recommended. No serologic testing or routine booster vaccination.
Travellers			
Individuals with mainly leisure related exposures by potential direct contact, particularly with carnivores or bats, during activities over an extended period e.g. backpackers, bicycle or	Remote settings where rabies is enzootic. Exposure typically episodic with source recognized. Bite or non-bite exposures. Partly remote settings where rabies is	Variable, mostly not Variable	PrEP recommended. Serologic testing unnecessary unless risk of exposure remains. Otherwise, test and boost if antibody titer falls below 0.5 IU/ml, or alternatively give a routine booster vaccination

motorbike riders, people visiting friends and relatives. Consider cumulative exposure in frequent travelers.	enzootic. Exposure typically episodic with source recognized. Bite or non-bite exposures.		before departure.
Individuals with leisure activities in caves leading to likely direct contact with bats.	Settings or areas where rabies is enzootic and where exposure may not be recognized. Presence of bats, particularly non-haematophagous bats. Bite, non-bite, or aerosol exposures.	Variable, mostly yes Variable	PrEP recommended. Serologic testing every ~2 years. Routine booster vaccination if antibody titer is below 0.5 IU/ml PrEP recommended. No serologic testing or routine booster vaccination.
Sub-populations			
Residents of remote areas where animal rabies control is impaired by difficult access, epidemiological and other factors	Settings or areas where rabies is enzootic, particularly in wildlife and where episodic exposure may not be recognized. Bite or non-bite exposures.	Variable, mostly not	PrEP recommended. No serologic testing or routine booster vaccination.
General population	Areas where rabies is enzootic or epizootic. Exposure always episodic with source recognized. Mostly bite, also non-bite exposures.	Yes	No PrEP recommended. PrEP for general populations is unlikely to be a cost-effective intervention and is usually more expensive than other measures to prevent human rabies deaths, such as post-exposure prophylaxis and dog vaccination campaigns.
In case of a WHO category II or III exposure to a rabid animal (or lyssavirus), post-exposure prophylaxis including thorough wound care is always required. People who have received PrEP should be instructed accordingly.			

[a] A primary course of pre-exposure immunization consists of either a two-site intradermal administration of 0.1 ml of vaccine on days 0 and 7 or one vaccine dose for intramuscular administration on days 0 and 7. Administration of booster doses of vaccine depends on nature and duration of the rabies exposure risk as above.

[b] Assessment of relative risk and any extra monitoring of immunization status of laboratory workers is the responsibility of the laboratory supervisor (as an example, see guidelines in the current edition of the United States Department of Health and Human Services' Biosafety in Microbiological and Biomedical Laboratories).

[c] A routine pre-exposure booster vaccination consists of one dose of modern cell culture vaccine, ID or IM (i.e., deltoid area).

[d] An acceptable antibody level is 0.5 IU/ml or 1:5 virus neutralizing antibody titer (complete inhibition in the RFFIT at a 1:5 dilution, approximately equivalent to 0.1 IU/ml). Boost if the titer falls below this level, as long as the person remains at risk of viral exposure.

[e] Human-to-human transmission of rabies has never been confirmed outside of the transplant setting. However, rabies virus can be found in saliva, tears, and nervous tissues of human rabies cases and represents a theoretical route of transmission. Therefore, pre-exposure immunization might be indicated and can alleviate the psychological burden of fear from infection of health care staff who are regularly attending to patients with clinical rabies.

Table 2: New evidence on routine boosters for potentially rabies-exposed professionals:

Author	**Year**	**Location**	**Study population**	**Study type**	**Cohort size**	**Vaccination Regimen**	**Time since PREP**	**Antibody titre** (in participants)
Lim, P.L. and T.M. Barkham	2010	Singapore	Government veterinarians and animal workers	Retrospective cohort study (convenience sample)	66	3 doses PVRV PREP	1y	>0.5 IU/ml: 60.6%
								<0.5IU/ml: 39.4%
					15	4 doses PREP (3 doses PREP + 1 booster dose)	Mean 10y (range 3-18y)	>0.5IU/ml: 100%
Cunha, R.S., et al	2010	Brazil	Healthy volunteers (veterinarians, biologists, students, researchers, municipal guards, zoonotic control workers)	Randomized controlled study	65	ID PREP	10d	>0.5IU/ml: 97%
							180d	>0.5IU/ml: 20-25%
					62	IM PREP	10d	>0.5IU/ml: 100%
							180d	>0.5IU/ml: 63-65%

Evidence Profile: Questions 3 & 4

Question 3: Can the duration of the entire course of current PREP regimens be reduced while maintaining immunogenicity and clinical protection?

Question 4: Can the number of doses administered in current PREP regimens be reduced while maintaining immunogenicity and clinical protection?

Population	Persons at high risk of rabies exposure
Intervention	Q3: Shortened duration (time frame, number of visits) of the PrEP regimen course Q4: Fewer doses of vaccine for the PrEP course
Comparator	Q3: Current duration of WHO-recommended PrEP regimen (IM or ID days 0, 7, and 21 or 28) Q4: Current number of doses of WHO-recommended PrEP regimen (IM or ID)
Outcome	Inadequate titres of neutralizing antibodies requiring standard PEP (with RIG) in case of a suspect rabies category III exposure, infection with rabies virus

Background:

PrEP can play an important role in protecting persons at high risk of rabies exposure. Reducing the time frame and number of doses required for PrEP would make it more simple and cost-effective to implement, particularly in sub-populations at high risk of rabies exposure or individuals who are occupationally or otherwise exposed. This is especially the case of people living in areas where control of disease in the animal reservoir (domestic or sylvatic) is virtually impossible or very difficult to implement, or where access to PEP and RIG is unreliable or non-existent. Fully immunized patients do not need a costly administration of scarce RIG in case of a category III rabies exposure. Additionally, shortened duration of or fewer visits for completing PrEP are also of high interest to travel medicine, as they may reduce the time span between the first travel clinic consultation and the patients' departure to a rabies endemic region.

Current position and practice:

Current WHO recommendations on PrEP encompass 3 visits for administration of vaccine within a timeframe of 21 to 28 days:

- ID doses of 1 ml or 0.5 ml (volume depending on the type of vaccine) to be given on days 0, 7 and 21 or 28.
- ID administration of a 0.1 ml one-site dose on days 0, 7, and 21 or 28

To confer significant savings, ID immunization sessions should involve enough individuals to utilize all opened vials within 6–8 hours.

New evidence:

A systematic literature review [1] lists 8 studies investigating the safety and immunogenicity of accelerated or revised PREP regimes. One-week duration (Kamoltham et al., 2007; Khawplod et al., 2008; Mills et al., 2011; Lau & Hohl, 2013) and one (Suandork et al., 2007; Khawplod et al., 2008, 2012) or two-visit (Suandork et al., 2007) regimens elicited protective antibody titres (≥ 0.5 IU) for up to one

year (Suandork et al., 2007; Khawplod et al., 2007, 2008, 2012; Mills et al., 2011; Lau et al, 2013), including in combination with JE vaccines (Jelinek et al., 2015). Evidence from two additional, unpublished studies on accelerated PrEP regimens were considered (Soentjens et al., Visser & Jonker). The detailed results of the cited studies are displayed in Table 1 below, including the total number of doses per regimen. Preliminary studies exploring accelerated or revised PrEP regimes have shown evidence that 1 week or even single day regimens may be non-inferior to the currently recommended 3-4-week regimens. Reducing the time frame and number of doses required for PREP would make it simpler and more cost-effective to implement, particularly in populations at high risk of rabies exposure. While fewer visits usually equal a lower number of doses for one site IM administration, ID administration does not automatically lead to use of fewer doses because in some regimens there is an increase or at equal number of doses, due to multi-site injections.

Unpublished work (in progress):

- A clinical study by Jonker & Visser (in press) randomly assigned 30 healthy rabies-naive volunteers to 4 study arms for a single visit primary rabies vaccination: 1 full dose PVRV IM, and 1, 2 and 3 site injection of 0.1 ml dose of PVRV ID in a single visit. 28 out of 30 subjects seroconverted one month after primary vaccination, one individual in the intramuscular arm and one in the 1-site dose intradermal arm did not. After one year, 22 out of 30 subjects had no longer titres above 0.5 IU/ml, with no discernible difference between groups. However, 100% of subjects mounted a robust booster response within 7 days after standard IM post-exposure booster (1 injection IM on day 0 and 3), with the highest titres found in the single dose IM group. The authors conclude that effective rabies pre-exposure vaccination may be achieved in a single visit using a modern PVRV vaccine, with 100% booster response after 1 year even in those who did not seroconvert after the experimental primary vaccination.
- Soentjens et al. conducted two clinical trials on shortened PrEP ID in adults: 1) to demonstrate clinical non-inferiority of the accelerated PrEP schedule comparing a two-visit, 2-site ID regimen (days 0 and 7) to the WHO recommended 3-visit ID PrEP regimen and 2) to determine if a 2-site 0.1 ml priming ID dose on day 0 results in an adequate level of rabies antibodies in all subjects after 1 year and to determine the lowest PEP dose needed to induce an acceptable boostability 1 year after initial vaccination (single day 2-site versus 4-site booster). Statistical reports are finalized and the results available in Table 1.

Conclusion:

The main goal of PrEP is to ensure sero-conversion at some time after the priming dose, rapid and effective recall of immunological memory if challenged and to avoid the necessity for RIG in case of exposure. The main barriers to comply with full PrEP regimens are the high cost of rabies vaccination, lack of awareness by travellers or residents of rabies endemic regions and the inconvenience or time limitations due to several clinic visits. A shortened regimen should be effective in all people despite giving vaccine within a few days, eventually on one day only. There is no general need to consider a booster vaccination (other than PEP following exposure) a certain time (e.g. 1 year) after primary vaccination, unless the patient faces increase risk of exposure as specified in the revised table on indications for PrEP (adapted from Müller et al 2015).

Considerations on the reduction of duration (and to some extent the number of doses) for PrEP regimens in healthy individuals at increased risk of rabies exposure:

a) The three-visit regimen in one week on days 0, 3, 7: Several studies showed that an IM or ID schedule on days 0, 3 and 7 is as immunogenic as the currently WHO recommended regimen (day 0, 7 a21 or 28). Advantage: Same days for clinic visits as the shortened PEP schedule under discussion. This schedule is recommended for severely immunocompromised patients.
b) The two clinic visits on days 0, 7: Australian studies (McGettigan, 2010; Mills et al., 2011; Wieten et al., 2013) presented data using only two ID injections on day 0 and 7, showing that this schedule produces similar consistent antibody responses as the current WHO-recommended PrEP regimen after priming (but boostability was not assessed). The larger randomized clinical trial from Belgium and Soentjens et all (manuscript in preparation) confirmed that 100% of the subjects seroconverted > 0.5 IU/ml and 100% had titres > 0.5 IU/ml if boosted ~1 year after primary vaccination. This regimen was considered non-inferior to the current WHO recommended PrEP regimen.
c) The single day PrEP regimen: Two ID or one IM injection(s) will result in an adequate antibody titre for at least one year and memory cells respond adequately to booster injections. This has been documented using WHO pre-qualified rabies vaccines with either IM or two ID injections (see also Table 1). The age range of the study participants, as well as the considered timeframes for boostability show limitations.

References:

Jelinek, T., et al., *Evaluation of rabies immunogenicity and tolerability following a purified chick embryo cell rabies vaccine administered concomitantly with a Japanese encephalitis vaccine.* Travel Med Infect Dis, 2015. **13**(3): p. 241-50.

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Suandork, P., et al., *Accelerated neutralizing antibody response to rabies vaccination six month after a single intramuscular pre-exposure dose.* Asian Biomedicine, 2007. **1**(2): p. 211-212.

Wieten RW, et al. Rabies vaccinations: are abbreviated intradermal schedules the future? Clin Infect Dis 2013; 56:414-419

Table 1: Published data from clinical trials and observational studies on accelerated or revised PREP regimes (adapted from Kessels et al.):

Study author	Year	Location	Study population	Methods / Study type (cohort size)	Vaccination/intervention			Total number of ID or IM vaccine doses (ml) per regimen	Relevant outcome		Comments
					Vaccine	Route	Regimen		Primary Antibody Response (GMT or IU/ml)	Antibody response following booster injection	
Kamoltham, T., et al.	2007	Thailand	schoolchildren 5-8 years old	Random prospective (96)	A-E: PVRV	ID/IM	A. 0.1ml @ 2 sites d 0, 7,28	6 (0.6 ml)	A.D 360 = GMT 0.96	A. D 374 = GMT 49.39	Booster: 0.1ml ID @ 2 sites d 360
							B. 0.1ml ID@ 2 sites d 0, 3, 7	6 (0.6 ml)	B. D 360 = GMT 1.12	B. D 374 = GMT 105.08	
							C. 1.0ml IM @ 1 site d 0, 3, 7	3 (3 ml)	C. D 360 = GMT 0.97	C. D 374 = GMT 125.00	
							D. 0.1ml ID @ 2 sites d 0	2 (0.2 ml)	D. D 360 = GMT 0.41	D. D 374 = GMT 51.96	
							E. 0.1ml ID @ 2 sites d 0, 3, 7, and @ 1 site d 28, 90	8 (0.8ml)	E. D 28 = GMT 5.84	E. Not tested	
					F: PCECV				F. D 28 = GMT 5.96	F. Not tested	
Suandork, P., et al	2007	Thailand	healthy volunteers	Random prospective (13)	PVCV	IM	1 IM dose @ d 0	1 (1 ml)	D 180 = >0.05	Accelerated immune response in all subjects	Booster: 1 IM dose @ d 0,3,7,14, 28
Mills, D.J., et al.	2011	Australia	travellers	Case series (420)	HDCV	ID	0.1ml ID @ 2 sites d 0, 7	4 (0.4 ml)	D 28 = >0.05 in 94.5% of subjects	Not tested	
Khawplod, P., et al.	2012	Thailand	healthy volunteers	Abbreviated prospective study (109)	PCECV	ID/IM	1) 0.1ml ID on days 0, 7 and 21	3 (0.3 ml)	D 360 (pre-booster):	D 7 post booster:	Accelerated immune response in all subjects within 7 days of booster
							2) 0.1 ml ID @ 2 sites on day 0	2 (0.2 ml)	1a) NAB = 0.49	1a. NAB = 11.27	
							3) 1.0ml IM on day 0	1 (1 ml)	1b) NAB = 0.30	1b. NAB = 42.49	
							Booster after 1 year: (a)1.0ml IM on d 0 (360), 3; (b) 0.1ml ID @ 4 sites d0		2a. NAB = 0.15	2a. NAB = 9.71	
									2b. NAB = 0.10	2b. NAB = 11.96	
									3a. NAB = 0.08	3a. NAB = 10.13	
									3b. NAB = 0.11	3b. NAB =13.33	
Lau, C.L. & N. Hohl	2013	Australia	travellers	Case series (54)	PCECV	ID	0.1ml ID @ 2 sites d 0, 7	4 (0.4 ml)	D28 = >0.05 (94.4% of subjects)	Not tested	
Wongsaroj, P., et al.	2013	Thailand	healthy subjects aged between 18 and 24 years	Random prospective (55)	PVRV	ID/IM	A. 0.1ml ID @ 2 sites d 0, 21	4 (0.4 ml)	A. D35 NAB = 4.51 IU/ml	D 14 post booster	Booster after 1 year: 0.1ml ID d 0 (365), 3
							B. 0.5ml IM d 0, 7, 21	3 (1.5 ml)	B. D35 NAB = 6.74 IU/ml	A. GMT= 14.38	
										B. GMT = 14.06	
Jelinek, T., et al.	2015	Germany, Austria, Switzerland	healthy adults (18 to ≤ 65 years)	Randomised, observer-blind multi-center study (661)	PCECV	IM	A. 1ml IM d 0, 7, 28 + JE standard	3 (3 ml)	D 57 = > 0.05 IU/ml (97-100% of subjects)	Not tested	An accelerated PrEP rabies and JE vaccination regimen is non-inferior to the standard 4-week rabies regimen
							B. 1ml IM d 0, 3, 7 + JE accelerated	3 (3 ml)			
							C. 1ml IM d 0,7, 28	3 (3 ml)			
							D. JE standard				
Jonker & Visser (in press)	2016	the Netherlands	30 healthy volunteers (18-28 years)	Randomized clinical trial, non-blinded comparative	PVRV (Verorab, Sanofi Pasteur)	ID/IM	A. 1ml IM d 0	1 (0.5 ml)	A.D 365 = GMT 0.35	D 7 post booster: A. D 372 = GMT 63.9	
							B. 1-site 0.1 mL	1 (0.1 ml)	B. D 360 = GMT 0.00	B. D 372 = GMT 22.6	
							C. 2-site 0.1 mL	1 (0.2 ml)	C. D 360 = GMT 0.22	C. D 372 = GMT 13.0	
							D. 3-site 0.1 mL	1 (0.3 ml)	D. D 360 = GMT 0.41	D. D 372 = GMT 20.1	
Soentjens (manuscript in preparation)	2017	Belgium	Belgian soldiers (18-47 years)	Randomized clinical trial, open-label	HDCV (Mérieux)	ID	1) 0.1ml ID @ 1 site d 0, 7 and 28	3 (0.3 ml)	Pre-booster: 1) D 365-1097 = GMT 2.0 1(a) GMT= 25	D 7 post booster: 1(a) GMT= 25	
							2) 2-site 0.1ml on d 0 and 7	2 (0.4 ml)	2) D 365-1097 = GMT 3.4 2(a) GMT = 37	2(a) GMT = 37	
					PCEV (Rabipur)		3) 2-site 0.1 mL d 0	1 (0.2 ml)	3(b) D 365 = GMT 0.29 3(b) GMT = 20	3(b) GMT = 20	
							Boosters: (a) 0.1ml ID @ 1 site on d 365- d 1097; (b) 0.1ml ID @ 4 sites d 365 ; (c) 0.1ml ID @ 2 sites d 365		3(c) D 365 = GMT 0.30 3(c) GMT = 14	3(c) GMT = 14	

GRADE Tables: Questions 3 & 4

Can the duration of the entire course of current PrEP regimens be reduced while maintaining immunogenicity?				
			Rating	Adjustment to rating
Quality Assessment	No. of studies/starting rating		4 RCT	4
	Factors decreasing confidence	Limitation in study design	Non-serious[1]	-1
		Inconsistency	Non-serious	0
		Indirectness	Serious[2]	-1
		Imprecision	Non-serious	0
		Publication bias	Non-serious	0
	Factors increasing confidence	Strength of association	Applicable[3]	1
		Dose-response	Applicable[4]	1
		Mitigated bias and confounding	Non-applicable	0
	Final numerical rating of quality of evidence			4
Summary of Findings	**Statement on quality of evidence**			Evidence supports a high level of confidence that the true effect lies close to that of the estimate of effect on health outcome
	Conclusion New evidence on modified PrEP regimens (1-week or 2 visits) indicates a protective level of neutralizing antibody titres of ≥ 0.5 I.U. and an accelerated immune response upon boosters/PEP equivalent to the current WHO recommended PrEP regimens.			

[1] Blinding is is not applicable for a usually fatal disease when trial is conducted in a rabies-endemic setting (2 studies in Thailand). Jelinek et al. did not mesure accelerated immune response (protective antibody levels) following a booster

[2] Jelinek et al study's main objective was the feasibility of simultanous administration of JE vaccine, accelerated PrEP is a secondary measurement.

[3] Modern rabies vaccines as used in the studies are highly immunogenic and there was no statistically significant difference between 1-week or 2-visit PrEP compared to current WHO PrEP regimens

[4] Results of modified PrEP regimens led to similar high levels of protective antibodies and immune response accross the different settings (rabies endemic and non rabies-endemic)

Can the duration of the entire course of current PrEP regimens be reduced while maintaining immunogenicity?				
			Rating	Adjustment to rating
Quality Assessment	No. of studies/starting rating		4 observational	2
	Factors decreasing confidence	Limitation in study design	Serious[1]	-1
		Inconsistency	Serious[2]	-1
		Indirectness	Non-serious	0
		Imprecision	Serious[3]	-1
		Publication bias	Non-serious	0
	Factors increasing confidence	Strength of association	Applicable[4]	1
		Dose-response	Applicable[5]	1
		Mitigated bias and confounding	Non-applicable	0
	Final numerical rating of quality of evidence			1
Summary of Findings	**Statement on quality of evidence**			Evidence supports a moderate level of confidence that the true effect lies close to that of the estimate of effect on health outcome
	Conclusion New evidence on modified PrEP regimens (1-week or 2 visits) indicates a protective level of neutralizing antibody titres of $\geq$ 0.5 I.U. and an accelerated immune response upon boosters/PEP equivalent to the current WHO recommended PrEP regimens.			

[1] Lau & Hohl and Mills et al. did not mesure accelerated immune response (protective antibody levels) following a booster; the study of Suandork et al. confirms only a time span between PrEP plus booster of < 7 months in (others ~ 12 months)

[2] Immunogenicity results of primary antibody response in the 1-visit PrEP (ID or IM) are not consistent between Suandork et al. and Khawplod et al. However, results of studies considering a 2-visit PrEP are consistent.

[3] The study of Suandork et al has limitations in terms of sample size

[4] Modern rabies vaccines as used in the studies are highly immunogenic and there was no statistically significant difference between modified PrEP regimens compared to current WHO PrEP regimens, particularly when comparing the immuneresponse after a booster or (mimicking PEP)

[5] Although neutralizing antibody levels differ after primary vaccination (dependant on PrEP regimen used), the magnitude of the recall of the immune response (neutralizing antibodies measured) following a booster (or PEP) is increasing with number of visits/doses received. All PrEP regimens (plus booster after 6-12 months) resulted in satisfactory immune resonses $\geq$ 0.5 IU/ml in close to 100% of the subjects

Evidence Profile: Question 5

Question 5: Which (operational) parameters affect cost-effectiveness of intradermal (ID) compared to intramuscular (IM) administration route of PEP? a. in urban settings; b. in rural settings.

Comparison of rabies post-exposure vaccination regimens

Background

A range of post-exposure vaccination regimens are recommended for preventing rabies (WHO, 2010). Intradermal (ID) regimens have been shown to be more cost-effective than intramuscular (IM) regimens (Hampson et al., 2011), but so far only a few countries have adopted ID post-exposure vaccination. ID vaccination is more economical because smaller volumes of vaccine can be used to elicit an equivalent immune response. But several considerations for ID administration may have contributed to slow adoption. Partially used vials must be discarded within 6 to 8 hours to minimize risks of bacterial contamination (current vaccines do not contain preservatives) (WHO, 1997; Quiambao et al., 2005), which may be perceived as waste. The ID route is commonly used for other vaccinations such as BCG, but inexperienced clinicians may consider ID vaccination to require more skill, and fear that smaller doses of vaccine are less protective.

Using standard syringes with mounted needles, clinicians often obtain 4 rather than 5 injections of 0.1mL from 0.5mL vials and 8 injections from 1mL vials, resulting in vaccine wastage of 20%. In contrast, all doses can be extracted from vials using insulin syringes with built in needles, which are more accurate but also more costly (see Annex 1). Injections using liquid-filled needles have been reported to be more painful and therefore in some settings, one needle is used to draw the vaccine from the vial and a second to inject the patient. A further advantage of finer insulin syringes is that the same syringe/needle is used for withdrawing the vaccine and injecting the patient.

Several new post-exposure vaccination regimens, both ID and IM, have been proposed and are currently under review. Here, we compare the cost-effectiveness of PEP regimens including existing approved regimens and new candidates subject to approval. We examine their cost-effectiveness from the perspective of the healthcare providers and the costs incurred by bite victims. We consider scenarios from low to high throughput clinics, different consumables used for vaccine administration (vial size, needle and syringe type and their implications for vaccine wastage) and the potential to treat more patients given limited vaccine availability.

Aims

To quantify the potential benefits and relative costs of delivering post-exposure vaccination in different settings and according to currently recommended and proposed rabies post-exposure vaccination regimens.

Methods

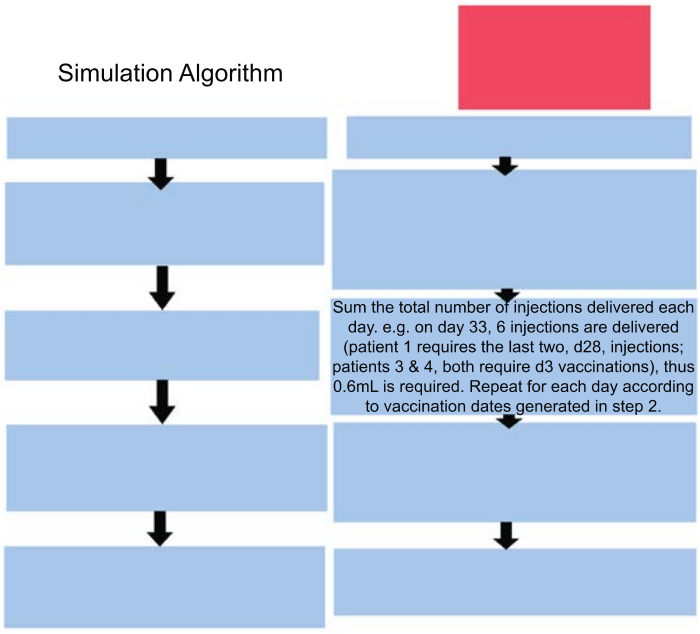
We used a simulation framework previously developed for evaluating vaccine use (Hampson et al., 2011) and compared vaccination regimens (Table 1). The algorithm for our simulations is detailed in Figure 1.

We used cost data reported from previous studies and after consultation with experts (Table 2). These include direct (medical) costs corresponding to rabies vaccines and their administration and indirect (non-medical) costs including transport to and from clinics. We assume that the time taken to vaccinate a patient is equivalent for all regimens and we did not include costs of RIG because most bite victims in Africa and Asia do not receive RIG (Mallewa et al., 2007; Hampson et al., 2008; Ly et al., 2009).

Table 1. Vaccination regimens analysed. *Neither the 5-visit TRC regimen or the 5-visit Essen regimens were examined as these are now replaced by the Updated TRC regimen (4-visits) and Essen 4-visit regimen respectively.*

Regimen	Clinic visits	Schedule (day)	Injections per visit	Vials required	Volume in ml (0.5 ml vials)	Route	Approval
Essen 4-visit	4	0,3 7,14	1,1,1,1	4	4(2)	IM	ACIP 2009-
Zagreb	3	0,7,21	2,1,1	4	4(2)	IM	WHO 1992-
Updated TRC	4	0,3,7,28	2,2,2,2	4	0.8	ID	WHO 2005-
1-week ID (4-site)	3	0,3,7	4,4,4	3	1.2-1.5	ID	pending
IPC	3	0,3,7	2,2,2	3	0.6	ID	pending
1-week IM	3	0,3,7	1,1,1	3	3(1.5)	IM	*for comparison*
2-visit IM	2	0,7	2,1	3	3(1.5)	IM	*for comparison*
4-site ID	3	0,7,28	4,2,1	3	0.7	ID	pending
4-site ID, 2-visit	2	0,7	4,2	2	0.6	ID	*for comparison*

Figure 1. Simulation framework for evaluating vaccine use under different PEP regimens.



We explored vaccine use according to the following inputs:

Clinic throughput: the number of bite patients presenting to a clinic for the first time in need of PEP. The overall number of patients that present to a clinic depends on the PEP regimen in use, its schedule requirements (Table 1) and the degree to which patients comply with the regimen.

Vial size: most rabies vaccines are sold in 0.5mL or 1mL vials, at equal cost, which affects the number of patients that can share the vial for ID vaccinations and the wastage of vaccine if standard 1CC syringes are used rather than insulin syringes (see vaccine wastage and Table 2).

Vaccine wastage: vaccine from opened vials must be used within 6-8 hours or be discarded. For regimens that use almost a complete vial (4 x 0.1mL injections from a 0.5mL vial) during a clinic visit (e.g. 4-site and 1-week ID regimens, Table 1), practitioners are assumed to use the entire vial to provide the four injections. For all ID regimens we assumed that 0.1ml injections per site were used, regardless of vial size (0.5mL or 1mL vials) and assumed use of WHO pre-qualified vaccines. However, for comparison, we tested 0.2mL injections from 1mL vials for the 4-site ID regimen (4-0-2-0-1).

Syringe type: For the 4-site regimens where an entire vial is assumed to be used on patients first visit and half a vial on their second visit (irrespective of whether the vial is 0.5ml or 1ml), we assumed use of standard syringes with two syringes used per visit. For other ID regimens we compared the costs of using insulin syringes that enabled clinicians to obtain 5 x 0.1mL injections from a 0.5mL vial and 10 x 0.1mL injections from a 1mL vial compared to the use of standard syringes (2 per visit).

Table 2. Costs for calculating the cost-effectiveness of post-exposure vaccination regimens per death averted. *Costs in bold were used as the default in simulations. Insulin syringes/needles were compared to standard 1CC syringes for ID regimens only. Non-medical costs were only considered when examining costs to patients and not from the health perspective of the health provider.*

Cost	Parameter	Unit cost estimate (USD)	Details
Medical	Material costs per injection (needles, syringes)	**$0.033-**0.4	Standard syringe - 2/consultation for ID regimens, 1/consultation for IM
		0.1455	Insulin needle – 1/consultation
	Overhead per clinic visit (staff salaries & administration)	$0.5-**1.2**	Depends no country/ setting
	Vaccine costs per vial	$6.6-20 **($10)**	Depends no country/ setting
Non-medical	Transport and accommodation costs per clinic visit	$2-14	Depends no country/ setting

Patient compliance: the probability of a bite patient returning to a clinic for subsequent PEP vaccination(s). Poor compliance has consequences for vaccine use, vial sharing and the efficacy of PEP. We investigated compliance in terms of the probability of returning for each visit rather than variability in the date of return. We assume patient compliance is affected by the cost of obtaining PEP and explore the implications of this.

We assumed high quality, pre-qualified rabies vaccines were used in our simulations. We ran 1,000 realisations (see Figure 1 example) for each scenario to capture variation in dates of patient presentation and consequences for vial sharing. We analyzed outcomes in terms of savings in vaccine

use and human rabies cases averted. We calculated cost-effectiveness from the perspective of the health provider and included only direct medical costs.

We compare the costs of PEP for bite-victims, depending upon pricing strategies for PEP, including the provision of PEP free-of-charge and under different assumptions about indirect costs (Table 3). Specifically, we assume that bite victims travel further to reach a clinic in rural rather than urban settings and incur correspondingly higher costs. We also examine the situation of limited vaccine supply. We assessed the maximum number of patients that could be treated with a given amount of vaccine under the different regimens, to understand the potential for preventing shortages and responding to outbreaks and surges in PEP demand.

Results

Of the currently approved regimens, the reduced 4-dose Essen and the Zagreb IM are equivalent in cost to the health provider. The Updated TRC ID regimen is more economical than either IM regimen particularly when administered from 1mL vials in high-throughput clinics (Figure 2).

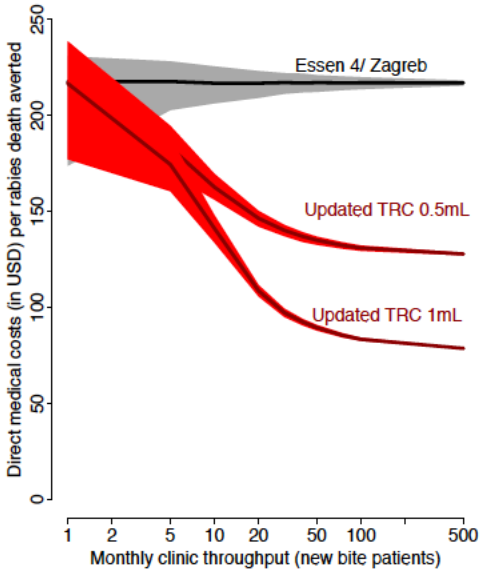


Figure 2. Direct medical costs per rabies death averted for currently used regimens in relation to clinic throughput. *Note that the cost-effectiveness of IM regimens does not change with clinic throughput whereas the cost-effectiveness of ID regimens improves with patient throughput as vials can be shared between patients.*

The comparative, investigational IM regimens that can be administered in 1 week (1-week IM and 2-visit IM) are more cost-effective per rabies death averted than currently used IM regimens (Figure 3), with the 2-visit IM regimen marginally more cost-effective than the 1-week IM regimen. In settings with limited vaccine supply, the proposed regimens would reduce vaccine use by 25% or potentially treat up to 25% more patients i.e. if vials are available to treat 1000 patients each year in a clinic with the Essen regimen, 1250 could be treated under the proposed IM regimens potentially savings lives of patients who might otherwise encounter shortages or might travel elsewhere to obtain vaccine. Overall, ID vaccination is more cost-effective than existing or proposed IM regimens and more dose sparing (see Figure 4). The currently used Updated Thai Red Cross regimen is shown in comparison to IM regimens

(Figure 3) and is more cost-effective than all currently used IM regimens in all settings and the proposed IM regimen in settings with at least 10 new patients per month.

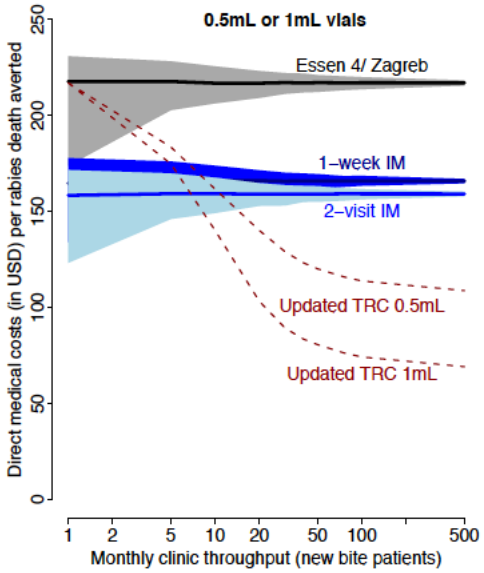


Figure 3. Direct medical costs per rabies death averted for IM regimens in relation to clinic throughput. *The Updated TRC regimen administered from 0.5mL and 1mL vials is also illustrated for comparison.*

In general ID regimens use less vaccine than currently approved IM regimens and cost less per rabies death averted (Figure 4). Clinic throughput generally increases the cost-effectiveness of ID vaccination, with high throughput clinics most cost-effective and low throughput clinics least cost-effective. The proposed IPC regimen is the most cost-effective of all regimens under all conditions and settings. The IPC regimen is most cost-effective when using 1mL vials and in settings with 10 or more new bite patients presenting per month, costing around $50 per life saved. In high throughput clinics the updated TRC ID regimen uses just 40% of the volume of vaccine in comparison to preferred IM regimens (Essen 4-dose and Zagreb) when 0.5mL vials are used and 20% of the volume when 1mL vials are used (Table 1). The IPC regimen uses just 30% or 15% of the volume of vaccine compared to preferred IM regimens from 0.5ml and 1mL vials respectively.

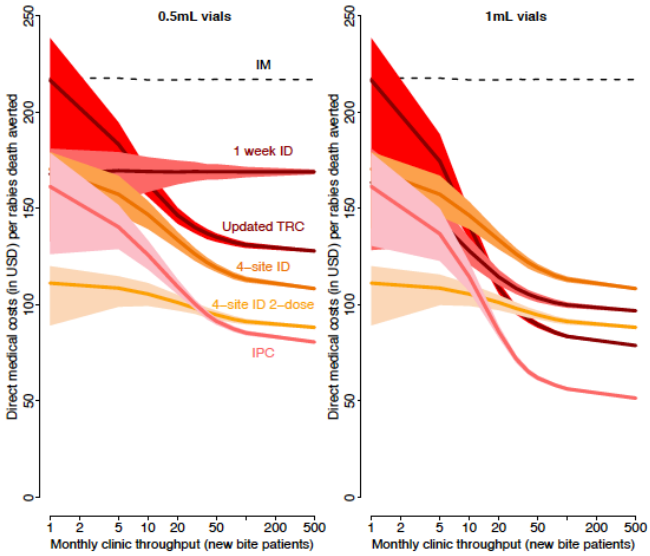


Figure 4. Direct medical costs per rabies death averted for selected ID regimens in relation to clinic throughput. *The Essen IM regimen is also illustrated for comparison. For 1ml vials (right) the solid orange line shows the cost-effectiveness of the 4-site ID regimen assuming use of 0.2 ml injections. For cost-effectiveness of the 4-site ID regimen assuming 0.1 mL injections from 1mL vials see comparison in Figure 5) .*

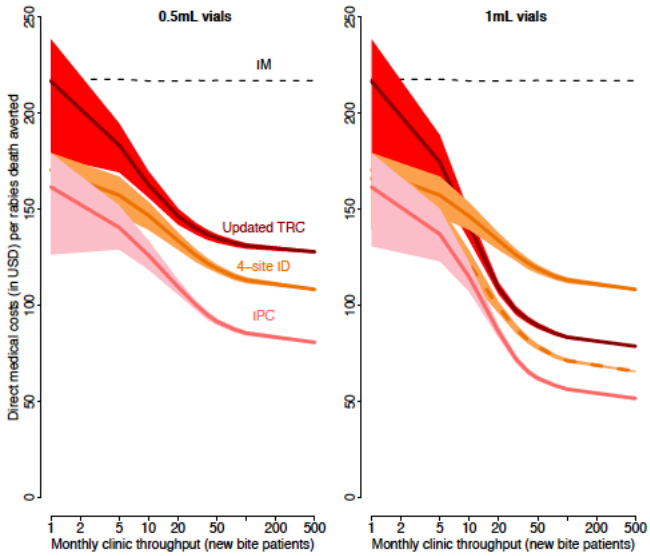


Figure 5. *Comparative modelling of changing ID dose for vials of 0.5mL versus 1mL vials of the 4-site ID regimen (other ID regimens for illustration with 0.1 mL doses): The dashed orange line shows the cost-effectiveness of the 4-site ID regimen assuming use of 0.1ml injections whereas the solid orange line shows the use of 0.2ml injections (in 1mL vials).*

The use of insulin syringes rather than standard syringes with mounted needles also results in minor cost savings (Figure 6). These savings become more apparent in clinics that receive more than 10 new bite patients presenting each month.

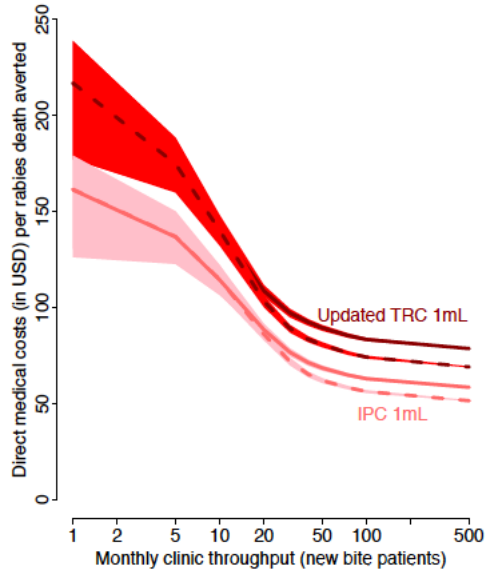


Figure 6. Direct medical costs per rabies death averted in relation to clinic throughput when using standard syringes (solid lines) versus insulin syringes (dashed lines). *Shown for the Updated TRC and the IPC regimens. Two standard syringes are assumed to be used for each visit whereas only 1 insulin syringe is used.*

Where PEP vaccination is provided free-of-charge, the Zagreb IM and 1-week ID regimens (1 week ID, IPC, 4-site ID 2-visit) are most preferable for patients, who incur only indirect costs (Table 3). This is because only 3 (or less) visits are required compared to the Essen IM and updated TRC regimens, which require 4 visits (Table 1). When patients are required to pay for PEP vaccination, the most preferable regimen for bite victims varies depending on pricing

strategies and relative travel costs. However, in terms of price, ID regimens are always preferable over IM regimens.

Table 3. Costs to the bite patients if PEP provided free of charge or if patients pay for PEP. *Pricing scenarios are detailed below. Any regimen that has reduced numbers of patient visits to clinics will have lower indirect costs, therefore reducing patient visits should be a priority for future regimens.*

	Travel costs only:		$2.5 per injection:		$15 full course:	
Regimen	Near	Far	Near	Far	Near	Far
Updated TRC	10	60	30	80	25	75
4-site ID	7.5	45	25	62.5	22.5	60
IPC	7.5	45	22.5	50	22.5	60
Essen 4-dose	10	60	50	100	50	100
Zagreb	7.5	45	47.5	85	47.5	85
1 week IM	7.5	45	37.5	75	22.5	60

An important consideration for equivalent vaccine regimens is their potential to improve access to patients, particularly when vaccine is in short supply. The number of patients that could be treated given limited vaccine supply is presented in Figure 7, and shows that a hypothetical 1-week IM regimen can treat 30% more patients that the Essen 4-visit regimen. However, the ID regimens are much more dose sparing and therefore have a much greater potential to treat more patients particularly as more vials become available. The IPC has the most capacity to treat large numbers of patients, and can treat 5x more patients than IM regimens when over 3000 vials are available (throughput of >70 patients per month). This makes ID regimens better able to prevent shortages during emergencies such as rabies outbreaks.

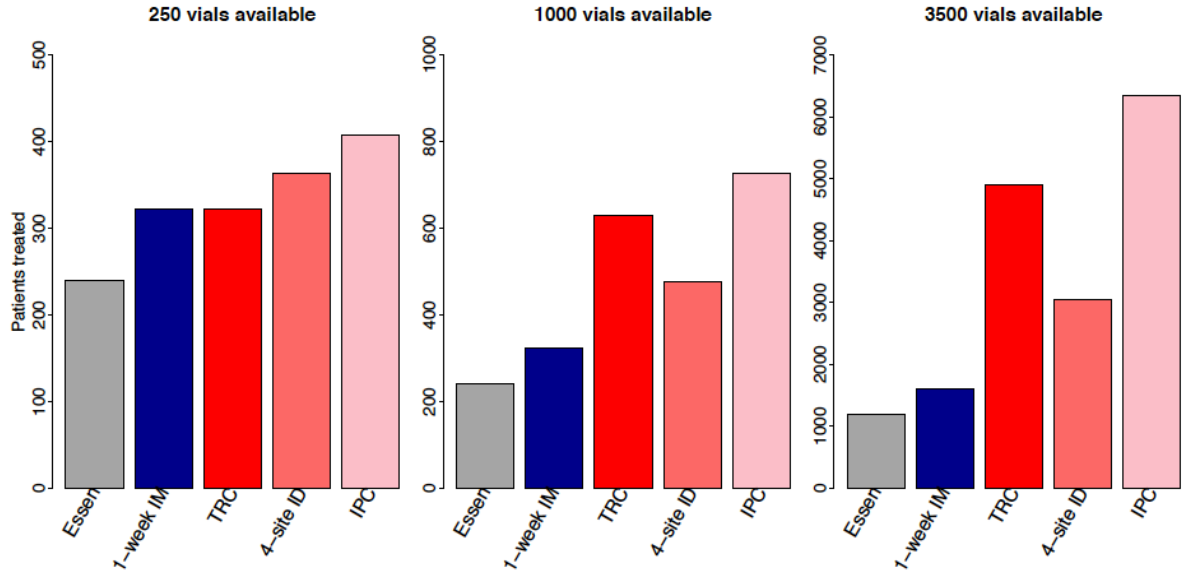


Figure 7. Patients treated under different, selected regimens given limited vaccine availability. *It was assumed that clinics had only 250, 1000 or 3500 vials available over a 1-year period. Note the different y-axis limits.*

Discussion

We found that ID delivery of PEP is more cost-effective than IM delivery in terms of averting rabies deaths. Clinic throughput affects the capacity for vial sharing, and therefore the cost-effectiveness of ID administration relative to IM. As throughput increases, ID regimens become increasingly cost-effective, using up to 85% less vaccine. Yet, even clinics with relatively low throughput (~10 new patients/month) would considerably reduce vial use by switching from IM to ID administration of PEP and even at lowest throughput ID administration is equivalent in cost to IM. Increased use of ID regimens could therefore prevent vaccine shortages and enable wider vaccine distribution, both increasing the number of patients that can be treated and the overall accessibility of PEP.

Our principal finding that ID administration of PEP is more cost-effective than IM administration and reduces the amount of vaccine used is important given the frequency with which PEP vaccine shortages occur at clinics in many developing countries. The potential impact on treating patients and buffering stock in times of emergencies is demonstrated across settings in Figure 7. Savings in vaccine use are substantially larger when using equivalently priced 1mL rather than 0.5mL vials, especially in high throughput clinics because of greater vial sharing. For safety reasons (potential for contamination) vial sharing is only possible on the day of vaccine reconstitution, even though potency remains high when properly stored (Kamoltham et al., 2002). Research into methods of preserving rabies vaccines and preventing contamination could therefore enable more economical use of vaccines, including production in larger volume vials.

Our model has several simplifications: we assume that the day of the week does not affect the likelihood of presenting for PEP vaccination. But patients may be less likely to present on Sundays (in many countries clinics providing PEP are not open on Sundays) and/or more likely to present on Mondays or other days of the week (e.g. after pay day), which may affect vial sharing. Moreover, we do not consider clustering of patient presentations as frequently occurs as a result of the same dog biting multiple people. In practice further savings would therefore be expected given the potential for vial sharing.

Our results provide evidence to show that a simplification to universal ID delivery of PEP could have advantages: streamlining guidelines, reducing the volume of vaccine use, mitigating vaccine shortages and making PEP more affordable to the most vulnerable. Health workers routinely deliver childhood immunizations intradermally, so there should be no technical difficulty in switching to ID administration. ID vaccination is as safe and efficacious as IM vaccination and is well-tolerated (WHO, 2007). The use of insulin syringes should provide clinicians with further confidence in vaccinating patients and reduce wastage as more accurate volumes of vaccine can be injected. If approved, the IPC regimen would be the most cost-effective rabies post-exposure vaccination regimen and would have considerable advantages for treating larger numbers of patients when vaccine is in short supply.

Conclusions

Overall, we find that ID vaccination is more economical that IM vaccination and that if approved, the IPC regimen could result in the greatest cost savings. We also suggest that insulin syringes be used to administer ID regimens efficiently and could provide re-assure clinicians that accurate doses have been administered.

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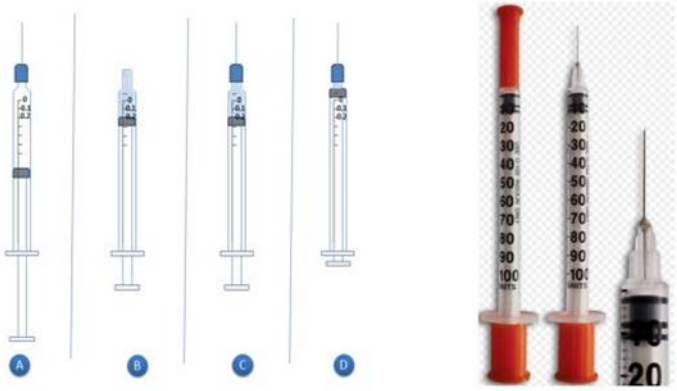
ANNEX 1

Syringes used for post-exposure vaccination, showing standard syringes with mounted needles and insulin syringes with inbuilt needles.

Annex 2: Rationale for change in syringes used at rpc@ipc



Syringes used until 2012 indusive were "classical" syringes with mounted needles that needed to be adapted to the syringes (A). The syringed was filled with two 0.1 ml dosed of rabies vaccine using another larger syringes to inject the vaccine into the pre-set space (B). A new needle was then mounted on the syringes (C). The result was that some vaccine remained in the dead space left over after the piston was fully engaged, thereby somewhat reducing the dose of vaccine administered to the patient (D).

A shift to insulin-type syringes with se needles and no dead space warrants more accurate vaccine dose administration.

Evidence Profile: Questions 6 & 7

Question 6:	**Can the duration of the entire course of current PEP regimens be reduced while maintaining immunogenicity and clinical protection?**
Population:	Immunocompetent rabies exposed patients (category II and III exposures)
Intervention:	Shortened duration of the full PEP schedule course
Comparison:	Current duration of WHO-recommended PEP schedules:
Outcome:	Rabies cases/deaths

Question 7:	**Can the number of doses administered in current PEP regimens be reduced while maintaining immunogenicity and clinical protection?**
Population:	Immunocompetent rabies exposed patients (category II and III exposures)
Intervention:	Reduced number of vaccine doses during the course of a full PEP schedule
Comparison:	WHO-recommended standard number of vaccines doses during the course of a full PEP schedule
Outcome:	Rabies cases/deaths

Background Information

Rabies is readily preventable through post-exposure prophylaxis (PEP). PEP should be initiated as early as possible following a potential rabies exposure. PEP includes rigorous wound washing with water, soap and disinfectants, rabies vaccination and administration of rabies immunoglobulins (RIG), if applicable. The PEP protocol depends on the exposure category (II or III), general immunological status of the patient and history of previous vaccinations for either pre- or post-exposure prophylaxis. Rabies vaccine can be administered by either the intradermal (ID) or intramuscular (IM) route, depending on the regimen utilised. Since 1992, WHO has promoted the use of ID administration, which confers up to 60-80% vaccine saving in rabies endemic countries, especially in high throughput clinics. Countries that have introduced policies for ID administration of rabies vaccine include Bangladesh, Bhutan, Cambodia, India, Madagascar, Pakistan, Philippines, Sri Lanka, Thailand, Uganda, United Republic of Tanzania and Viet Nam.

Although rabies vaccines are safe and highly immunogenic, the currently approved vaccine regimens (PEP regimens) require approximately a month to complete. Due to the long duration of the regimen, many animal bite victims exposed to rabies do not complete the full course of vaccination, which can leave them unprotected and susceptible to fatal, clinical rabies. The high cost of rabies PEP and potential loss of income due to frequent travel to the clinic are often a barrier, particularly in low- and middle-income countries (Mohammad et al., 2013; Sambo et al., 2013). Furthermore, healthcare workers may be hesitant to fractionate vials of rabies vaccine for patients if they cannot guarantee the full volume will be used before it should be discarded (6 to 8 hours), which often delays the initiation of PEP schedules. Stock outs of rabies vaccines (and RIG) frequently occur, particularly in small clinics in rural areas. For these reasons, it would be advantageous to reduce the duration of the entire PEP course and number of doses administered, while maintaining immunogenicity. Abbreviating the rabies PEP regimen is expected to improve patient compliance and be potentially cost saving.

Current Practice

The PEP regimens currently approved by WHO in previously unimmunized individuals are summarized in Table 1.

Table 1: Summary of Currently Recommended Regimens

Schedule	Route	Sites	Days	Clinic visits	Duration (days)	Notes
Essen	IM	(1-1-1-1-1)	0, 3, 7, 14, 28	5	28	Suitable for immunocompromised patients
Zagreb 2-1-1	IM	(2-0-1-0-1)	0, 7, 21	3	21	
4-dose Essen	IM	(1-1-1-1-0)	0, 3, 7, 14	4	14	Only if immunocompetent + PQ vaccine (+ high quality RIG, if applicable)
Updated TRC-ID	ID	(2-2-2-0-2)	0, 3, 7, 28	4	28	

Rabies vaccines are considered safe and highly effective in preventing rabies. A rabies virus neutralizing antibody (RVNA) concentration ≥ 0.5 IU/ml on day 14 post-immunization is considered protective. This threshold is a clinical endpoint used to indirectly measure the protective effect of vaccination in studies of rabies vaccine efficacy and effectiveness. The regimens in Table 1 are currently recommended for those with category II or III rabies exposure (plus RIG, if applicable). For people with a category II or III exposure who have previously had PrEP or PEP (even when the rabies virus neutralizing antibody titre is $\underline{\geq}$0.5 IU/ml), shorter regimens without RIG are recommended: 1 dose of vaccine on days 0 and 3, either by IM or ID route. Alternatively, the patient may be offered a single visit 4-site ID regimen on day 0.

WHO's current position states that: "*New PEP regimens, particularly those using ID administration, even if shown to be safe and efficacious, must have clear practical or economical advantages, or both, over existing regimens if they are to be endorsed.*"

New Evidence

Investigational PEP regimens were reviewed and cross-referenced with the current evidence on vaccine regimen potency. Hampson et al. 2011 analyzed different PEP vaccination regimens and evaluated their relative costs and benefits to bite victims and healthcare providers. The same model was used to simulate wastage, direct and indirect costs and potential public health impact of investigational PEP regimens, details are available in evidence profile of Question 5.

Table 2: Investigational PEP Regimens. See Annex 1 for comprehensive table

References	Regimen	Route	Sites	Days	Clinic visits	Dosage (ml)	Duration (days)

Narayana et al. Shantavasinkul et al. Sudarshan et al.	1-week / 4-site ID	ID	(4-4-4-0-0)	0, 3, 7	3	0.4-0.4-0.4	7
Huang et al.	1-week / 2-1 IM	IM	(2-0-1-0-0)	0, 7	2	1.0-0.5*	7
Warrell et al. Quiambao et al. Ambrozaitis et al.	'modlified'# 4-site ID	ID	(4-0-2-0-1)	0, 7, 28	3	0.4-0.2-0.1	28
Tarantola et al. (manuscript in preparation)	'IPC' 1-week / 2-site ID	ID	(2-2-2-0-0)	0, 3, 7	3	0.2-0.2-0.2	7

* for 0.5 ml vaccine vials (2.0-1.0 for 1ml vials), # see detailed explanations in the text below

1-week IM regimen (2-0-1-0-0)

Huang et al. evaluated the immunogenicity and safety of a one-week IM regimen (2-0-1-0-0) compared to the 5 dose Essen IM regimen in 79 and 102 healthy veterinary school students (aged 19-23 years), respectively. It was reported that the 2-1 IM regimen demonstrated the same immunogenicity and safety profile as the 5-dose Essen regimen. The regimen elicited adequate and protective RVNA concentrations ≥ 0.5 IU/ml from day 14 onwards until day 180. No RIG was administered. The use of this regimen could not only reduce individuals' expenditure, but also improve PEP compliance rates through fewer (2) clinic visits and shorter course (7 days). Moreover, this schedule utilizes only 3 injection sites, so it is likely to reduce the frequency of adverse events. However, Huang et al. concluded that further investigation is necessary to continue to assess the immunological and clinical efficacy of the 2-1 IM regimen before making new policy recommendations to change the current immunization protocols.

1-week 4-site ID regimen (4-4-4-0-0)

Shantavasinkul et al. evaluated the safety and immunogenicity of a one-week ID regimen in healthy volunteers. This study included 3 arms: 1) a 4-site 1-week ID regimen in healthy volunteers; 2) a 4-site 1-week ID regimen plus eRIG in healthy volunteers; and 3) a full TRC-ID regimen in patients that presented with category III exposures from suspected rabid animals. The 1-week ID regimen was found to be safe and immunogenic. All participants had protective RVNA concentrations ≥ 0.5 IU/ml on days 14 and 28. The proportion of subjects that had antibody concentrations ≥ 0.5 IU/ml on day 360 were similar across the three study arms. The 1-week, 4-site ID regimen showed increased immunogenicity compared to the TRC-ID regimen. These findings suggest that this reduced regimen could be an alternative ID regimen and is convenient because it consumes an entire vaccine ampoule volume (of a 0.5 ml vial) in one visit, which reduces wastage.

Sudarshan et al. evaluated the safety and immunogenicity of a one-week ID regimen in healthy volunteers. Sudarshan et al. confirmed that the one-week ID regimen was immunogenic and the immune response was comparable to that induced by the currently approved PEP regimens. All participants (100%) had adequate protective RVNA concentrations until day 180. However, after one-year post immunization, only 62.5% in the PVRV group and 78.9% in the PCEC group were protected. The regimen also induced strong immunological memory, demonstrated by the quick anamnestic response observed after boosting (in participants with titers that dropped below 0.5 IU/mL after one

year). The regimen was also well-tolerated and adverse event rates were relatively low. They concluded that further studies are needed in individuals exposed to confirmed rabid bites.

Narayana et al. evaluated the immunogenicity and safety of a one week, 4-site ID regimen in suspected rabid animal bite cases. This regimen might be preferable to the current WHO-approved regimen because it reduces the number of clinic visits to 3, thus reducing logistic costs and duration of the PEP course improving patient compliance. The regimen requires a total of 1.2 mL of rabies vaccine per course, which is 0.4 ml more than the 2-site updated TRC regimen. The schedule elicited adequate and protective RVNA concentrations ≥ 0.5 IU/ml from day 14 onwards until day 365 as per WHO criteria indicative of protection against rabies. The incidence of local and systemic reactions in this study was comparable to that of rates reported for WHO approved regimens.

An ongoing clinical trial in the Philippines includes patients exposed to a suspected rabid animal. This trial will compare the TRC regimen with a 4-site and 3-visit regimen and may strengthen the body of evidence.

'Modified' 4-site ID regimen (4-0-2-0-1)

Warrell et al. evaluated a randomized controlled trial of a simplified 4-site ID regimen. This schedule proposes vaccination 4-sites ID injection on day 0, 2-site on day 7 and 1-site on day 28. Although this trial proposed a 90-day schedule, this article has been provided as evidence for a 4-site ID regimen with 2 sites on day 28 and omitting the dose on day 90 as this modification was made to the TRC-ID regimen without compromising immunogenicity. The trial compared the modified 4-site ID regimen to 3 WHO-approved regimens: 1) 2-site ID updated TRC regimen; 2) the Oxford 8-site ID regimen; 3) Essen 5-dose IM regimen. Participants in all study arms had RVNA concentrations ≥ 0.5 IU/ml. The IM Essen regimen elicited the lowest geometric mean antibody titers of all regimens studied. Compared to the TRC regimen, the modified 4-site ID regimen requires fewer clinic visits, is potentially more practical in small clinics and provides a wider margin of safety, if the patient does not return after the first session. The study was conducted in health individuals.

Ambrozaitis et al. 2006. The two-arm study conducted in Lithuania used the modified 4-site ID regimen A) with PCECV in 91 healthy people and B) with PVRV vaccine in 89 healthy people. By day 7, 3% in arm A) and 6% in arm B) had titres >0.5 IU/ml. GMTs on day 7 were higher for PCVC than for PVRV. By day 14 all participants had adequate titres until day 105 (99-100%), both vaccines used with this regimen are immunogenic.

Quiambao et al. 2008 conducted a five arm study on a total of 339 healthy individuals and patients who consulted for a category I or II exposures (to healthy dogs or cats) in the Philippines. A) 96 patients received an 8-site Oxford regimen; B) 96 patients the modified 4-site ID regimen; C) 97 patients full Essen IM and; D) 99 patients received a full TRC ID regimen (plus eRIG or hRIG IM). PVRV was used. By day 14, all subjects had seroconverted. The GMT of all groups on day 14 was above 0.5 IU/ml, with the GMT of the 8-site ID group significantly higher than all other groups on day 7 (arms B-D equal). There was no follow up of patients beyond day 90.

Compared to the 8-site ID regimen (Sirikwin et al., 2009), this regimen is more convenient for both, patients and health personnel and can be used with vaccines formulated in 1.0 ml and 0.5 ml vials limiting wastage of vaccine. However, this regimen was not tested in patients exposed to confirmed

rabid animals. Further, the changing number of doses may be considered complicated by the health personnel and difficult to handle in large clinics.

1 week 2-site ID regimen, 'IPC regimen' (2-2-2-0-0)

A prospective study was conducted in Cambodian patients received at the rabies treatment centre at Institut Pasteur Cambodge (IPC) between 20 May 2016 to June14, 2017. The eligible 105 study participants of all ages were all patients with a category III exposure to laboratory confirmed rabid dogs and received the updated TRC regimen (PVRV) and RIG. Serology was conducted for all patients, results are currently available for 88 patients, the remaining 15 patients analysis is pending. Blood samples were collected on days 0, 7, 28 and 42. The mean titre of day 7 was 1.9 IU/ml (min 0.11, max 28 IU/ml), mean titre of day 28 was 38.5 IU/ml (min. 1.1, max. 148.5). All participants were protected after 3 sessions of 2 ID doses, including underweight patients (around 30%) or individuals with other diseases (e.g. parasitoses, other infections, etc). The GMT of day 28 higher than GMT of day 42. Twenty-two of these patients were also explored for B-cell phenotyping and no statistical difference was found between the two groups. The patients were followed up for at least 6 months, no rabies-related death was observed. Another arm of the study in similarly managed patients after exposure to rabies-suspect, but untested dogs, found no deaths among 155 who received only three sessions of the updated TRC regimen, versus 904 patients who received four sessions at least, as per recommendations (100% survival in both groups).

The studies support the PEP effectiveness on short- and medium-term protection, including clinical outcome data and support the removal of the fourth session of the updated TRC regimen on day 28. The countries implementing ID PEP administration are currently using the updated TRC regimen, so the new regimen would be easy to adopt, in that it follows the same schedule but without the fourth dose.

As an example for additional immunogenicity data in support of the above from different settings and use of PCECV.

Sudarshan et al. 2005 enrolled 91 healthy people in a 2-arm study in India with PCECV comparing: A) 45 people subject to the full TRC and B) 46 people who received the Essen IM regimen. All subjects had titres ≥0.5 IU/ml at all follow-up visits including serological testing (day 14, 28, 90, 180, 365). The GMTs of day 14 (=3 sessions for ID and IM completed) were (ID) 4.17, (IM) 6.89 and for day 28 (ID) 7.60, (IM) 11.53, respectively.

Conclusions Overview

The available evidence suggests that the current PEP regimens can be reduced in duration, in some cases also in number of doses while maintaining efficacy, effectiveness and immunogenicity. However, the quality of available evidence is weakened by the small samples sizes of the reviewed studies and limited geographic representativeness, as most trials are conducted in (South East) Asia. Moreover, most of the recent trials have only studied abbreviated PEP regimens in healthy volunteers, instead of patients of rabies endemic settings, with potential or confirmed rabies exposures from animal bites. This review indicates that more studies with larger samples sizes may be needed to improve the quality of

evidence and impact of the results. Trials conducted on the African continent would also be valuable, as the rabies burden is large but rabies vaccine trials are underrepresented in the current literature.

Although the available new evidence is limited, there is the greatest amount of evidence supporting the 4-site ID and 1-week ID regimens. The tolerable and practically feasible number of injection sites per visit has been subject to debate since 2008. Therefore the 4-site injections (and above) were not fully supported by clinicians in rabies endemic countries, despite promising other advantages of these regimens.

The modified 8-site ID regimen may also be an immunogenic alternative to the 5-dose Essen regimen. However, this regimen would require 8 injections at each visit, which is reported to be disagreeable to patients and particularly difficult to handle in children. Immunogenicity data from patients who did not complete the approved PEP schedules suggest that IM schedules could also be reduced while maintaining the protective effect seem to suggest that IM regimens could be reduced while maintaining the protective effect (Robertson et al.). Observational studies including contact tracing of patients who did not complete the recommended PEP regimens (e.g. Sambo et al. 2013) indicate a similar trend, if solely looking at survival rates. The limitations of most such observational country data are that the rabies status of the biting animal is virtually unknown and it is usually impossible to assess immunogenicity in the patient. The abbreviation of IM schedules has unfortunately not been the focus of recent studies.

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Annex 1: Overview evidence and limitations investigational PEP regimens

Author	Year	Schedule	Route	Sites	Days	Clinic Visits	Total Dosage (mL)	Duration (days)	Sample Size	Sample Specifics	Vaccine Used	Serology Results	Potency (IU/mL)	Limitations and Concerns	General or Lab Confirmed Rabid
Huang et al.	2014	1-week / 2 and 1 site IM	IM	(2-1)	0, 7	2	1.5 (0.5 ml vial) 3.0 (1ml vial)	7	181	1. 79 in test group and 102 in control group (Essen) 2. 919 blood samples obtained out of 1086 sampling events scheduled due to poor compliance by control group at days 180 and 360 3. All had no prior antirabies vaccination	Purified Vero cell	1. On day 14, all study subjects exhibited RVNA titres >0.5 IU/mL 2. RVNA titres were maintained in both groups through days 45 and 180 before gradually declining 3. The percentage of subjects positive for RVNA on day 7 was not statistically different between the test and control groups 4. On day 360, the percentage of subjects positive for RVNA in the variable and control groups were 93.9% and 100% respectively, which was statistically significant	5.5	1. Schedule tested in healthy subjects 2. All study subjects were young adults between 18 and 26 years of age 3. The long-term persistence of immunity was slightly reduced following the 2-1 schedule compared with the five-injection schedule therefore clinical research is needed for comparison 4. Did not include vaccine combined with RIG	healthy volunteers
Naranya et al.	2015	1-week / 4-site ID	ID	(4-4-4-0-0)	0, 3, 7	3	0.4-0.4-0.4 (total 1.6 ml)	7	90	1. eRIG administered to all category III exposures 2. Randomized into 2 groups to receive either Rabipur or Verorab 3. Sociodemographic characteristics between groups were similar 4. All had no prior antirabies vaccination	Purified chick embryo cell (Rabipur) or purified Vero cell (Verorab)	1. Serum samples were collected on days 0, 14, 90, and 365 2. In the Rabipur group, the GMT of RVNA was 14.5, 11.78, and 5.96 IU/mL on days 14, 90, and 365 respectively 3. In the Verorab group, the GMT of RVNA was 14.43, 11.93, and 5.67 IU/mL on days 14, 90, and 365 respectively 4. 100% of the subjects had adequate >0.5 IU/mL RVNA concentrations from day 14 to day 365 5. Both the vaccines with or without eRIG had similar GMT RVNA concentrations	6.9 - 7.5	1. Confirmation of rabies in the biting animals was not possible due to practical difficulties 2. Was not tested in children, pregnant, or lactating women	category II or III animal bites/exposures from suspected rabid animals (not lab confirmed)
Shantavasinkul et al.	2010	1-week / 4-site ID	ID	(4-4-4)	0, 3, 7	3	0.4-0.4-0.4 (total 1.6 ml)	7	131	1. All had no prior antirabies vaccination 2. The characteristics of subjects in each group were similar in terms of age and sex 3. Group A received test schedule Group B received test schedule and eRIG, and Group C were those with category III rabies exposures and received TRC-ID schedule and eRIG	Purified Vero cell	1. RVNA levels were tested on days 0, 7, 14, 28, 90, 180, and 360 2. The overall pattern of antibody response was similar in each study group; highest on days 14 and 28 and slowly decreased up to day 360 3. All subjects who received the 4-site ID schedule had RVNA levels >0.5 IU/mL on days 14 through 90 4. The GMT of RVNA in the 4-site ID schedule with and without eRIG were significantly higher than the GMTs from the TRC-ID schedule on days 14 and 28 5. On day 180, subjects receiving the TRC-ID schedule had significantly higher GMTs than did the subjects receiving the 4-site ID schedule with or without eRIG; explained by the day 90 booster 6. The percentages of subjected who had RVNA levels >0.5 IU/mL were not significantly different among the 3 groups from days 0 through 360	9.6	1. Schedule tested in healthy subjects 2. Confirmation of rabies in the biting animals in the control group was not possible	healthy volunteers category III exposed patients from suspected rabid animals in the control group (not lab confirmed)
Sudarshan et al.	2012	1-week / 4-site ID	ID	(4-4-4)	0, 3, 7	3	0.4-0.4-0.4 (total 1.6 ml)	7	80	1. All had no prior antirabies vaccination 2. The sociodemographic characteristics of the two groups were similar 3. Subjects were allocated randomly to PCECV or PVRV test groups	Purified chick embryo cell (Rabipur) or purified Vero cell (Verorab)	1. Blood samples were collected on days 0, 7, 14, 28, 180, and 365 2. All subjects in both groups had adequate RVNA concentrations >0.5 IU/mL from day 14 to 180 and the difference of GMT between the two groups was not significant 3. ID booster was given to those who did not have adequates RVNA concentration on day 365 and resulted in a quick and enhanced RVNA concentrations	> 2.5	1. Schedule tested in healthy subjects 2. Small sample size 3. Did not include vaccine combined with RIG	healthy volunteers
Warrell et al.	2008	modified 4-site ID	ID	(4-0-2-0-1)	0, 7, 28	3	0.4-0.2-0.1 (total 0.7 ml)	28	254	1. The characteristics of subjects in each group were similar 2. Subjects received one of four PEP schedules the 2-site ID, the 8-site ID, the 4-site ID, or standard 5-dose IM 3. All had no prior antirabies vaccination	Purified Vero cell	1. RVNA responses were measured on days 0, 7, 14, 90, and 365 2. All ID schedules showed similar immunogenicity 3. The IM schedule gave the lowest GMTs 4. On day 14, all subjects had antibody levels >0.5 IU/mL 5. The 4-site PEP schedule is supported as immunogenic as current regimens	5.3 - 8.4	1. Regimen tested in healthy subjects 2. Did not included vaccine combined with RIG 3. Day 90 dose	healthy volunteers
Quiambao et al.	2008	modified 4-site ID	ID	(4-0-2-0-1)	0, 7, 28	3	0.4-0.2-0.1 (total 0.7 ml)	28	339	1. The characteristics of subjects in each group were similar 2. Subjects received one of four PEP schedules the the 8-site ID no RIG, modified 4-site ID no RIG, 2-site ID TRC with RIG, or a 5-dose Essen IM without RIG 3. All had no prior antirabies vaccination	Purified Vero cell	1. RVNA responses were measured on days 0, 5, 7, 14, 28, before the booster at 1 year and 2 weeks after the booster 2. By day 14, all subjects in the 8-site ID and modified 4-site ID groups had antibody levels >0.5 IU/mL, 1 patient each for the other groupups did not show a titre > 0.5 IU/ml 3. The GMT of all groups on day 14 was above 0.5 IU/mL, the modified 4-site regimen showed GMTs slightly above the TRC, but this was not statistically significant 4. Although the 8-site ID regimen resulted in higher antibody titers than the other 3 groups, seroprotection did not occur any earlier 5. The 4-site PEP schedule is supported as immunogenic as current regimens	≥5 IU/mL	1. Regimen tested in healthy subjects 2. Did not included vaccine combined with RIG for investigational regimen (modified 4-site) 3. Day 90 dose 4. Non-peer-reviewed journal	healthy volunteers category I and II patients exposed to healthy animals
Ambrozaitis et al.	2006	modified 4-site ID	ID	(4-0-2-0-1)	0, 7, 28	3	0.4-0.2-0.1 (total 0.7 ml)	28	180	healthy volunteers were randomized to receive a modified 4-site regimen with either 0.1mL volumes of PCECV or PVRV	Purified chick embryo cell and Purified Vero cell	1. RVNA responses were measured on days 0, 7, 14, 90, and 104 2. By day 14, all 173 subjects reached RVNA titers above 0.5 IU/mL 3. For 171 of the 173 subjects RVNA titers remained above 0.5 IU/mL throughout the study with a trough on day 90 when the last ID dose was given	PCECV 5.53 IU/ml, PVCV 17.86 IU/ml	1. Regimen tested in healthy subjects 2. Did not included vaccine combined with RIG 3. Day 90 dose	healthy volunteers
Tarantola et al. (Manuscript in preparation)	2017	1 week 2-site ID 'IPC regimen'	ID	(2-2-2-0-0)	0, 3, 7	3	0.2-0.2-0.2 (total 0.6 ml)	7	112	Cambodian patients of all ages presenting at the IPC rabies clinic for cat. rabies III exposures, including underweight subjects.	Purified Vero cell	1. RVNA responses were measured on days days 0, 7, 28 and 42 2. By day 28, all 88 subjects reached RVNA titers above 0.5 IU/mL 3. The mean titre of day 7 was 1.9 IU/ml and 38.5 IU/ml for day 28, respectively 4. The GMT of day 28 was higher than GMT of day 42, supporting a 3-session regimen	≥2.5 IU/mL	1. Serological results of 15 subjects pending 2. Not yet published	subjects bitten by laboratory confirmed rabid dogs

GRADE table

Question 6 & 7 Shortened duration/fewer doses compared to currently recommended duration/number of doses for immunocompetent rabies exposed patients (categories II and III)

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	shortened duration/fewer doses	currently recommended duration/number of doses	Relative (95% CI)	Absolute (95% CI)		
2-1 IM regimen: Huang et al. (follow up: mean 1 years; assessed with: RVNA levels)												
1	randomised trials	not serious [a,b,c,d,e]	not serious	serious [f,g,h,i]	serious [j,k]	none	79/181 (43.6%)	102/181 (56.4%)	RR 21.90 (19.06 to 25.17)	1,000 more per 1,000 (from 1,000 more to 1,000 more)	⊕⊕◯◯ LOW	
1-week 4-site ID regimen: Narayana et al. (follow up: mean 1 years; assessed with: RVNA levels)												
1	randomised trials	not serious [a,c,l]	not serious	not serious [m,n,o]	serious [j,p]	none	89/89 (100.0%)	0/0	RR 89.00 (13.41 to 15.57)	89 fewer per 1,000 (from 13 fewer to 16 fewer)	⊕⊕⊕◯ MODERATE	
1 week ID regimen: Shantavasinkul et al. (follow up: mean 360 days; assessed with: RVNA levels)												
1	randomised trials	not serious [a,c]	not serious	serious [f,h,q]	serious [j,raa]	none	45/131 (34.4%)	86/131 (65.6%)	RR 45.00 (14.21 to 24.29)	1,000 more per 1,000 (from 1,000 more to 1,000 more)	⊕⊕◯◯ LOW	
1 week ID regimen: Sudarshan et al. (follow up: mean 1 years; assessed with: RVNA levels)												
1	randomised trials	not serious [a,c]	not serious	serious [f,g,o]	serious [j,s]	none			RR 78.000 (11.240 to 12.755)	78 fewer per 1,000 (from 11 fewer to 13 fewer)	⊕⊕◯◯ LOW	
4-site ID regimen: Warrell et al. (follow up: mean 1 years; assessed with: RVNA levels)												

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	shortened duration/fewer doses	currently recommended duration/number of doses	Relative (95% CI)	Absolute (95% CI)		
1	randomised trials	not serious [a,c,e]	not serious	serious [f,g,h]	serious [j,t]	none	173/254 (68.1%)	56/254 (22.0%)	RR 173.00 (161.99 to 442.08)	1,000 more per 1,000 (from 1,000 more to 1,000 more)	⨁⨁◯◯ LOW	

a. sociodemographic characteristics between groups were similar

b. exclusion factors: fever, alcoholism, pregnancy, diabetes, anemia, kidney and liver diseases, chronic diseases, blood sampling difficulties

c. exclusion factors: previous rabies vaccination or RIG reception, and immunosuppression

d. randomized (cluster random sampling method)

e. blinding (analyst single-blind)

f. persons healthy volunteers, not animal bite victims

g. did not include RIG administration, as would be recommended in full PEP courses

h. tested only PVRV (not PCECV)

i. small age range (18 to 26 years)

j. fewer than 300 participants

k. 181 subjects total, 919 blood samples obtained total

l. randomized (randomized, active-controlled, parallel assigned, comparative, open-label)

m. subjects exposed to suspected rabid animals

n. administered alongside RIG

o. tested both PVRV and PCECV

p. 90 events total

q. compared both with and without RIG

r. 131 total events

s. 80 events total

t. 254 subjects total

Evidence Profile: Question 8

Question 8: Does novel evidence support recommendations on modified PEP protocols versus current PEP protocols for specific risk groups of rabies exposed patients, such as: Immuno-compromised patients (e.g. HIV-infected); patients concurrently using antimalarial drugs; pregnant women; bat exposures (i.e. for bat lyssavirus)?

Population:	Subsets of category II or III rabies-exposed patients with specified health risk
Intervention:	Updated protocols for PEP or switch to use of standard PEP protocols, if applicable
Comparison:	Current PEP protocol in use for specific risk groups of patients
Outcome:	Fewer rabies cases/deaths

Background:

Rabies is readily preventable through post-exposure prophylaxis (PEP). PEP should be initiated as early as possible following potential rabies exposure. PEP includes rigorous wound washing with water, soap and disinfectants, rabies vaccination and administration of rabies immunoglobulins (RIG). PEP protocol depends on the exposure category, general immunological status and previous vaccination for either pre- or post-exposure prophylaxis. Rabies vaccine can be administered by either the intramuscular (IM) or intradermal (ID) route, depending on the schedule utilised. The PEP schedules currently approved by the World Health Organization (WHO) in previously unimmunized individuals are summarized in Table 1.

Table 1: PEP schedules currently approved by WHO

Regimen	Route	Sites	Days	Clinic visits	Duration (days)	Notes
Essen	IM	(1-1-1-1-1)	0, 3, 7, 14, 28	5	28	Recommended for immunocompromised patients
Zagreb 2-1-1	IM	(2-1-1)	0, 7, 21	3	21	
4-dose Essen	IM	(1-1-1-1)	0, 3, 7, 14	4	14	Immunocompetent only
Updated TRC-ID	ID	(2-2-2-0-2)	0, 3, 7, 28	4	28	

Rabies vaccines are considered safe and highly effective in preventing rabies. A rabies virus neutralizing antibody (RVNA) concentration ≥ 0.5 IU/ml on day 14 post-immunization is considered protective. This standard is a clinical endpoint used to indirectly measure the protective effect of vaccination in studies of rabies vaccine efficacy.

Current position and practice:

“Because rabies is a lethal disease, no contraindications exist to PEP following high-risk exposure. This is also the case for PEP during infancy or pregnancy, and for immunocompromised individuals, including children with HIV/AIDS. People taking chloroquine for malaria treatment or prophylaxis may have a

reduced response to ID rabies vaccination. These patients should receive the vaccine intramuscularly" (WHO Position Paper, 2010).
Currently available recommendations on PEP schedules for specific risk groups as stated above are summarized in Table 2.

Table 2: PEP recommendations for subsets of risk patients exposed to rabies:

	Risk group	**Route**	**Doses**	**Days doses are given**	**Duration of schedule**	**Remarks**
	Immunocompromised	IM	5	0, 3, 7, 14, 28	28	Full series
	Antimalarial drugs	IM only				
	Pregnant women	Same recommendations as general population				
	Bat exposures	Category II and III rabies exposures from a bat are all treated as a category III exposure and require RIG*				

* Additionally, as stated in the 2nd report of the WHO Expert Consultation on Rabies (2013): "PEP should be considered when contact between a human and a bat has occurred, unless the exposed person can rule out a bite or scratch or exposure of a mucous membrane."

New evidence:

a) Immunocompromised individuals
Rahimi et al. evaluated the immune responses of the 5-dose Essen schedule, currently recommended for immunocompromised individuals, in Iranian patients with specific medical conditions with potential rabies exposures, compared to healthy volunteers. On day 14 post-immunization, all subjects had neutralizing antibody concentrations ≥ 0.5 IU/ml. GMTs were 16.2 IU/ml and 8.73 IU/ml in immunocompetent and immunocompromised participants, respectively. On day 35, all subjects in both groups were also protected. The GMTs were 30.3 IU/ml (8.3-45.5 IU/ml) and 20.7 IU/ml (8-30.2 IU/ml) in immunocompetent and immunocompromised participants, respectively. Although the average antibody titers were greater for the immunocompetent participants, the GMT ranges overlap and are above the protective threshold in both groups, which suggests that the immune responses are comparable. Therefore, if immunocompromised patients mount comparable immune responses to the 5-dose Essen regimen, it suggests that other regimens may be suitable for specific risk groups. This is especially pertinent considering that ID regimens have been shown in other studies to elicit a stronger immune response compared to IM regimens.

Individuals with HIV infection
General considerations:
HIV patients under treatment and monitoring would most likely react like not severely immunocompromised patients or HIV uninfected individuals as observed in studies conducted for routine vaccines (Simani et al. 2014). A Kenyan study in severely immunosuppressed HIV-infected children with very low CD4 counts (Farquhar et al. 2009) showed that after 6 months, the majority of the children who received highly active antiretroviral therapy, had a booster response to measles vaccine. The IM route is still the recommended rabies vaccine administration to HIV infected, immunocompromised patients. Studies involvig patients with low CD4 counts who received inactivated influenza vaccines showed that ID immunization with viral antigen was as immunogenic as IM administration (Garg et al 2016, Seo et al 2016).
Rabies-specific updates:

Sirikwin et al. evaluated the immunogenicity of a modified 8-site ID regimen in HIV-infected patients, a risk group that is known to have reduced immune responses to vaccination. Individuals whose CD4+ cell counts both below and above 200 cells per microliter were studied. The timing of this schedule was based off the Essen regimen. All patients had adequate antibody concentrations ≥ 0.5 IU/ml on day 14 after immunization. There was no statistically significant difference between individuals with CD4+ cell count < 200 and CD4+ cell count ≥ 200 up to day 360. Sirikwin et al. concluded that PCECV is immunogenic in HIV-infected patients with CD4+ cell counts below 200 when administered in a modified 8-site ID regimen.
Older studies did not confirm that a higher antigen dose results in a more adequate immune response in seriously immunocompromised individuals.

Malnourished Children (and Adults):
The large majority of PEP recipients live in the South and South East Asia region, despite decreasing prevalence, mild to severe malnutrition or even hunger, are unfortunately still present. The long years' clinical experience and a very low number of true PEP failures (Wilde 2007) may indicate that current PEP regimens in use are efficacious in the average population of these regions, including patients suffering from (not reported) malnutrition. In an older study from India (Sampath et al. 2005), 45 malnourished children aged 8 months to 16 years who received PEP (5-dose Essen IM regimen, WHO pre-qualified vaccine) were investigated for their immune response. All children had developed RVNA levels ‡0.5 IU/ml by Day 14. There was no significant difference in antibody concentrations between the malnutrition categories.

End Stage Renal Failure, Receiving Dialysis
Tanisaro et al. evaluated the use of an ID regimen in hemodialysis patients with end stage renal failure, receiving adequate dialysis, using the complete TRC-ID regimen (2-2-2-0-1-1). Chronic hemodialysis patients have been shown to express suboptimal immune responses to vaccines. Tanisaro et al. reported that all subjects (n=14) had protective antibody responses against rabies 14 days after vaccination. At day 90, 13 of the 14 patients had protective antibody levels, resulting in a 92.8% response rate. These results suggest that ID rabies vaccine administration is immunogenic in hemodialysis patients and may be suitable for use in immunocompromised individuals. Even though this regimen is longer in duration than the 5-dose Essen regimen, it is dose sparing and would be relevant in low-resource settings. Moreover, considering that this regimen was well tolerated in hemodialysis patients, the updated TRC-ID regimen may also be immunogenic in this population. However, due to the small sample size of the study, more evidence may be needed before a recommendation be made.

b) Antimalaria drugs (to be treated under Pre-exposure prophylaxis)
There are no new published studies investigating the reduced response to ID rabies vaccination of people under antimalarial drug treatment. Almost all sources can be traced back to an older randomized clinical trial on PrEP and ID administration route by Pappaioanou *et al.* of 1986 (cited in the current WHO position paper). This study did only investigate the potential effect of chloroquine on efficacy of ID rabies primary immunization. There has been a surge of chloroquine-resistant malaria since the 1960's and many countries affected changed their official policy to no longer use chloroquine as a first line drug against *P.falciparum* malaria. However, chloroquine is still in use: Officially recommended to treat *P.vivax* malaria in most of Asia (India, Pakistan, etc.) and Latin America[1] or illicit trade over the counter /

[1] WHO, World Malaria Report 2016, Annex 1 data sources, http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/

street (in many countries, no figures). Unfortunately, there are no other data confirming or discarding any interference between antimalarial drugs (other than chloroquine and derivates) and rabies immunization via ID administration mode.
Forthcoming data: An exploratory trial[2] to evaluate the effect of antimalarial drugs on the immune response generated by rabies vaccine, when mimicking PEP started in April 2016. This randomized, open label, trial in healthy US adults (age 18-60 years), has four study arms: PEP as concomitant factor with Chloroquine, Atovaquone & Proguanil and Doxycycline versus no antimalarial drug treatment. However, there will be no ID administration of vaccines, only IM (Essen 4 versus 5 doses IM).

c) Pregnant women

Huang et al. evaluated the safety of PEP using the Essen 5-dose regimen among pregnant women with potential rabies exposures. All of the infants exhibited normal development and both PVRV and PCECV were supported as safe for use in pregnant women. No rabies cases were reported for any of the subjects or babies. The authors highlight that educational gaps exist about the safety of PEP during pregnancy and highlight that pregnancy terminations are common in China due to concerns about rabies vaccination risk. As such, it would be beneficial to include a stronger recommendation for the use of PEP during pregnancy in the updated rabies position paper and provide evidence that vaccination does not interfere with the development of the fetus or infant. Reviews on vaccines and pregnancy including reference to rabies are available from de Martino et al. and Crowcroft et al.

d) Bat-mediated exposures

Published literature on bat-mediated rabies exposures outside the Americas is scarce. While bats in the Americas transmit RABV only, bats in other parts of the world can transmit a variety of lyssaviruses. Exposures and reported bat-associated lyssavirus infections in humans are extremely rare in the 'old world'. The number of discovered bat lyssaviruses has increased over the years (Afonso et al., 2016). At present, three phylogroups of lyssavirus species are recognized in bats. Rabies virus is in phylogroup I lyssavirus. Experimental evidence indicates that currently available rabies vaccine strains are ineffective against lyssaviruses in phylogroup II and phylogroup III (Rupprecht 2016, Warrell et al 2010). Udov et al found that patients with bat-acquired rabies were 3 times more likely to have had no known exposure site compared to patients with dog-acquired exposures. Bat bites or scratches are not easily visible and bat exposures not always result in a (detectable) wound, e.g. to inject RIG. Thus, for direct exposures with physical contact with a potentially rabid bat and where there is no visible wound, RIG should be injected around the site of exposure to the degree which is anatomically feasible and possible. In absence of novel, pertinent evidence on improvements of PEP in individuals who experienced a bat-mediated rabies or lyssavirus exposure, the group concluded that recommendations on PEP regimens for bat exposures remain unchanged.

[2] https://clinicaltrials.gov/ct2/show/NCT02564471

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GRADE Table: **Question 8.**

Updated data compared to current WHO PEP protocol for specific risk groups of rabies exposed patients (excluding literature on bat-mediated rabies)

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	updated data	current WHO PEP protocol	Relative (95% CI)	Absolute (95% CI)		
Immunocompromised individuals: Rahimi et al. (follow up: mean 35 days; assessed with: RVNA levels)												
1	randomised trials	not serious [a]	not serious	not serious [b]	serious [c,d]	none [e,f]	50/80 (62.5%)	30/80 (37.5%)	not estimable		⨁⨁⨁◯ MODERATE	
Pregnant women: Huang et al. (follow up: range 6 months to 15 months; assessed with: RVNA levels)												
1	observational studies	not serious	not serious	not serious [b]	serious [c,g]	none [e,h,i]		-	-	-	⨁◯◯◯ VERY LOW	
End stage renal failure: Tanisaro et al. (follow up: mean 90 days; assessed with: RVNA levels)												
1	randomised trials	not serious [a]	not serious	serious [j]	serious [c,k]	none [f,j]			not estimable		⨁⨁◯◯ LOW	
Individuals with HIV/AIDS: Sirikwin et al. (follow up: mean 1 years; assessed with: RVNA levels)												
1	randomised trials	not serious [a]	not serious	serious [j]	serious [c,l]	none [i,m]			not estimable		⨁⨁◯◯ LOW	

a. subjects with no history of rabies or detectable RVNA concentrations
b. animal bite victims
c. fewer than 300 participants
d. 80 participants
e. rabid status of biting animal could not be confirmed
f. just PVRV, not PCECV
g. 72 participants
h. PVRV and PCECV
i. no use of RIG
j. subjects not exposed to rabies
k. 14 participants
l. 27 participants
m. just PCECV, not PVRV

Evidence Profile: Question 9

Question 9: Does a change in route of administration (IM or ID) during a single course of a PEP regimen affect immunogenicity of PEP?

Population:	Subsets of category II or III rabies-exposed patients who received part of the PEP course via IM and ID administration routes
Intervention:	Allow for a switch of vaccine administration route during an entire course of PEP
Comparison:	Maintain vaccine administration route throughout the entire course of PEP
Outcome:	Comparable immunogenicity, improved practicability in some settings

Background:

PEP should be initiated as early as possible following possible rabies exposure; PEP includes wound washing with soap and water, rabies vaccination and administration of rabies immunoglobulin (RIG) if indicated in previously unimmunized individuals. Rabies vaccine can by administered by either the intramuscular (IM) or intradermal (ID) route, depending on the schedule utilized.

Rabies vaccines are considered safe and highly effective in preventing rabies. A rabies virus neutralizing antibody (RVNA) concentration ≥ 0.5 IU/ml on day 14 post-immunization is considered adequate. This standard is a clinical endpoint used to indirectly measure the protective effect of vaccination in studies of rabies vaccine efficacy and effectiveness.

Although rabies vaccines are safe and highly immunogenic, the currently approved vaccine schedules require approximately a month to complete. Therefore, PEP schedules are often left incomplete and changes in route of administration (IM to ID or vice versa) are likely to occur in practice. Especially considering the current global shift towards ID administration, patients receiving a first vaccine administration in a small clinic in a rural area (likely IM), patients returning to rural areas for the remainder of vaccine administration after consultation in a larger clinic (likely ID) or travelers getting first boosters abroad and completing the PEP course back home, etc.. Therefore, it is important to assess the adequacy of the immune response conferred by changes in the route of administration for PEP.

Current position and practice:

The current WHO position paper on rabies vaccination states that “when it is impossible to complete post-exposure prophylaxis with the same CCEEV, another CCEEV should be used instead. However, since no study has been done yet on vaccine immunogenicity following changes in the route of vaccine administration (for example, from intramuscular to intradermal) during post-exposure prophylaxis, such changes should be the exception” (2010).

New evidence:

Ravish et al. provided supporting evidence that changes in the type of CCEEV (n=43) or the route of administration (n=47) of rabies vaccines (n=24 from ID to IM and n=23 from ID to IM) are safe and immunogenic. All participants had RVNA titers greater than 0.5 IU/mL on day 14 post-immunization. This study suggests that changes in the CCEEV and/or the route of administration should be allowed in unavoidable circumstances to promote completion of the lifesaving PEP regimen. Detailed immunogenicity data are available in Table 1.

In a slightly different context Sudarshan et al. conducted a study on n=20 volunteers who previously received a full course of PEP. The immune response was assessed by mimicking PEP for previously immunized people and forcing a change in route of administration. The study used purified chick embryo cell rabies vaccine (PCECV). It showed that these are safe and immunologically efficacious following booster vaccination, even after cross-over from the ID to the IM route and vice versa.

Table 1. Rabies virus neutralizing antibody titers following a:

	a) change in route of administration during the same PEP course					
	Ravish et al.					
Day	Switch from intradermal route to intramuscular route or vice versa					
	Geometric mean titer	95% CI	Range			
14	14.83	13.58-15.63	7.5–22.5			
	b) change in route of administration for booster vaccination (days 0 and 3)					
	Sudarshan et al.					
Day	Intradermal route*			Intramuscular route†		
	Geometric mean titer	95% CI	Range	Geometric mean titer	95% CI	Range
0	0.59	0.50–0.68	0.5– 0.8	0.59	0.50–0.71	0.5–1.0
14	8.84	7.58–10.30	7.4–12.4	9.17	7.84–10.70	6.9–12.7

* previous vaccination by intramuscular route † previous vaccination by intradermal route CI confidence interval

† value for test of significance of geometric mean titre between days 0 and 14 was 79.26 for the intradermal and 24.87 for the intramuscular group. The degrees of freedom were 9 and p value <0.0001 for both the groups.

Conclusion:

Although the practice of changing administration route during a single PEP course probably occurs, there is only a single new study in one setting which assesses the associated immunogenicity data.

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Evidence Profile: Question 10

Question 10: Are there novel approaches to RIG (-sparing) injection versus current practice as part of PEP for category III exposed patients? Such as:

a. **discontinuation of calculation of RIG dose needed according to body weight?**
b. **RIG only into or around the biting wound(s) compared to additional administration of remaining RIG to other body parts?**

Population	Category III exposed patients receiving PEP
Intervention	Simplification of recommendations: a. RIG volume calculation based on factors other than patient body weight b. RIG administration to wound area without remaining RIG injected at distant site
Comparator	Current recommendations: a. RIG volume calculation based on body weight: 20 IU/kg body weight for hRIG and 40 IU/kg body weight for eRIG b. RIG administration into or around the wound sites with remaining RIG injected intramuscularly at a site distant from the site of vaccine administration
Outcome	Sustained or increased patient survival; more efficient use of RIG; improved cost-effectiveness

Background:

The high cost, low availability and supply, batch to batch variation affecting efficacy, uncertain quality (no WHO prequalification) and short shelf life of RIG are barriers to implementing the gold standard set by WHO for PEP in category III bites. RIG is often a barrier for attaining public health impact because of a hesitation to use vaccine without RIG and therefore manufacturers and countries often do not want to make vaccines available without RIG, which means no PEP at all. The simplification of WHO's recommendations on RIG based on new evidence available is important considering the aspects above. The individuals in rabies-endemic settings most often affected are those who can least access and afford PEP. Additionally, RIG is in scare availability, compared to the other components of the PEP regimen, so its efficient use is important for ensuring maximal availability to the patients bearing the highest risk. Indeed, ''less than 3% of at-risk dog bite cases [globally] receive RIG'' (Bharti *et al*., 2016). Additionally, the current recommendations increase procedure complexity for care providers; procedures such as weighing the patient, completing calculations, performing multiple injections at different sites and diluting the vial content all require additional resources and knowledge. Updated recommendations should aim to lower the cost per patient, avoid RIG wastage and simplify practices for physicians.

If new evidence shows that RIG dose and volume for administration can be adjusted for factor(s) other than body weight, then recommendations can be made to determine RIG-saving administration practices that are as efficient as or more efficient than current recommendations. Similarly, if it is shown that administration of RIG (as part of PEP) to only the wound area is more efficient than additional, non-local intramuscular administration, then recommendations can be

strengthened and discourage additional administration of RIG to other body parts. However, limitations regarding objectivity and standardization must be considered.

Current position and practice:

The current WHO recommendations state that ‘‘rabies immunoglobulin should be administered in all people with category III exposure and to those with category II exposure who are immunodeficient;’’ this summary focuses on immunocompetent individuals with category III exposure. The current WHO position paper continues that ‘‘rabies immunoglobulin for passive immunization is administered once, preferably at, or as soon as possible at, the initiation of post-exposure vaccination. Beyond the seventh day after the first dose, rabies immunoglobulin is not indicated because an active antibody response to the vaccine is presumed to have built up. The recommended dose of hRIG is 20 IU/kg body weight; for eRIG and $F(ab')_2$ products, it is 40 IU/kg body weight. All of the rabies immunoglobulin, or as much as anatomically possible (but avoiding possible compartment syndrome), should be administered into or around the wound site or sites. The remaining immunoglobulin, if any, should be injected intramuscularly at a site distant from the site of vaccine administration. RIG may be diluted to a volume sufficient for all wounds to be effectively and safely infiltrated’’ (WHO, 2010).

New evidence:

Evidence regarding new data and improved quality of RIG suggest that the recommendations for the administration of RIG may be simplified. Specifically, these data support the discontinuation of both RIG dose calculation based on patient’s body weight and of RIG administration at sites distant from the wound(s). However, limitations regarding objectivity and standardization as well as questions of data transferability must be considered. First, it is currently unclear how dose calculation could be standardized objectively if not by body weight; a lack of standardization would confer clinician judgement, which is prone to errors and imprecise volume determination. Second, administration of RIG into small wound spaces (*e.g.* fingers, toes, ears, noses) is limited and may not provide a sufficient dose of RIG.

a) <u>Body Weight Calculation</u>

The recommendation on body weight dosage was originally derived from studies in which unpurified eRIG was administered systemically; therefore, the body weight recommendation gave ‘‘consideration for biological half-life of heterologous proteins and extent of distribution and dilution in the body’’ (Bharti *et al*., 2016; Madhusudana *et al*., 2013). However, these recommendations lack empirical support and appear outdated in light of the newer, more efficacious ‘‘highly purified and enzyme refined [immunoglobulins] containing only antigen binding components’’ (Madhusudana *et al*., 2013). Yet, it is important to consider that the antigen-binding immunoglobulin fragments (F(ab’)2) have a shorter half-life *in vivo* than intact immunoglobulins; effective neutralization with F(ab’)2 products may wane in the critical period before active immunity and RVNAs appear.

Madhusudana *et al*. performed a laboratory-based study that investigated the neutralization efficacy of reduced RIG in BHK21 cells and mice (2013) (Table 1). No vaccine was given to the mice that received RIG, yet the experimental groups of mice that received at least 0.025 IU/100 µl of RIG had a 100% survival rate, compared to 100% mortality in the control group (Madhusudana *et al*., 2013) (Table 1). This study reduced the RIG dose by ‘‘at least 16 times the presently advocated dose,’’ in

mice (Madhusudana *et al.*, 2013). However, murine models alone are not ideal models for evaluation of rabies biologicals because of their great variability, limited reproducibility and limited comparability. Moreover, these data are in conflict with studies conducted before the scope of this systematic review (e.g. Hanlon *et al*., 2002). Further clinical studies are needed to relate this notion to larger mammals or observational data in humans.

Table 1: Reduced RIG in BHK21 cells and mice (Madhusudana *et al*., 2013).

	***In vitro* (BHK21 Cells)**			***In vivo* (Mice)**		
	[Virus] (FFD_{50}/µl)	Amount of RIG (IU)	Neutralization (%)	[Virus] (LLD_{50}/µl)	Amount of RIG (IU)	Neutralization (%)
eRIG	10^4	0.025	100	10^3	0.025	100
	10^3	All dilutions	100			
hRIG	10^3	All dilutions	100	10^3	0.025	100

b) <u>RIG Infiltration Methods</u>

Following the calculation of RIG dose by body weight, often there is too small a volume of RIG to be distributed to the wound(s), or too large a volume of RIG to be infiltrated into the wound space (Bharti *et al*., 2016 and 2017). When too small a volume of RIG is allotted, it is often diluted with saline so that the volume may be spread between all wounds; this action decreases the concentration of RIG (Bharti *et al*, 2016; Madhusudana *et al*., 2013). If these spaces are areas that contain many peripheral nerve endings, such as the face and fingertips, this further increases patients' risk (Behera *et al*., 2012). When the amount of RIG allotted is too large a volume to be infiltrated into the wound, the excess is administered intramuscularly (Bharti *et al*., 2016; Madhusudana *et al*., 2013). Data suggest that this practice is wasteful and inefficient (Madhusudana *et al.*, 2013; Wilde, 2016).

Saesow *et al*. report that hRIG administered intramuscularly was retained locally, experimentally in eight adult rabbits (2000). The RIG, labelled with Iodine 131, ''could still be detected at the [intramuscular] site 24 hours later'' yet was undetectable in blood samples (Saesow *et al.*, 2000). As the RIG remained in its injection site, it failed to produce circulating protective serum antibody levels at the ''minimum acceptable level of 0.5 IU/mL'' (Bharti *et al.*, 2016; Madhusudana *et al.*, 2013; Saesow *et al.*, 2000). Furthermore, some maintain that systemically inoculated RIG may interfere with the natural immune response, decreasing vaccine efficacy (Madhusudana *et al*., 2013; Wilde, 2015). As data show that neutralization by RIG occurs at the site of infection, its injection into and around the wound(s) is likely to be the most efficacious and efficient method (Bharti *et al*., 2016; Wilde, 2016).

Annex 1 provides calculations based on PEP patient demographics and weight from Institut Pasteur Cambodge. The theoretical estimates on resulting circulating antibody titres after administration of ½ of the eRIG dose intramuscularly and ½ of the eRIG dose was used for local wound injection. The resulting circulating antibody titres would be beyond the adequate threshold of 0.5 IU/ml in all groups.

Synergy of Both RIG Administration Changes; Cost-Effectiveness

Simplified recommendations as above would work synergistically to reduce the average cost per patient and improve the efficiency of RIG administration, as the excess RIG from the body weight calculated dose would no longer be available to inject intramuscularly. The simplification of recommendations is exemplified in two recent studies by Bharti *et al*. 2016 and 2017.

First, Bharti *et al*. investigated cost-effective alternatives to the current RIG standards (2016). The study group included 269 patients with category III rabies exposure (in a confirmed rabies-endemic area), to which all were administered RIG volumes ‘‘just sufficient to infiltrate wounds […] irrespective of body weight’’ (Bharti *et al*., 2016). All patients were administered with the same batch of eRIG, with a titre of 550 IU/mL throughout the study period; the doses of RIG ranged from 0.25 mL to 8 mL, with an average volume of 1.26 mL (Bharti *et al*., 2016). In total, 42 vials were used to treat all patients, compared to 363 vials had the doses been calculated according to body weight; rabies-exposed patients had between 60% to 80% reductions in RIG dose volume compared to those of the body weight standards’ group (Bharti *et al*., 2016). Additionally, no administered dose exceeded the dose calculated by body weight as currently recommended, therefore avoiding concerns of interference of RIG with vaccine-induced rabies-neutralizing antibodies; this notion was supported by serological tests done on 20 of the patients, of which none detected immunosuppression (Bharti *et al*., 2016). Within the 82% follow-up rate for a time period of over 9 months, there was a 100% survival rate (Bharti *et al*., 2016).

Second, Bharti *et al*. investigated a rabies outbreak in which 18 people suffered from category III exposures (2016). In a similar manner to the above study, RIG volumes not calculated by body weight were infiltrated only locally (Bharti *et al*., 2016). In total, 8.3 vials (41.5 mL) of RIG were used to treat all patients, compared to 24 vials (120 mL) had the doses been calculated according to body weight (Bharti *et al*., 2016). There was a 100% follow-up rate with a 100% survival rate (Bharti *et al*., 2016). Bharti *et al*. go so far as to state that ‘‘if use of RIG is to increase, as is clearly needed, it is surely incumbent on the WHO Expert Committee to recommend the lowest dose of RIG that is likely to be effective’’ (2016). The most recent study of Bharti *et al*. 2017 using the same methodology, investigated 26 WHO category III rabies exposed patients who had been bitten by laboratory confirmed rabid dogs. The patients were followed for over one year and all survived.

In each of these studies, data supported that the combined abandonment of body weight calculation and intramuscular administration of RIG improved cost-effectiveness while maintaining safety and efficacy, particularly in clinics with several patients in need of RIG every day. Unfortunately, these conclusions require broad assumptions, as most studies failed to confirm the rabies status of the biting animals. Lastly, objective and quantifiable standardization of new volume and infiltration practices would be critical to ensure efficacy and reproducibility.

Modelling results:

Comparing current recommendations for RIG administration and a proposed change in strategy to administer RIG at the site of the wound only, results in considerable savings in RIG use (Figure 1 & 2), if we assume that bite patients are comparable to those from Himachal Pradesh, India in terms of the body weight distribution and types of wounds (data provided by Bharti et al). These savings increase with patient throughput as vials can be more effectively shared between patients (we estimate 1.1 vials of RIG per patient in low throughput clinics and 0.87 vials per patient in high throughput clinics when administered according to body weight versus 0.92 vials and 0.32 vials per patient in low and high throughput clinics if infiltrated only at the wound site(s). Moreover, when available vials are limited then many more patients can be treated if RIG is only administered at the site of the wound. In the clinic in Himachal Pradesh, India where data on RIG were generated, around 270 patients are seen per month requiring approximately 262 vials of RIG each month if injected at the site of the wound, or 370 vials/month if following current recommendations, a 40% saving of RIG.

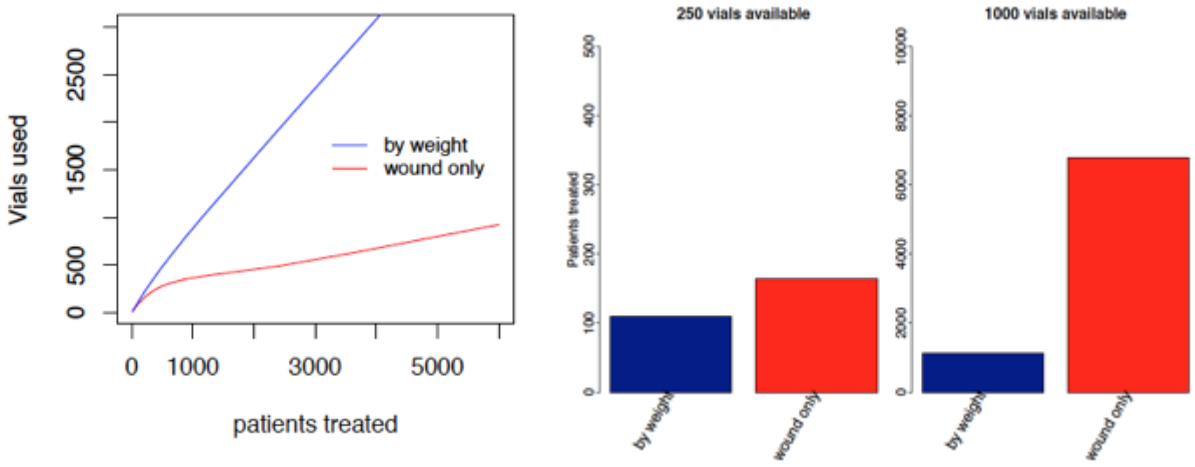

Figure 1 &2. Patients treated with RIG when administered according to current WHO recommendations (blue) and at the site of the wound only (red). *We compared vials used under different levels of patient throughput (left), and also how many patients could be treated given limited vial availability (right) with examples shown for 250 vials and 1000 vials per year.*

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ANNEX 1

Question 10: Supplementary data and calculations

The table below provides data on patients seeking PEP (source Rabies Prevention Center Pasteur Institute of Cambodia (IPC)).

The last section of the table is the titer, which is the remaining dose (determined by 50% of the dose per weight * weight) over blood volume (also determined by volume per weight * weight in our formula). As expected, the ratio is therefore a fixed one (dose per weight / volume per weight) so I showed only data for all. I can try to get the data from a small series (n=100) of patients being studied, but remaining doses (set at 50%) in my calculations) show clearly that much of RIG is being wasted.

These computations are for equine RIG, not hRIG which is not used at IPC. To our knowledge there are no reference values for circulating eRIG titres that are considered protective. The only reference titres considered protective were set by clinical trials on rabies vaccines in humans, usually evaluated with concurrent use of human RIG.

ERIG is thought to be half as effective as HRIG per volume (hence recommended doses of 40 UI/kg for eRIG instead of 20 UI/kg for hRIG), although this does not translate into clinical findings because the necessary RIG dose in a wound is probably very small. This table can therefore theoretically be done for eRIG but the HRIG (not used in Phnom Penh) doses (and consequent titres) would therefore need to be divided by two.

Table: Estimates of circulating antibody titres after administration of ½ of the eRIG dose remaining after local wound injection in 4131 eRIG recipients, by gender, Rabies Prevention Center, Institut Pasteur du Cambodge 2000-2015, Phnom Penh, Cambodia.

	Male	Female	Total
N documented*	2243	1888	4131
Body weight			
Mean weight ± SE	35.13 ± 22.38 kg	36.37 ± 19.34 kg	35.70 ± 21.05 kg
Median	25 kg	40 kg	31 kg
Range	3 – 99 kg	6.5 – 87 kg	3 – 99 kg
Interquartile range	15.5 – 55.4 kg	16 – 52.4 kg	15.5 – 54 kg
Blood volume (in mL)			
Est. blood volume per kg body weight**	75 mL/kg	65 mL/kg	65 mL/kg
Mean volume ± SE	2635.26 ± 1678.30	2364 ± 1257.21	2511.31 ±
Median	1875	2600	2210
Range	225 – 7425	422.5 – 5655	225 - 7425
Interquartile range	1162.5 - 4155	1040 – 3406	1125 – 3737.5
eRIG dose (IU) taken from 3000 IU vial			
Est. eRIG dose	40 IU/kg	40 IU/kg	40 IU/kg
Mean dose ± SE	1405.47 ± 895.09	1454.80 ± 773.67	1428.02 ±

Median	1000 IU	1600 IU	1240 IU
Range	120 - 3960	260 - 3480	120 - 3960
Interquartile range	620 - 2216	640 - 2096	620 - 2160
50% remaining RIG dose after wound			
Mean titre ± SE	702.74 ± 447.55	727.40 ± 386.83	714.01 ± 421.02
Median	500	800	620
Range	60 – 1980	130 – 1740	60 - 1980
Interquartile range	310 - 1108	320 – 1048	310 - 1080
Circulating blood eRIG titres (IU/mL)*			
Mean titre ± SE	-	-	0.28 ± 0.02
Median	-	-	0.27
Range	-	-	0.27 – 0.31
Interquartile range	-	-	0.27 – 0.31

* eRIG recipients only, no hRIG for which recommended injected dose volumes (and consequent circulating titres in case of injection of the remaining dose) are half of those of eRIG

** http://reference.medscape.com/calculator/estimated-blood-volume based on Morgan, Mikhail, and Murray. Clinical Anesthesiology. 3rd Edition.

GRADE Table: Question 10

Question 10: Simplification of RIG recommendations compared to current recommendations for category III rabies patients

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	simplification of RIG recommendations	current recommendations	Relative (95% CI)	Absolute (95% CI)		
Body weight calculation: Madhusudana et al 2013												
1	randomised trials	not serious	not serious	serious [a]	serious [b]	none			not estimable		⊕⊕◯◯ LOW	
RIG infiltration: Saesow et al 2000												
1	randomised trials	not serious	not serious	serious [a]	serious [b]	none			not estimable		⊕⊕◯◯ LOW	
Simplification: Bharti et al 2016 (1)												
1	observational studies	serious [c]	not serious	not serious	serious [b]	none			not estimable		⊕◯◯◯ VERY LOW	
Simplification: Bharti et al 2016 (2)												
1	observational studies	serious [c]	not serious	not serious	serious [b]	none		-	-	-	⊕◯◯◯ VERY LOW	

CI: Confidence interval

a. animal model

b. fewer than 300 events

c. did not control for confounding

Evidence Profile: Questions 11 & 12

Question 11: Is there clinical equivalence in the safe use of eRIG compared to hRIG in category III exposed patients?

Question 12: Is there clinical equivalence in the efficacious use of eRIG compared to hRIG in category III exposed patients?

Population	Category III rabies exposed patients (focus on dog-mediated rabies)
Intervention	The use of eRIG as an safe and efficacious alternative to hRIG
Comparator	The use of hRIG as the preferred product, as data from 2010 Rabies Vaccination Position Paper suggest that eRIG carries a small risk (1/45000) of anaphylactic reaction
Outcome	Safety of PEP process (*e.g.* adverse effects); efficacy of PEP (*e.g.* patient survival); cost-effectiveness

Background:

Updated data regarding the safety and efficacy of equine rabies Immunoglobulins (eRIG) and human rabies immunoglobulins (hRIG) are of practical importance because eRIG is frequently a less expensive and more accessible option than hRIG, especially in canine rabies-endemic areas. The safety perception of the earlier, poorly refined eRIG preparations may continue to limit the wider acceptance of the new generation of immunoglobulins of equine origin. If it is shown that the differences between eRIG and hRIG are negligible, then the current recommendation of eRIG as a safe, efficacious alternative to hRIG can be strengthened. Yet, safety and efficacy should not be compromised considering the potentially high mortality rate in rabies virus-exposed patients.

Current position and practice:

The current WHO recommendations state that ‘‘rabies immunoglobulin should be administered in all people with category III exposure and to those with category II exposure who are immunodeficient. […] If hRIG and eRIG (and F(ab’)2 products) are correctly administered they eliminate the virus at the wound site within a few hours. hRIG is often perceived as the preferred product, particularly in cases of multiple severe exposures and bites on the head, face and hands. However, hRIG is scarce and available mainly in industrialized countries. Where it is not available or affordable, eRIG or F(ab’)2 products of eRIG be used, although the F(ab’)2 have a faster clearance than hRIG. Most of the new eRIG preparations are potent, highly purified, safe and considerably less expensive than hRIG. However, they are of heterologous origin and carry a small risk of anaphylactic reaction (1/45,000 cases). There are no scientific grounds for performing a skin test prior to administering eRIG because testing does not predict reactions, and it should be given whatever the result of the test. The treating physician should be prepared to manage anaphylaxis which, although rare, could occur during any stage of administration. […] The dose of hRIG is 20 IU/kg body weight; for eRIG and F(ab’)2 products, it is 40 IU/kg body weight’’ (WHO, 2010).

New evidence:

Updated evidence on eRIG and hRIG further supports the safety and efficacy of these products. In view of many more years of experience in countries and additional data available now, the use of eRIG is supported to be a safe and efficacious alternative in the many areas where hRIG is unavailable or unaffordable.

The high cost, low availability and supply, batch to batch variation affecting efficacy, uncertain quality (no WHO prequalification), short shelf life and correct administration of RIG are barriers to implementing the gold standard set by WHO for PEP in category III bites. RIG is often a barrier for attaining public health impact because of a hesitation to use vaccine without RIG and therefore manufacturers and countries often do not want to make vaccines available without RIG, which means no PEP at all. For example, in Cambodia, eRIG (which is consistently less expensive than its sole alternative, hRIG) costs ''between US$20 and US$30 per dose [yet] a Cambodian farmer's monthly salary is between US$60 and US$80'' (Tarantola *et al*., 2012). In other words, a dose of RIG can drain up to half of one's monthly salary. Similar discrepancies between income and RIG price exist throughout Asia and Africa (Madhusudana *et al., 2013;* Tarantola *et al.*, 2015; Tenzin *et al.*, 2012). The issues of cost and availability are even more prominent for hRIG, and thus it is impractical for use in areas with limited resources (Anderson, 2007). Therefore, new data regarding eRIG safety and efficacy is relevant to most WHO category III rabies-exposed individuals.

Safety

Data support the safety of eRIG. In the past, unpurified eRIG conferred high rates of serum sickness, anaphylaxis and other severe adverse reactions (Madhusudana *et al*, 2013). But now, eRIG is highly purified and enzyme-refined and contains over 85% antigen-binding immunoglobulin fragment (F(ab')2) (Madhusudana *et al*., 2013; Shantavasinkul & Wilde, 2011; Quiambao *et al*., 2008, Kittipongwarakarn *et al.* 2011, Reveneau *et al.* 2017). Through purification techniques such as heat treatment, pepsin digestion and enzyme refinement, the crystallisable/constant (Fc) fragment is removed and the nonspecific protein content of the purified sera is decreased to less than 3% (Behera *et al.*, 2011; Chawan *et al.*, 2007). As the Fc fragment in unpurified eRIG ''is responsible for direct complement activation and anaphylactic reactions,'' the high F(ab')2 content and low Fc protein content allow for increased safety and specific activity (Chawan *et al.*, 2007; Madhusudana *et al*., 2013; Quiambao *et al*., 2008). eRIG treatment has even been shown to be safe for pregnant women, as F(ab')2 is not shown to cross the placenta (Dixit *et al*., 2016). Both, eRIG and hRIG are efficacious in eliminating the virus at the wound site within a few hours, therefore differences in half-life of the products seem not to impact the success of the treatment.

Studies suggest that severe adverse reactions, such as serum sickness and anaphylaxis, are infrequent (Adverse Reaction Rates to eRIG, see annexe). Other adverse reactions recorded tend to be mild, not life-threatening and easily resolved, such as local pain, redness, induration, fever and itching (Table 1). Indeed, data show that adverse reaction rates for eRIG are similar to that of penicillin (Wilde, 2012).

Despite the safety of eRIG, adverse reactions still occur more frequently in those who receive eRIG than those who receive hRIG (Table 2). However, these data may not be perfectly representative as more data were available for patients who received eRIG.

Table 2: Comparison of eRIG and hRIG Adverse Reaction Rates

Authors and Year	eRIG	hRIG
Dixit *et al*., 2016	1.83%, total adverse events	0.09%, total adverse events
	0.72%, serum sickness	0.007%, serum sickness
Suwansrinon *et al*., 2006	0.05%, serum sickness, in those under 10 years	0.01%, serum sickness, in those under 10 years
Warrell, 2012	1.83%, total adverse reactions	0.09%, total adverse reactions
	0.73%, serum sickness	0.007%, serum sickness

Regardless of its comparison to hRIG, the safety of eRIG is supported, especially in light of the price and scarcity of hRIG and the 100% mortality rate of clinical rabies.

Efficacy

In addition to increased safety, it is also suggested that modern, purified eRIG is efficacious (Madhusudana *et al*., 2013). Conversely, it is important to consider that the antigen-binding immunoglobulin fragments (F(ab')2) have a shorter half-life *in vivo* than intact immunoglobulins; effective neutralization with F(ab')2 products may wane in the critical period before active immunity and RVNAs appear. Quiambao *et al*. discuss that, while the clearance of F(ab')2 eRIG is faster than that for unpurified eRIG and hRIG, the F(ab')2 fragments have a higher specificity and instance of antigen-binding reactions, and therefore its efficacy is preserved (2009). Both *et al*. state that while purified eRIG ''is generally highly effective, the reduced half-life of these experimentally induced antigen-binding fragment products might have contributed to a few anecdotal PEP failures, and related data derived from animal studies have shown that intact immune globulin products are more effective for rabies PEP than are derived F(ab')2 fragments'' (2012). In a similar manner to the discussion of its safety and following WHO standards for PEP in category III bites, the efficacy of eRIG is supported, considering the price and scarcity of hRIG and the 100% mortality of clinical rabies.

For example, a study by Madhusudana *et al*. investigated the neutralization efficacy of reduced eRIG and hRIG in BHK21 cells and mice (2013). *In vitro,* percent neutralization for eRIG and hRIG were identical (Madhusudana *et al*., 2013). *In vivo,* full protection was conferred by both eRIG and hRIG (Madhusudana *et al*., 2013). No vaccine was given to the mice that received RIG, yet the experimental groups of mice that received at least 0.025 IU/100 µl of either eRIG or hRIG had a 100% survival rate, compared to 100% morality in the control group (Madhusudana *et al*., 2013).

Other studies suggest holistic success rates with eRIG in humans (Bharti *et al*., 2016; Bharti *et al*., 2017; Quiambao *et al*., 2008). Deaths despite the reception of eRIG have been attributed to deviation from the WHO PEP guidelines or causes unrelated to rabies exposure or treatment (Dixit *et al*., 2016; Quiambao *et al*.; 2008, Salahuddin *et al*., 2014 & 2016).

Conclusions:

Despite the data that show its safety and efficacy, eRIG is not always used, even when available, due to individuals' concerns (patients or medical personnel). Evidence allows the strengthening of the current WHO recommendations for the use of eRIG. First, the abandonment of skin testing before eRIG administration is supported. Second, eRIG use is promoted as an alternative to hRIG and as a life- and cost-saving option. Third, continued education and awareness for eRIG are encouraged. Lastly, it is recommended that WHO develop a quality-assurance process (similar to pre-qualification for vaccines) for immunoglobulins.

Annex 1 is a suggestion elaborated by working Group Members for an improved, generic classification of adverse effects after RIG administration. It could be used e.g. by countries to report back to international organisations and RIG producers. Examples for reporting forms from other vaccines are available from the "Global Manual on Surveillance of Adverse Events Following Immunization " http://www.who.int/vaccine_safety/publications/aefi_surveillance/en/

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Table 1: Adverse Reaction Rates to eRIG

Authors and Year	Setting	Number of Patients	Time Frame	Serum Sickness	Anaphylaxis	Other Adverse Reactions	Notes
Behera *et al*., 2011	Orissa, India majority of cases from rural areas	1,494 (children)	100 days	3%	0%	91.8%, induration 43.1%, erythema 29.8%, local pruritus 19.9%, pain 34.8%, fever 29.5%, malaise 6.8%, general pruritus	
Behera *et al*., 2012	Orissa, India	195	Not specified	1.53%	0%	49.74%, local reactions (induration, pain, pruritus) 12.3% systemic (low grade fever)	Injection into fingers and toes specifically 0%, compartment syndrome
Bharti *et al*., 2016	India	269	Over 9 months	0%	0%	60%, local pain 40%, redness	
Chawan *et al*., 2007	Mumbai, India	168	30 days	0%	0%	31.5%, local adverse reactions (pain, swelling, pruritus, induration, erythema)	None who were sensitive to the skin test developed serious side effects
Dixit *et al*., 2016	Thailand	70,000	Not specified	0.72%	0%	1.83%, all adverse reactions	Broad range included eRIG produced both before and after modern purification techniques
Quiambao *et al*., 2009	The Philippines	7,660	35 days to 29 months			0.46%, all adverse reactions	
Shantavasinkul & Wilde, 2011	Bangkok, Thailand	150,000	Not specified		2 / 150,000		
Sudarshan *et al*., 2011	Bengaluru, India	2,008	26 months			1.5%, all adverse reactions	

Annex 1

Types of reactions after rabies immunoglobulin administration

The classical presentation of immune-mediated adverse effects proposed by Gell and Coombs[1] does not address the protean and interpenetrating forms of immune-mediated reactions described following the injection of equine or human-derived rabies immunoglobulin.

We therefore propose to start from the clinical aspects of the reaction rather than the immunological classification, as shown in the table below.

Reaction after RIG injection	Signs	Frequency / Severity	Delay	Mechanism
Local	Local redness, tenderness and swelling	High / Benign	Immediate or within hours	Local trauma or inflammation due to injected volume
Serum sickness-like reaction	Fever, myalgia, epigastric pressure, rash, thrombo-cytopenia, anorexia, arthralgia	Medium / Medium	Usually within days, sometimes within hours	Type III hypersensitivity reaction, mediated by IgA/IgM
Hypersensitivity reaction (urticaria)	Rash, urticarial, wheezing, dyspnea, hypotension, swelling, tachycardia, dizziness, chest pain, nausea	Medium / Medium	Immediate in previously sensitized patients, minutes in others	Type I hypersensitivity reaction mediated by IgE
Anaphylaxis	Skin itching, sweating, faintness, dizziness; nausea and vomiting, diarrhea, are inconstant; Cardio-respiratory collapse then shock is possible.	Rare / Severe	Within minutes	Type I hypersensitivity reaction mediated by IgE

Types of reactions which may occur after rabies immunoglobulin administration (Adapted from [2–5])

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GRADE Table: Questions 11 & 12

Question 11 & 12: Safe use and efficacy of eRIG compared to safe use and efficacy of hRIG in category III exposed patients

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**eRIG events**	**hRIG events**	**Relative (95% CI)**	**Absolute (95% CI)**		
eRIG adverse events: Behera et al 2011												
1	observational studies	serious [a]	not serious	not serious	not serious	none			not estimable		⨁◯◯◯ VERY LOW	
eRIG adverse events: Behera et al 2012												
1	observational studies	serious [a]	not serious	serious [b]	serious [c]	none			not estimable		⨁◯◯◯ VERY LOW	
eRIG adverse events: Bharti et al 2016												
1	observational studies	serious [a]	not serious	not serious	serious [c]	none			not estimable		⨁◯◯◯ VERY LOW	
eRIG adverse events: Chawan et al 2007												
1	observational studies	not serious	not serious	not serious	serious [c]	none			not estimable		⨁◯◯◯ VERY LOW	
eRIG adverse events: Dixit et al 2016												
1	observational studies	very serious [a,d]	not serious	serious [e]	not serious	all plausible residual confounding would reduce the demonstrated effect			not estimable		⨁◯◯◯ VERY LOW	
eRIG adverse events: Quiambao et al 2009												

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	eRIG events	hRIG events	Relative (95% CI)	Absolute (95% CI)		
1	observational studies	not serious	not serious	not serious	not serious	none			not estimable		⨁⨁◯◯ LOW	
eRIG adverse events: Shantavasinkul and Wilde 2011												
1	observational studies	serious [a]	not serious	not serious	not serious	none			not estimable		⨁◯◯◯ VERY LOW	
eRIG adverse events: Sudarshan et al 2011												
1	observational studies	serious [a]	not serious	serious [f]	not serious	none			not estimable		⨁◯◯◯ VERY LOW	
eRIG vs hRIG adverse events: Dixit et al 2016												
1	observational studies	serious [a]	not serious	serious [e]	not serious	all plausible residual confounding would reduce the demonstrated effect	1.83/100 (1.8%) [g]	0.09/100 (0.1%) [g]	not estimable		⨁◯◯◯ VERY LOW	
eRIG vs hRIG adverse events: Suwanrinon et al 2006												
1	observational studies	very serious [a,f]	not serious	not serious	not serious	none	0.05/100 (0.1%) [g]	0.01/100 (0.0%) [g]	not estimable		⨁◯◯◯ VERY LOW	
eRIG vs hRIG adverse events: Warrell 2012												
1	observational studies	serious [a]	not serious	not serious	not serious	none	1.83/100 (1.8%) [g]	0.09/100 (0.1%) [g]	not estimable		⨁◯◯◯ VERY LOW	
neutralization efficacy: Madhusudana et al 2013												

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	eRIG events	hRIG events	Relative (95% CI)	Absolute (95% CI)		
1	randomised trials	not serious	not serious	serious [h]	serious [c]	none			not estimable		⨁⨁◯◯ LOW	

a. did not control for confounding
b. injection into fingers and toes specifically
c. fewer than 300 events
d. varied follow-up times

Evidence Profile: Question 13

Question 13: Can monoclonal antibodies (mAb) be safely and efficaciously administered in category III rabies-exposed patients compared to standard RIG?

Population	Rabies exposed patients (category III exposures)
Intervention	A safe and more cost-effective mAB is used over hRIG or eRIG
Comparator	Administration of eRIG or hRIG according to standard protocol
Outcome	Sufficient passive immunity to protect patients from clinical rabies/death, less expensive and more available PEP for category III exposed patients

Background:

Rabies immunoglobulin (RIG), derived from the blood of humans or horses, is currently used as a component of rabies post-exposure prophylaxis (PEP) as a method of passive immunization. RIG neutralises the rabies virus in the time before the immune system responds to the vaccine, which prevents the rabies virus from infiltrating the central nervous system. While RIG is an effective and life-saving product, the barriers of high cost, low availability, limited access and continual high demand suggest the need for an alternative to RIG. Moreover, concerns with RIG purification, interference with rabies vaccine and weakened efficacy against non-RABV Lyssaviruses further support the need for a RIG alternative (Benedictis et al., 2016; Franka et al., 2013; Hefferon, 2013).

A prospective alternative or supplement to RIG is an anti-rabies monoclonal antibody (mAb) cocktail. While mAbs were initially used for diagnostic and experimental purposes, they are now often employed as treatment and therapeutic agents in a variety of clinical settings (Silva et al., 2013). For example, there are over 40 licensed monoclonal antibodies in use for a range of diseases, although the majority of these are for noncommunicable disease such as cancer or inflammatory disorders. Only a few mAbs are licensed for infectious diseases, such as respiratory syncytial virus (RSV) and anthrax (Sparrow et al., 2017). Efficacious and safe anti-rabies mAbs would increase access and affordability of PEP and subsequently decrease rabies deaths.

Current position and practice:

As the first product has only been recently licensed, there are currently no WHO recommendations about the use of monoclonal antibodies for passive immunization against rabies. The 2010 Position Paper for Rabies Vaccines and Immunizations focuses solely on rabies RIG with respect to passive immunization. However, due to circumstances of affordability and access to RIG, finding alternatives to RIG is strongly encouraged. The current WHO information available on anti-rabies mAbs is available here and is also discussed below (Sparrow, 2016).

The first mAb against rabies (a single monoclonal antibody) was recently licensed by the Serum Institute of India (SII) and is expected to be launched in India for use in PEP in 2017 (personal communication from SII, 19 August 2016). SII has shared confidential data on the clinical trials of this product with WHO and this has been reviewed by an independent expert and the SAGE working group on rabies.

New evidence:

A review of available evidence (published literature, meeting reports and expert knowledge) on monoclonal antibodies for use in rabies PEP was compiled.
Evidence regarding anti-rabies mAbs suggests characteristics that would likely increase the safety and efficacy of a therapeutic rabies mAb cocktail, proposes mAbs as candidates for inclusion in therapeutic cocktails and addresses common concerns.
Studies of anti-rabies mAb cocktail candidates provide insight into safe and efficacious mAb combinations. Central to these studies is the rabies glycoprotein: as it extends through the virion surface and may mediate entry into host cells, the antigenic sites targeted by the mAbs are most frequently components of the glycoprotein (Buthlezi et al., 2016; Duan et al., 2012; Ellison et al., 2012). Targeting the appropriate antigenic epitopes is necessary to ensure breadth and depth of efficacy.
Furthermore, the compatibility of two mAbs used together in a cocktail is influenced by the antigenic sites targeted by each (Both et al., 2015). The anti-rabies mAbs included in a cocktail must recognise different epitopes, so that escape mutants from one antibody can be neutralised by its counterpart. It has been suggested that mouse mAbs frequently recognise antigenic site II, yet human mAbs generally recognise antigenic sites I and III (Sloan et al., 2007; Sun et al., 2012).
The efficacy, safety and affordability will determine a cocktail's utility in actual clinical practice. The desired characteristics for an anti-rabies mAb cocktail are summarised in Table 2.

Table 2: Summary of mAb Cocktail Desired Characteristics

Characteristic	Reasoning
Recognition of distinct, non-overlapping epitopes	1. To ensure no binding competition between individual mAbs 2. To increase breadth of reactivity 3. To mitigate escape mutants
High breadth of reactivity	1. To address natural variation between strains 2. To increase possibility of use against phylogroup I non-RABV isolates 3. To neutralise strains likely encountered in relevant geographical areas
High potency	1. To require only a small volume 2. To ensure noninferiority to RIG
Nonimmunogenic	1. To reduce the risk of adverse effects
Noninterfering with immune response to vaccine	1. To increase safety, efficacy and flexibility of PEP schedule
Affordable cost	1. To permit accessibility to those in low-resource communities

(Benedictis et al., 2016; Buthlezi et al., 2016; Hanlon et al., 2001; Nagarajan et al., 2008)

The studies of anti-rabies mAb cocktails since 1990 are summarised in Annex 1. In general, these studies suggest that anti-rabies mAb cocktails (a) can display noninferiority to RIG when they are comprised on mAbs with non-overlapping targets, (b) most potently neutralise Lyssaviruses from Phylogroup I rather than Phylogroups II and III, (c) may not negatively impact the immune response to vaccine as is suggested with RIG and (d) have similar adverse reaction rates to RIG.
However, limitations and concerns regarding anti-rabies mAb cocktails exist. First, as the rabies RNA-polymerase is not capable of genetic proofreading and repair, resistant mutants may quickly form under selective pressure. Sloan et al. palliate this concern, citing that "patients receiving PEP are not the primary source of virus to others" and that PEP failure is uncommon (2007). It is also stated that "very similar concern was raised for preventing RSV with a mAb, which like rabies is an RNA virus with different strains circulating [and] escape mutants for RSV had been generated in cell culture" (Sloan et al., 2007). Yet, an anti-RSV mAb has now replaced its counterpart polyclonal antibody, with "no reported incidences of resistant strains despite the worldwide use [since] 1998" (Sloan et al., 2007).

The cost of production of mAbs is of concern, with production costs using mammalian cell culture estimated to be 100 USD per gram (Kelly, 2009). However, the expected amount of monoclonal antibody required for rabies PEP is expected to be quite low and it is anticipated that products would be available at a lower cost than HRIG (Kelly, 2009). Many studies have emphasized the possibility of production in transgenic plants. It is suggested that plant-based systems are advantageous due to high efficiency and yield, easier processing, increased ability to carry out modifications, greater safety and lower production costs (Girard et al., 2006; Ko and Koprowski, 2005).

Lastly, the ethical implications of phase III studies of anti-rabies mAbs on exposed patients has been debated. Many countries have considered these studies unethical, considering the 100% fatality rate of rabies (Blaise and Gautret, 2015).

WHO and the Collaborating Centres for Rabies Research are currently facilitating mAb cocktail developments. Through the Collaborating Centres, a range of anti-rabies mAbs and their hybridomas have been evaluated and transferred under Material Transfer Agreement to respective manufacturers for further development (Table 3).

Table 3: Current WHO Anti-Rabies mAb Projects

Product Name	Manufacturer	Trial Stage
Rabimabs (comprising 2 mAbs: M777-16-3 and62-71-3)	Zydus Cadila Healthcare Ltd., India	Phase I/II completed Phase III to be initiated in 2017 ongoing
RabiVir (comprising 2 mAbs: E559 and 62-71-3)	Council for Scientific and industrial Research (CSIR), South Africa	Late preclinical (development on hold)
Cocktail of 3 mAbs (62-713, M777-16-3, E559.9.14)	Span Biotherapeutics, India	Early preclinical (development on hold)

According to a review of clinical trial registries (clinicalTrials.gov and WHO ICTRP) using the search term "rabies AND monoclonal" conducted in May 2017, there are several mAbs which have been or are being evaluated in clinical trials (Table 4).

Table 4: Products in Clinical Trials

Product Name	Manufacturer	Trial Stage/ Registration Number	Comment
CL184 (cocktail of 2 mAbs)	Crucell, Johnson & Johnson	Phase I (USA) completed ISRCTN18660493	Further clinical trials are not planned (communication from Crucell 2016).
		Phase I/II (India) completed ISRCTN12693237	
		Phase II (USA) completed NCT00656097	
		Phase II (India) completed NCT01228383	
		Phase II (Philippines) completed NCT00708084	

RMAb (single mAbs)	Partnership between MassBiologics and Serum Institute of India	Phase I (India) completed CTRI/2009/091/000465 Phase II/III (India) completed CTRI/2012/05/002709	Product was licensed in India in August 2016
Rabimabs (cocktail of 2 mAbs)	Zydus Cadila	Phase I/II (India) completed CTRI/2012/12/003225 CTRI/2015/06/005838	Phase III to be initiated in 2017
SYN 023 (cocktail of 2 mAbs)	Synermore Inc.	Phase II (currently recruiting) NCT02956746	

Conclusions:

The available data on the first single mAb licensed for clinical use support the concept that this single mAb is at least as safe and of comparable potency against rabies infection in category III exposed patients as standard RIG in the vast majority of naturally occurring rabies strains. The caveat is that this mAb product is known to be unable to neutralize a few naturally occurring strains of rabies virus in in vitro assays. However, the possible benefits to human health with the use of mAbs alone in place of standard RIG should be considered a great potential and valuable avenue to overcome short supply of RIG. With growing importance and as cost associated with the production of mAbs decreases it would enable the mAb's use in developing countries at a frequency higher than currently seen with standard RIG (~2% of infected individuals are provided RIG).

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*Included in Annex 1

ANNEX 1

Historical data on the safety and efficacy of mAbs

Authors	Year	mAb or Cocktail	Type	Origin	Efficacy and Safety Notes	Antigenic Site
Benedictis et al.	2016	RVC20 and RVC58	whole	human	1. *In vitro,* neutralized all 35 rabies virus strains and 25 non-rabies Lyssaviruses 2. Showed higher potency and breadth compared to antibodies currently under clinical development (CR57, CR4098, and RAB1) and commercially available RIG 3. *In vivo,* protected Syrian hamsters from a lethal rabies virus challenge and did not affect the post vaccination response 4. Suggests that the cocktail is not inferior to HRIG	I, III
Tsekoa et al.	2016	E559 and 62-71-3	whole	chimeric	1. *In vitro,* neutralized diverse rabies virus variants 2. *In vivo,* exhibited enhanced protection compared to HRIG from a post-exposure lethal rabies virus challenge in hamsters	I, II
Both et al.	2015	62-71-3	whole	chimeric	1. *In vitro,* demonstrated strong neutralization of rabies virus strains CVS11, BBLV, KLEV, and an E559 mAb escape mutant 2. Demonstrated weak neutralization of pasteur virus 3. Did not neutralize Duvenhage Virus or LBV	I
Zhuang et al.	2015	zipFv57S	scFv, dimer	human	1. Dimer showed higher binding ability and affinity than it's monomer 2. *In vitro,* dimer showed improved neutralizing activity and stability 3. *In vivo,* showed a similar protective rate from a lethal rabies virus challenge in mice	III
Duan et al.	2012	mutants of CR57: scFV57, ds-FV57, and ds-FV57-	scFV, disulfide-stabilized	human	1. All provided efficient protection against rabies virus for cells *in vitro* and mice *in vivo* 2. The stability and *in vitro* neutralizing potency of ds-FV57-VL8Ser was improved compared to the other mutants	III

		VL85Ser				
Turki et al.	2014	scFv50AD1-Fd	scFV, trivalent	human	1. Neutralized rabies virus for cells *in vitro* and for mice *in vivo* 2. Showed high neutralization activity up to 75-fold compared to the monovalent scFv form and RIG	III
van Dollerweerd et al.	2014	E559	whole	chimeric	1. E559 exhibited the broadest neutralization spectrum and greatest potency of the mAbs identified that recnogize antigenic site II 2. *In vitro,* E559 neutralized phylogroup I Lyssaviruses but not phylogroup II Lyssaviruses 3. *In vivo,* hamsters that received a lethal rabies virus challenge and no vaccine had 50% survival rates for both E559 and HRIG after 14 days. After 28 days, the survival rates were 11% for E559 and 0% for HRIG 4. Suggests that the mAb is not inferior to HRIG	II
Duan et al.	2014	mutants of CR57: scFV57	scFv, disulfide bond mismatched	human	1. Mismatched disulfide bond conferred deleterious effects on its neutralizing activity against rabies virus 2. *In vivo,* correctly matched disulfide bonds provided an additionaly 30% efficacy against a lethal challenge of rabies virus in mice	III
Gogtay et al.	2012	SII RMab	whole	human	1. Was well-tolerated with similar frequency of local injection site reactions to HRIG 2. When administered alongside vaccine *in vivo*in hamsters, the RVNA titres for the mAb and HRIG were comparable 3. *In vivo,* provided superior protection compared to ERIG and HRIG in hamsters faced with a lethal challenge of emerging Asian Lyssaviruses	not given

Papaneri et al.	2012	CL184 cocktail: CR4098 and CR57	whole	human	1. Alanine scanning used to evaluate the possibility of mutated rabies viruses escaping neutralization from mAbs 2. Neutralized all the viruses tested 3. Indicated that single amino acid exchanges in the antigenic sites do not affect the broad neutralizing capability of the cocktail	I, III
Sun et al.	2012	RV01, RV03, RV05, RV08, RV09	Fab	human	1. *In vitro,* RV03, RV05, RV08, and RV09 were shown to neutralize all three strains of rabies virus tested, but RV01 showed lower neutralization for one strain 3. *In vivo,*each of the mAbs protected hamsters from a lethal rabies virus challenge equally as well as CR57	II
Wang et al.	2012	scFv-Fc, "fusion protein"	single chain scFv fused with IgG1 Fc	chimeric	1. *In vivo,* neutralized rabies virus in mice in a comparable manner to HRIG 2. Incubating the fusion protein and virus together before inoculation resulted in a 50% survival rate compared to a 0% survival rate in the control group	not given
Wang et al.	2012	3B12 and 4A12	whole	mouse	1. Samples from supernatant and ascitic fluid for both mAbs neutralized rabies 2. Study assessed primarily diagnostic applications but considered therapeutic candidacy	not given
Zanluca et al.	2012	10 antibodies, including MAb8D11 and 68H	whole	mouse	1. 10 antibodies tested with varied results 2. *In vitro,* mAb8D11 neutralized rabies virus and mAb6H8 was reactive against all rabies virus isolates tested, including all virus strains present in human and veterinary commercial vaccines	not given
Li et al.	2011	Fab091	Fab, transferred from an scFv	human	1. *In vitro,* neutralized rabies virus sample 2. *In vivo,* mice treated with vaccine and mAb showed protection against rabies compared to the control group 3. However, survival rates for groups with vaccine and mAb were lower than that of vaccine and HRIG	not given

Wang et al.	2011	RAB1	whole	human	1. Neutralized all indentified rabies isolates 2. Two distinct mutations in the glycoprotein required for abrogation of neutralization 3. *In vivo,* hamsters were protected from a lethal rabies challenge with mAb alone and mAb and vaccine together	III
Muller et al.	2009	E559.9.14, 1112-1, 62-71-3, M727-5-1, and M777-16-3	whole	mouse	1. *In vitro,* the cocktails neutralized a broad spectrum of Lyssaviruses except for those belonging to phylogroups II and III, specifically MOKV, WCBV, and LBV 2. *In vivo,* hamsters were protected from a lethal rabies challenge by the mAb cocktail as a component of PEP in a comparable way to PEP with HRIG 3. All three novel cocktail combinations were shown to have equal efficacy to HRIG 4. The cocktail neutralizing activity was as much as 2,000 times higher than that for HRIG	II, III
Bakker et al.	2008	CL184 cocktail: CR4098 and CR57	whole	human	1. First, subjects received a single IM dose of CL184 or placebo in a double blind, randomized, dose-escalation trial 2. Second, open-label CL184 was co-administered with rabies vaccine 3. Pain at the injection site was reported by less than 40% of subjects 4. No fever, local induration, redness or swelling was observed 5. RVNA was detectable from day 1 to day 21 after a single dose 6. All subjects had adequate RVNA levels from day 14 when combined with rabies vaccine	I, III

Muhamuda et al.	2007	2C5H9, 2C5F8, 2C5E8, 2C5D10, 2C5F5, 2C5A10, 2C5D8, 2C5D8, 2C5F7, 2C5B2, and 2C5H2	whole	mouse	1. *In vivo,* 70%-100% of mice and guinea pigs were protected from a lethal rabies challenge, the percentage depending on the strain of the virus 2. *In vivo,* 100% of guinea pigs given a mAb survived a challenge with CVS and six street rabies virus strains 3. The mAbs were found to be 2,000 times more potent than commercial ERIG in terms of effective protein concentration and neutralizing titer 4. Time point experiments conducted in mice showed that the mAbs are effective up to 72 hours post infection, whereas ERIG was only effective up to 48 hours post infection	not given
Sloan et al.	2007	HuMAb 17C7	whole	human	1. *In vitro,*neutralized rabies virus variants from a broad panel of isolates of public health significance fromm Asia, Africa, Europe, and the Americas 2. HuMab17C7 neutralized all rabies virus isolates tested 3. *In vivo,*hamsters were protected from a lethal rabies challenge by HuMab17C7 as a component of PEP	III
Zhao et al.	2007	T166 and F21	scFv	human	1. The mAbs recognized a site that is highly conserved among a large panel of street rabies viruses 2. *In vitro,* T166 had high specificity and reasonable affinity against varied rabies virus strains 3. It is suggested that T166 could be a good candidate to complement RD9 in a therapeutic cocktail	III
Goudsmit et al.	2006	CL184 cocktail: CR4098 and CR57	whole	human	1. *In vitro,* the cocktail neutralized all viruses from a panel of 26 representative street virus isolates, including those of canine and bat origin 2. *In vivo,* Syrian hamsters were protected against a lethal rabies challenge by vaccine and mAb cocktail, in a comparable way to vaccine and HRIG 3. The cocktail did not interfere with the vaccine, differently than HRIG 4. The cocktail and HRIG have comparable coverage of phylogroup I	I, III

					Lyssaviruses	
Bakker et al.	2005	CL184 cocktail: CR4098 and CR57	whole	human	1. The cocktail had a high *in vitro* and *in vivo* neutralizing potency and broad neutralization spectrum 2. The components recognize nonoverlapping epitopes, so escape mutants for one antibody are neutralized by the other antibody 3. *In vitro,* exposure to the cocktail yielded no escape mutants 4. Glycoprotein sequence analysis of natural rabies virus isolates revealed that the majority of strains contain both intact epitopes and the few remaining strains contain at least one of the two epitopes	I, III
Ando et al.	2004	EP5G3 and GD2D12	Fab	human	1. *In vitro,* EP5G3 exhibited neutralizing activity against rabies virus strain CVS with a reduction in the infected cell count of 76% at a 1:2 dilution and 20% at a 1:4 dilution 2. *In vitro,* GD2D12 exhibited neutralizing activity against the same strain with a 57% reduction at 1:2 and 41% at 1:4	II, III
Marissen et al.	2004	CR57 and CRJB	whole	human	1. The competition between CR57 and CRJB, the *in vitro* escape profile, and the apparent overlap between the recognized epitopes argues against including both CR57 and CRJB in a mAb cockatail 2. CR57 escape mutants were only partially covered by CRJB 3. CRJB-resistant variants completely escaped neutralization by CR57	I
Ko et al.	2003	mAbP, plant derived	whole	human	1. *In vitro,* mAbP was as effective at neutralizing the activity of the rabies virus as the mammalian-derived antibody and HRIG 2. mAbP had a shorter half life than the mammalian mAb 3. *In vivo,* mAbP was as efficient as HRIG for PEP against a lethal rabies virus challenge in hamsters	not given

Prosniak et al.	2003	rhuMAb cocktail	whole	human	1. *In vitro,* the cocktail neutralized several fixed and street wild-type rabies viruses 2. *In vivo,* mice and hamsters faced with a lethal dose of rabies virus were protected against infection after one treatment with the cocktail 3. In the mouse models, the efficacy of the cocktail was comparable to that of HRIG	not given
Hanlon et al.	2001	JA-3.3A5, JF-2.1G11, JF-2.1H8, JF-2.1F1, JB.1	whole	human	1. *In vitro,* the mAbs neutralized representative rabies virus variants 2. Phylogroup II viruses were not neutralized by the mAbs 3. *In vivo,* hamsters faced with a lethal rabies virus challenge were protected by JB.1 in a comparable way to RIG	not given
Hanlon et al.	2001	1112	whole	human	1. *In vivo,* 70% of hamsters were protected from a lethal rabies virus challenge by mAb without vaccine in a similar way to RIG 2. Two separate purified ERIG products confered less than 10% survival when used without vaccine	not given
Dietzschold et al.	1990	mab57 (now CR57)	whole	human	1. *In vitro,* neutralized all rabies virus strains tested but did not neutralize the Duvenhage or Mokola viruses 2. *In vivo,* mice faced with a lethal rabies virus challenge were protected by pretreatment with the mAb	I
Lafon et al.	1990	HUM1, HUM2, and HUM3	whole	human	1. The mAbs reacted with the rabies glycoprotein 2. *In vitro,* rabies virus and Mokola virus were neutralized, but European Bat Lyssaviruses were not neutralized, suggesting that some common antigenicity exists between the glycoproteins of phylogroups I and III 3. The lytic activity of the mAbs was equivalent to that of HRIG	VI

Evidence Profile: Question 14

Question 14: In cases of RIG shortage and constraints, can subcategories of patients be identified who should be given highest priority for RIG administration?

Population	Category III exposed patients
Intervention	PEP without RIG administration under clearly specified circumstances
Comparator	PEP with RIG under all category III circumstances
Outcome	Patient survival; cost-effectiveness of PEP, increased affordability

Background:

The high cost, low availability and supply, batch to batch variation affecting efficacy, uncertain quality (no WHO prequalification), short shelf life and correct administration of RIG are barriers to implementing the gold standard set by WHO for PEP in category III bites. RIG is often a barrier for attaining public health impact because of a hesitation to use vaccine without RIG and therefore manufacturers and countries often do not want to make vaccines available without RIG, which often means no PEP at all. The individuals in rabies-endemic settings most often affected are those who can least access and afford RIG. Therefore, guidance on its prudent use is important for ensuring its maximal availability to the patients bearing the highest risk. In cases where there is not enough RIG to be distributed to all category III patients, a best practice statement may suggest which patients are objectively of the highest priority for RIG administration and what measures should be taken for those who do not receive RIG.

Current position and practice:

The current WHO recommendation states that ‘‘rabies immunoglobulin should be administered in all people with category III exposure and to those with category II exposure who are immunodeficient. […] Rabies immunoglobulin for passive immunization is administered only once, preferably at, or as soon as possible after, the initiation of post-exposure vaccination. […] RIG should never be administered in the same syringe or in the same anatomical site as the first vaccine dose. However, subsequent doses of vaccine in the four-dose series can be administered in the same anatomic location where the HRIG dose was administered. Beyond the seventh day after the first dose, RIG is not indicated because an active antibody response to the CCEEV is presumed to have occurred’’ (WHO, 2010). This summary focuses on individuals with category III exposure who are not known to be (seriously) immunocompromised.

New evidence:

Published evidence regarding the administration of PEP without RIG discusses the scarcity and high cost of RIG that result in low provision of RIG to patients with category III exposure. Data show the rarity of incidences of RIG administration on both global scales and specific studies, and elucidate case factors that potentially raise or lower the priority of RIG administration. This information, together with observational data from countries may be used in the generation of a best practice statement for prioritization of RIG allocation to selected patients and under clearly specified circumstances .

The scarcity and cost of RIG result in its expansive unavailability and underuse. As RIG is of low availability, clinics in canine rabies-endemic areas ‘‘wait months for orders to be filled, and some remain unfilled’’ (Wilde *et al.*, 2002), particularly on the African continent (Dodet et al.2009) . Even when RIG is available, many patients cannot afford to purchase it (Hossain *et al*, 2010, Sambo *et al.*, 2013) or it is allocated to patients who can afford it, but who not necessarily bear the highest risk.

In Cambodia, eRIG (which is consistently less expensive than its sole alternative, hRIG) costs ‘‘between US$20 and US$30 per dose [yet] a Cambodian farmer’s monthly salary is between US$60 and US$80’’ (Tarantola *et al.*, 2015). In Cameroon, one eRIG vial costs about 55$ and the monthly minimum salary amounts around the same amount. In other words, a dose of RIG can drain up to half of one’s monthly salary. Similar discrepancies between income and RIG price exist throughout Asia and Africa (Tarantola *et al.*, 2015; Tenzin *et al.*, 2012). Therefore, many individuals forego RIG purchase and are treated solely with wound cleansing and vaccine. Worse, in cases where care providers only adhere strictly to WHO recommendations, some category III patients must refuse PEP entirely, as vaccine will not be administered without RIG. More comprehensive recommendations would suggest that patients who cannot afford RIG should still be able to receive rabies vaccine.

Globally, estimates of RIG availability for category III exposed patients range from 2% to 10% of actual demand (Khawplod *et al.*, 2002; Warrell, 2012). In rabies-endemic, low-income countries RIG is estimated to be available for less than 1% of category III exposed patients (Warrell, 2012). Multiple studies document region-specific instances of the underuse of RIG (Table 1). Differences within countries, and between rural and urban areas, are also present; for instance, one study in India recorded 2.70% RIG reception in urban areas, whereas another study in India recorded 90% RIG reception in rural areas (Table 1) (Gogtay *et al*., 2011; Samanta *et al*., 2016).

Table 1: RIG Received by Category III Exposed Patients in Specific Studies

Authors, Year	Study Location	Rural / Urban	Number of Patients Total	Number of Patients Who Received RIG	Percentage of Patients Who Received RIG
Gogtay *et al.*, 2011	Mumbai, India	Urban	783	21	2.70%
Hossain *et al.*, 2010	Dhaka, Bangladesh	Urban	794	7	0.80%
Poorolojal *et al.*, 2015	Tehran, Iran	Urban	14,083	3660	25.98%
Samanta *et al.*, 2016	North / West Bengal, India	Rural	84	76	90%
Tarantola *et al.*, 2015	Cambodia	Both	6,362	591	9.30%
Uwanyiligira *et al*., 2012	Lausanne, Switzerland *	Urban	99 *	53	53.53%

**Only 54 of 90 travellers potentially exposed were seeking advice from a physician while abroad*

In addition to vaccines given on a completed WHO-recommended regimen, patients ‘‘may still require additional passive immunity […] before vaccine-generated virus-killing antibody appears in circulation,’’ as PEP failures are often attributed to lack of RIG (Deshmukh *et al.*, 2011; Wilde *et al*., 2013). Shantavasinkul and Wilde maintain that ‘‘none of the vaccine regimens can substitute for the use of RIG’’ (2011). A cost-effectiveness study in Tanzania by Shim *et al*. 2009 ‘‘indicates that

investing in supplies of RIG would be very cost-effective'' when RIG is provided with all PEP administrations of the Essen regimen. However, in light of the small percentage of patients who actually receive RIG, its prudent use and prioritization is pertinent (2011).

Evidence identifies risk factors that may raise a patient's need for RIG. These risk factors include (but are not limited to) the use of a nerve tissue vaccine[3] (instead of a recommended CCEEV), injuries to the head, neck, face, hands, or other places with a high density of peripheral nerve endings (Table 2, Cleaveland *et al.,* 2002), immunocompromised patient and a confirmed laboratory diagnosis of rabies from the animal's brain tissue (Dimaano *et al.*, 2011; Hossain *et al*., 2011; Tarantola *et al.*, 2015; Shim *et al*., 2009; Wilde *et al.*, 2013).).

A study by Khawplod *et al*. aimed to obtain an early immune response using an accelerated intradermal vaccination schedule, to decrease patients' window of need for RIG (2002). However, the serological titres of antibodies obtained from the accelerated schedule were not sufficient to discontinue the use of RIG, as RNAb levels were ''undetectable […] by day 5 in nearly all subjects'' and ''NAb titers barely approaching the 0.5 IU/mL level were seen only in 9%-10% of subjects by day 7'' (Khawplod *et al*., 2002). Significant differences were not seen between vaccination schedules in both one- and two-week periods'' (Khawplod *et al.*, 2002). The authors recommend continued use of RIG.

A study by Zhang *et al*. discusses the ''ineffectiveness of rabies vaccination alone for post-exposure protection against rabies infection'' in the animal models of beagles, golden hamsters and Kunming mice (2016). In their trials, an average of ''100% of animals survived after administration of traditional rabies vaccines and rabies immunoglobulin, 80% of animals survived with [hRIG] alone, [and] only 20-40% of animals inoculated with PCECV alone survived'' after 45 days (Zhang *et al*., 2016). This study also tested a new vaccine, PIKA-RV, which conferred a mean 80% survival rate in the experimental group (Zhang *et al*., 2016). Similar results have been shown by former studies with the use of other experimental vaccines, especially recombinant rabies virus vaccines (personal comments WHO CC).

Conversely, other data show that PEP without RIG is sufficient in most cases. Proper wound care with ''scrupulous cleaning and deep irrigation, followed by application of a potent antiseptic agent'' and timely administration of the first CCEEV dose are a key factor for increasing survival in cases which RIG is unavailable (Shantavasinkul and Wilde, 2011; Wilde *et al.*, 2002). It is also supported that the availability of highly immunogenic vaccines and vaccination schedules increase survival in cases where RIG is unavailable (Wilde *et al*., 2002).

Unpublished data from Cambodia and Tanzania on survival of category III patients exposed to either confirmed or probable rabid dogs without RIG administration and varying PEP completion rates confirm these findings:

In Cambodia (data 2003-2014) no rabies related deaths were observed in 68 and 203 patients bitten by laboratory confirmed or sick looking dogs, respectively. This despite varying PEP completion rates of 1-4 sessions of the Thai Red Cross regimen.

In Tanzania, from 2,196 persons bitten by animals which were subsequently traced and classified as clinically suspect rabid animals, 88 human rabies deaths were identified. The vast majority of these patients did not receive any PEP and none of these bite victims received RIG. Amongst the bite victims that started PEP promptly, only one death occurred and that was the result of the patient, who was bitten on the head, receiving only the first dose of vaccine (5-dose Essen or Tanzanian 3-visit IM regimen (d 0, 7, 21) and no RIG. Among the patients that had delayed PEP, 4 deaths occurred out of 261 patients who presented 1 day late, 5 deaths occurred out of 319 patients that presented

[3] NTV *still in use for humans in Bolivia, Algeria, Argentina and Ethiopia (plans to phase out)*

3 or more days late, and 1 death occurred out of 130 patients that started PEP >7 days late. Plausibly all of the deaths that occurred could have been avoided had PEP been initiated promptly and completed by patients. Notably these data also highlight that most patients do not seek or obtain PEP promptly – 19% of rabid bite victims fail to obtain PEP, 36% of bite victims obtain PEP at least one day late and 9% obtain PEP 1 week late. From an additional 10,700 patients who attended clinics in Southern Tanzania (of whom we expect ~30% to be due to rabid animals), 147 were treated with RIG. Forty-seven deaths occurred in patients that did not receive either RIG or vaccine - there was no evidence for PEP failures.

Table 2 Transmission risk of rabies (Cleaveland et al. 2002)

Area of bite	Transmission risk
Head/Neck	30-60%
Arm	15-40%
Hand	15-40%
Finger	15-40%
Genitalia	15-40%
Trunk	0-10%
Leg	0-10%
Foot	0-10%

New Evidence from Experimental Studies (still in the animal model):

Morimoto *et al.* investigated local vaccination in hamsters as a possible alternative to RIG (2016). The results suggest that ‘‘vaccine injection at the wound site in the same manner as administration of RIG provided protective efficacy that was not inferior to [the] combination of vaccination and RIG. [Yet], further study is needed to determine whether it can replace the use of RIG’’ (Morimoto *et al*., 2016). The data show that vaccination at the site of infection had lower mortality than the administration of the current PEP regimen (vaccination and RIG) and of a contralateral leg vaccination (Morimoto *et al*., 2016). Indeed, it was the most efficacious regimen in the trial (Table 3).

Table 3: Results from Different Vaccination Regimens in Hamster Model (Morimoto *et al*., 2016).

Regimen Number	Regimen Description	Percent Survival After 30 Days
1	Current PEP (Vaccination + RIG)	70%
2	Contralateral Leg Vaccination	53%
3	Infection Site Vaccination	93%
4	Control	27%

The authors explain that ‘‘vaccine injected in the vicinity of [the] RABV exposure site may trigger the local innate immunity more strongly, thus reducing the viral load and replication rate and retarding viral entry to nerve endings’’ (Morimoto *et al.*, 2016). However, this paper has many limitations, may not be directly compared to infection site vaccination in humans, and these data contradict

experiences from former studies. First, as there was a 27% survival rate in the control group, the immunological challenge conferred appears to be low. Second, ‘‘although the idea of taking advantage of the innate immune [response] at the site of exposure [may be] worthwhile to explore, [there] are better ways to trigger an innate response than a rabies vaccine’’. For example, in most rabies exposures, the bite wounds are additionally inoculated with immunogenic bacteria. Furthermore, components of the innate response, such as interferons, in turn can inhibit activation of the adaptive immune system..

If local vaccination is explored further, it will be important to mitigate confusion that may occur with the current PEP recommendations, as vaccine and RIG administered at the same site can result in immunological interference. The distinctions between these regimens must be reinforced if research on site-specific vaccination is continued.

Conclusion:

There is need for more systematic evidence generation on this subject. Available publications to date do not converge and are not 100% consistent on priority allocation of RIG in low-resource or shortage settings.

In practice, prioritization is happening due to shortage, cost, age, severity of exposure, etc. and clinicians are confronted daily on how to allocate scare RIG to patients at highest risk. An algorithm for more prudent and equitable use of RIG is therefore needed to support clinical management of bite patients potentially exposed to rabies. A decision support for clinicians for most appropriate use of biologicals and patient care, would also ease ethical and logistical challenges.

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ANNEX 1

Access to rabies post-exposure prophylaxis (field data)

Background

The WHO-recommended PEP protocol includes immediate wound washing, prompt administration of rabies vaccine, and for severe categories of exposure, infiltration of purified rabies immunoglobulin (RIG) in and around the wound (WHO, 2010). RIG is rarely administered in low-income countries, as it is usually in short supply (see the following examples The Asian Rabies Expert Bureau, 2006; Mallewa et al., 2007; Hampson et al., 2008; Ly et al., 2009)) and often not affordable for bite victims, with the cost ranging from USD$25 to over USD$200 depending on whether it is of equine or human origin. Therefore, it is usually only post-exposure vaccination (without RIG) that is administered to protect a bite victim from succumbing to rabies (Hampson et al., 2008). Rabies vaccine shortages are common in low-income countries and due to limited availability bite victims often travel long distances to obtain vaccine. Thus, patients often incur substantial costs and face dangerous delays in securing PEP and avoidable human rabies deaths occur as a direct result of poor access to affordable PEP (Mallewa et al., 2007; Hampson et al., 2008; Ly et al., 2009; Sambo et al., 2013). To understand how delays in post-exposure vaccination, incomplete post-exposure prophylaxis including a lack of RIG contribute to rabies deaths we examined data on the outcomes of persons bitten by clinically suspect rabid dogs.

Methods

Data were compiled on the outcomes of persons bitten by rabid dogs in Tanzania and according to whether they received PEP, how many doses and whether PEP was started promptly or after some delay. Additional data were compiled from health facilities in Southern Tanzania where rabies PEP access was improved.

Results

From 2,196 persons bitten by animals which were subsequently traced and classified as clinically suspect rabid animals we identified 88 human rabies deaths. The vast majority of these patients did not receive any PEP and none of these bite victims received RIG. Amongst the bite victims that started PEP promptly, only one death occurred and that was the result of the patient, who was bitten on the head, receiving only the first dose of vaccine. No further doses were obtained due to a lack of funds (Table 1). Among the patients that had delayed PEP, 4 deaths occurred out of 261 patients who presented 1 day late, 5 deaths occurred out of 319 patients that presented 3 or more days late, and 1 death occurred out of 130 patients that started PEP >7 days late (Table 2). Plausibly all of the deaths that occurred could have been avoided had PEP been initiated promptly and completed by patients. Notably these data also highlight that most patients do not seek or obtain PEP promptly – 19% of rabid bite victims fail to obtain PEP, 36% of bite victims obtain PEP at least one day late and 9% obtain PEP 1 week late.

From an additional 10,700 patients who attended clinics in Southern Tanzania (of whom we expect ~30% to be due to rabid animals), 147 were treated with RIG. Forty-seven deaths occurred in patients that did not receive either RIG or vaccine - there was no evidence for PEP failures.

These data sets providing a starting point for estimating how reduced compliance and delays in initiating PEP reduce the effectiveness of PEP in preventing rabies. In this setting, over 90% of patients had to pay for vaccine, typically costing around $50 to complete a course. Costs of travel

and or accommodation are also high and in the majority of cases included costs for an accompanying family member.

Table 1. Probability of developing rabies following a bite by a rabid dog and according to the number of PEP doses received for patients who started PEP was started promptly (same day as bite)

Number of doses:	0 doses	1 dose only	2 doses only	3 doses only	4 or 5 doses
P(death)	0.13	0.03	0.00	0.00	0.00
Suspect bites	682	40	23	114	217
Rabies deaths	88	1	0	0	0

Table 2. Probability of developing rabies following a bite by a rabid dog and according to the delay in starting PEP (vaccination only, no patients received PEP). It should be noted that the person who died after starting PEP on day 0 only completed 1 dose overall and was bitten on the head.

Late PEP	PEP started day 0	Day 1	Day 2	Day 3+	Day7+
P(death)	0.00	0.02	0.00	0.02	0.01
Suspect bites	425	261	144	319	130
Rabies deaths	1	4	0	5	1

Conclusions

Rabies post-exposure vaccination is essential for preventing this fatal disease but can be out of the financial reach of many bite victims. Incidence in resource poor countries is directly affected by the inability of bite victims to obtain PEP and obtain it promptly. Reducing the cost of PEP and preventing administration delays is therefore particularly important in resource-limited settings. Lowering the cost of PEP regimens, ideally making PEP entirely free of charge, as well as using regimens that require less hospital visits could improve patient health-seeking behaviour and prevent human deaths from rabies. These data also show that in the vast majority of cases late or incomplete PEP still provides very high levels of protection against rabies even in the absence of RIG. The vast majority of human rabies deaths are a direct result of bite patients not obtaining PEP at all. Given the poor accessibility and high cost of PEP in this setting, it is recommended that improvements in vaccine access should be prioritized to save lives most effectively and cost-effectively. Many more patients could be treated with the same volume of vaccine if ID versus IM vaccination was used in Northern Tanzania. This could plausibly prevent vaccine shortages in this area and prevent future deaths from rabies.

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GRADE Table: Question 14

Question 14: PEP without RIG compared to PEP with RIG for category III rabies exposed patients under clearly specified circumstances for risk-based priority allocation

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	PEP without RIG	PEP with RIG	Relative (95% CI)	Absolute (95% CI)		
Dimaano et al. 2011												
1	observational studies	not serious	not serious	serious [a]	not serious	none			not estimable		⊕◯◯◯ VERY LOW	
Gogtay et al. 2014												
1	observational studies	serious [b]	not serious	serious [a]	not serious	none			not estimable		⊕◯◯◯ VERY LOW	
Hossain et al. 2011												
1	observational studies	not serious	not serious	serious [a]	not serious	none			not estimable		⊕◯◯◯ VERY LOW	
Morimoto et al. 2016												
1	randomised trials	not serious	not serious	very serious [c]	serious [d]	none			not estimable		⊕◯◯◯ VERY LOW	
Poorolajal et al. 2015												
1	observational studies	not serious	not serious	serious [a]	not serious	none		-	-	-	⊕◯◯◯ VERY LOW	
Samanta et al. 2016												

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	PEP without RIG	PEP with RIG	Relative (95% CI)	Absolute (95% CI)		
1	observational studies	not serious	not serious	serious [a]	serious [d]	none			not estimable		⨁◯◯◯ VERY LOW	
Shantavasinkul et al. 2011												
1	randomised trials	not serious	not serious	serious [a]	serious [d]	none			not estimable		⨁⨁◯◯ LOW	
Shim et al. 2009												
1	observational studies	not serious	not serious	serious [a]	not serious	none			not estimable		⨁◯◯◯ VERY LOW	
Tarantola et al. 2015												
1	observational studies	not serious	not serious	serious [a]	not serious	none			not estimable		⨁◯◯◯ VERY LOW	
Uwanyiligira et al. 2012												
1	observational studies	not serious	serious [e]	serious [a]	serious [d]	none			not estimable		⨁◯◯◯ VERY LOW	
Wilde et al. 2002												
1	observational studies	not serious	not serious	not serious	serious [d]	none			not estimable		⨁◯◯◯ VERY LOW	
Wilde et al. 2013												

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	PEP without RIG	PEP with RIG	Relative (95% CI)	Absolute (95% CI)		
1	observational studies	not serious	not serious	not serious	serious [d]	none			not estimable		⨁◯◯◯ VERY LOW	

CI: Confidence interval
a. indirect comparison to RIG requirement
b. did not control for confounding

Evidence Profile: Intradermal Vaccine Potency

Question: Currently required potency of cell culture and embryonated egg-based rabies vaccines is above 2.5 IU per dose, does this need review based on the current practice of vaccination?

Background:

Intradermal (ID) regimens for rabies vaccines have been recommended by WHO since 1992 (WHO Expert Committee on Rabies 1992). While intramuscular (IM) administration requires an entire vial, ID administration requires a fraction. This decreased volume allows for the sharing of vials of vaccine between patients, which results in increased vaccine availability and lower vaccination cost. The ID post-exposure prophylaxis (PEP) regimen has been reported to allow for a reduction in vaccination costs of 60 to 80% when compared to an IM schedule with the same vaccine (see for instance Tarantola 2015 or Salahuddin 2016). Increased availability and affordability are particular advantages for many rabies-endemic areas and these factors have allowed for the discontinuation of nerve tissue vaccines in many places (Chutivongse 1990, Chowdury 2015).

The WHO rabies vaccine position paper (Rabies vaccines: WHO position paper 2010) recommends a potency ≥ 2.5 IU per IM dose. No WHO potency recommendation exists per ID dose. However, an additional WHO recommendation sets the volume of ID injection to 0.1 mL, thereby constraining the potency per ID dose.

The need to define a minimum potency per ID dose, in addition to the recommended volume, has been a topic for discussion among experts in the past years. Such discussion was in part prompted by the observation that the 0.5 mL Purified Vero Cell Vaccine (PVRV) vaccine vials provide approximately 5 intradermal doses with potency per dose ≥ 0.50 IU, whereas other vaccines supplied in 1.0 mL vials provide around 10 intradermal doses with potency ≥ 0.25 IU.

WHO also recommends new vaccines to have shown their immunogenicity in humans using WHO recommended ID regimens (WHO Expert Consultation on Rabies, 2004). Concerns have been raised following the observation that virtually all such clinical trials using ID route were conducted using vaccines with potency much higher than the 2.5 IU per IM dose threshold.

Some countries in Asia have implemented their own national potency standards for ID doses (Dodet 2011). In The Philippines it is recommended that rabies vaccines must contain at least 0.5 IU per ID dose; in Thailand and Sri Lanka, the minimum antigen content per ID dose is 0.7 IU.

The present document reviews the available evidence to determine the immunogenicity and effectiveness of current vaccines administered in lower volume by ID route. Would any data suggest situations of suboptimal performance of such vaccines, then a review of the current WHO recommendation will be indicated.

PART 1: EVIDENCE REVIEW

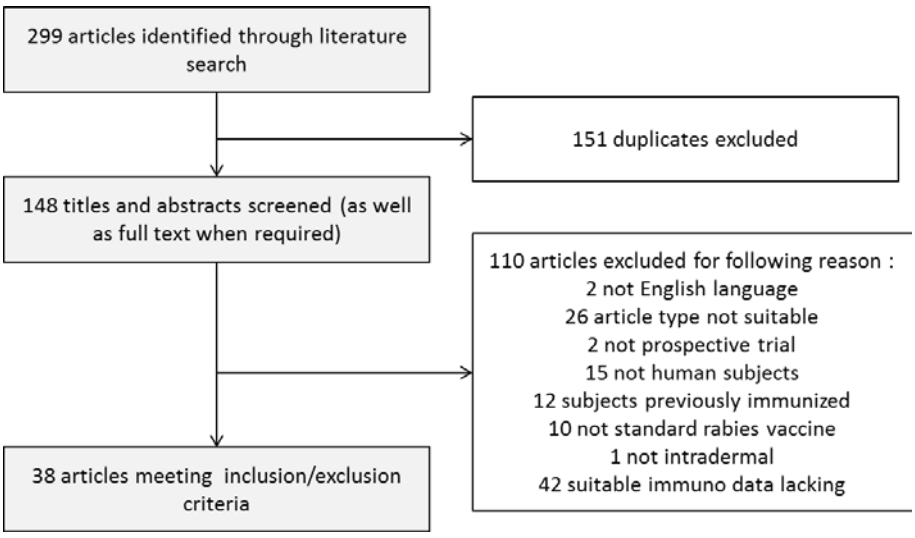
Search strategy

A. Immunogenicity and vaccine potency

The antibody response following vaccination by ID route was studied by Sudarshan and colleagues in a meta-analysis of immunogenicity studies (Sudarshan 2010). The authors concluded that increasing vaccine potency above 5 IU per IM dose did not affect the antibody response. The objective of the present review was to extend this analysis using a more systematic identification of available data and covering publications from 2007 that were not included in the 2010 meta-analysis.

As searches using the term "potency" appeared too restrictive, the less specific search string [rabies AND vaccine AND [intradermal OR ID] AND [trial OR study]] in article Title, Abstract or Keywords was preferred. The search was conducted in PubMed, Scopus, Cochrane and Science Direct databases on 6 and 7 February 2017. It was limited to papers published during the period from 1997 to 2017. A total of 299 articles were identified. Selection was subsequently performed as indicated in Figure 1.

Figure 1. Flow diagram of first literature search strategy (immunogenicity)



Titles and abstracts were reviewed for relevance. When required, the full text of the publication was also screened. Inclusion criteria for this systematic review were as follows :

Type of studies :

Prospective trials, irrespective of blinding and randomization

Types of participants :

- Human subjects irrespective of their age
- Subjects not previously immunized against rabies
- Healthy subjects as well as patients exposed to animal bite and/or subjects with underlying medical condition

Types of intervention :

- Standard rabies vaccine (inactivated)
- Vaccination by intradermal route

Types of outcome :

Immunogenicity as measured by Rabies Virus Neutralizing Antibody (RVNA) titers, in the time frame of 7 to 90 days following the first vaccine injection.

We excluded publications that were not written in English language, as well as publications such as case reports, conference reports and review papers, as they did not provide the expected data. From the total 299 search hits, only 38 publications meeting these criteria were retained.

Table 1. List of selected publications and short description (immunogenicity)

1	Ambrozaitis 2006	Immunogenicity of a 4-site ID PEP schedule (4-0-2-0-1-1) in healthy adult subjects
2	Ashwath Narayana 2014	Evaluation of an Indian vaccine administered to healthy adult subjects according to an ID PEP schedule
3	Beran 2005	Immunogenicity of vaccine dilutions in healthy adult subjects receiving simulated ID PEP
4	Bose 2016	Comparison of ID vs. IM PEP schedules in subjects 5-77 years of age with cat. II or III animal bite/exposure
5	Briggs 2000	Immunogenicity of ID and IM PEP in patients from 2 years of age with cat. II/III animal bites/exposures
6	Cunha 2010 Jun;44(3):548-54.	Immunogenicity of ID and IM PrEP schedules in healthy adult subjects
7	Jaijaroensup 1999	Immunogenicity of ID or IM PrEP in healthy students and evaluation of anamnestic responses after subsequent simulated PEP
8	Khawplod 2002a	Comparison of different ID or IM PEP schedules in healthy young adults
9	Khawplod 2002b	Immunogenicity of ID or IM PrEP in healthy subjects and evaluation of anamnestic responses after subsequent simulated PEP
10	Khawplod 2002c	Immunogenicity of ID PEP in healthy subjects using vaccine immediately after reconstitution or refrigerated storage
11	Khawplod 2006	Immunogenicity of ID PEP schedules with or without day 90 injection in patients with cat. III animal bite/exposure
12	Khawplod 2012	Comparison of different ID or IM PrEP schedules in healthy adults
13	Kulkarni 2013	Evaluation of an Indian vaccine administered to healthy adult subjects according to ID or IM PrEP schedule
14	Lang 1999	Immunogenicity of ID and IM PrEP schedules in healthy infants
15	Laurent 2010	Immunogenicity of a rabies vaccine administered with an ID device of varying needle length in healthy adult subjects according to PrEP schedule
16	Madhusudana 2001	Comparison of 2 ID PEP schedules in adult subjects with cat. I animal bite/exposure
17	Madhusudana 2002	Evaluation of ID PEP with or without RIG in patients with cat. III animal bite/exposure
18	Madhusudana 2004	Immunogenicity of ID PEP in healthy adult subjects
19	Madhusudana 2006	Immunogenicity of ID PEP in patients with cat. III animal bite/exposure
20	Magpantay 2010	Immunogenicity of an Indian vaccine administered to healthy subjects 5 to 50 years of age according to ID PEP or PrEP schedule
21	Miranda 2014	Immunogenicity of ID PEP or PrEP in healthy subjects 7 to 65 years of age
22	Masthi 2014	Immunogenicity of ID PrEP in schoolchildren and other subjects, in the context of a more global project for the prevention and control of rabies in a rural area (one health concept)
23	Narayana 2015	Immunogenicity of 2 rabies vaccines administered by 4-4-4-0-0 ID PEP schedule to adult subjects with cat. II or III animal bite/exposure (phase III trial)
24	Pengsaa 2009	Immunogenicity of ID or IM PrEP in healthy children 12 to 18 months of age and evaluation of anamnestic responses after subsequent simulated PEP by the same route. Co-administration with Japanese Encephalitis vaccine
25	Quiambao 2005	Evaluation of ID PEP in patients 5 to 60 years of age with cat. III animal bite/exposure confirmed as rabid
26	Ravish 2014	Immunogenicity of ID PEP in adult subjects with cat. II/III animal

		bites/exposures
27	Sampath 2010	Evaluation of the immunogenicity of Indirab in 3 different studies : IM PrEP in healthy subjects, IM PEP in patients aged 5 to 55 years, and ID PEP in healthy subjects
28	Saraya 2010	Evaluation of antibody and plasma cytokine responses in patients 18 to 25 years of age with cat. II animal bite/exposure
29	Shantavasinkul 2010	Immunogenicity of a 4-site ID PEP schedule in healthy adult subjects with or withour RIG, compared to standard ID PEP in patients with cat. III animal bite/exposure
30	Shiota 2008	Evaluation of a Japanese vaccine administered to young healthy adult subjects according to an ID PrEP schedule
31	Sirikwin 2009	Immunogenicity of an 8-site ID PEP schedule in HIV patients
32	Sudarshan 2005	Comparison of ID vs. IM PEP schedule in healthy adults, with 1 year follow-up
33	Sudarshan 2012	Immunogenicity of 2 rabies vaccines administered by 4-4-4-0-0 ID PEP schedule to healthy adult subjects
34	Tantawichien 2001	Immunogenicity of a double dose ID PEP schedule in HIV patients
35	Tantawichien 2014	Evaluation of different vaccines administered to healthy adult subjects according to ID or IM PrEP or ID PEP schedule
36	Warrell 2008	Comparison of different ID or IM PEP schedules in healthy adults
37	Wongsaroj 2013	Evaluation of a 2-dose ID PrEP regimen in healthy young adults
38	Yanagisawa 2012	Evaluation of a Japanese vaccine administered to healthy subjects according to a PrEP schedule

The following data were then extracted from the publications in order to build the tables supporting our analyses :

- Type of study
- Information on the vaccine : tradename (or manufacturer), volume, potency
- Type of vaccination : route and schedule
- Information on the subjects : number, age, healthy subjects or patients, possibly using anti-malarial drugs or not, receiving rabies immunoglobulin (RIG) or not
- Immunogenicity data : neutralizing antibody titer on days 7, 14, 28 and 90; percentage subjects with titer ≥ 0.5 IU/ml on days 7 and 14

The quality of the 38 publications was also assessed as presented in Table 2 below.

Table 2. GRADE Table (immunogenicity)

Quality assessment							Summary
No. of studies	**Design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	
3	single-blind randomised	serious*	not serious**	no	not serious†	-	⊕⊕⊕◯ MODERATE
22	open randomised	serious*	no	no	not serious‡	-	⊕⊕⊕◯ MODERATE
13	prospective	serious*	no	no	serious††	-	⊕⊕◯◯ LOW

* study protocol not available; detailed list of inclusion/exclusion criteria rarely available; variable amount of information disclosed in the publications; data frequently not analyzed per age group, while elderly subjects for instance are known to mount lower immune responses than younger subjects

** RVNA titers observed in the 2008 paper by Warrell much higher than seen in other studies; observation remaining unexplained

† RVNA titers expressed in IU/mL (international serum standard available); narrow limits of the 95% confidence interval around RVNA geometric mean titers observed and/or number of subjects per group >75

‡ RVNA titers expressed in IU/mL (international serum standard available); narrow limits of the 95% confidence interval around RVNA geometric mean titers and/or number of subjects per group >75 for 16 /22 studies, while 4 studies had a size per group <30 subjects

†† RVNA titers expressed in IU/mL (international serum standard available); mouse neutralization test used in 2 of the studies to measure RVNA titers (RFFIT for all other studies); narrow limits of the 95% confidence interval around RVNA geometric mean titers for 4/13 studies; number of subjects per group <30 for 6 studies;)

B. Recent observations in terms of efficacy / effectiveness

Rabies vaccine efficacy can be assessed by investigating survival after exposure. This is ideally done by looking at survival after initiation of proper PEP following category II or III exposure to a laboratory-confirmed rabid animal (Rabies vaccines: World Health Organization position paper 2010). Such survival data analyzed after administration of Rabipur to rabies-exposed patients were reviewed by Giesen (2015). The review included data from 380 subjects who received PEP by ID route, including 300 cases of exposure to a proven rabid animal, and reported 100% survival rate. A similar review that analyzed survival after administration of Verorab (Toovey 2007) identified another group of 40 subjects with severe bites inflicted by confirmed rabid animals, who received ID PEP and were still alive 3 years later.

As both reviews included publications issued up to 2006, the present literature search was performed on February 9 2017 to identify data published from 2007 and thereby analyze more recent trends. The search string [rabies AND vaccine AND [intradermal OR ID] AND [exposure OR bite]] in article Title, Abstract or Keywords was applied to the period from 2007. The search was conducted in PubMed, Scopus, and Cochrane databases on February 9, 2017. In addition, the search string [rabies AND [post-exposure OR [post AND exposure] AND prophylaxis AND patient AND immunoglobulin] was applied to capture additional publications that would not use the term vaccine in Title, Abstract and/or Keywords. A total of 227 search hits was obtained. Inclusion criteria for this systematic review were then applied as follows:

Type of studies:

Prospective trials as well as observational studies

Types of participants:

- Human subjects irrespective of their age, previously vaccinated or not
- Subjects with category II or III exposure to a suspected or confirmed rabid animal

Types of intervention :

- Standard rabies vaccine (inactivated)
- Vaccination by intradermal route, irrespective of vaccination regimen

Types of outcome :

Survival

We excluded publications that were not written in English language, as well as publications such as case reports, conference reports and review papers. 84 duplicate papers were excluded as well as 132 publications not meeting the inclusion/exclusion criteria. This process resulted in the selection of 11 papers.

Table 3. List of selected publications and short description (efficacy)

1	Salahuddin 2016	Study describing experience with ID vaccination in a hospital in Karachi, Pakistan
2	Barthi 2016	Study in cat. III patients, evaluating local infiltration of RIG into the wound without any systemic administration
3	Tarantola 2015	Analysis of rabies management data from databases in Cambodia
4	Narayana 2015	Safety immunogenicity study of ID PEP in subjects exposed to suspect rabid animal,

		with one-year follow-up
5	Ravish 2014	Safety immunogenicity study of ID PEP in subjects exposed to suspect rabid animal, with one-year follow-up
6	Ravish 2014b	Safety immunogenicity study of UD PEP in subjects exposed to suspect rabid animal, with 6-month follow-up
7	Satapathy 2011	Evaluation of ID PEP in patients with category III animal bites
8	Behera 2011	Evaluation of the safety of RIG and ID PEP in children <15 years of age with category III exposure to animal bite
9	Shantavasinkul 2010b	Retrospective study involving previously vaccinated patients who had proven or possible exposure to rabies virus and were given a single-visit, 4-site ID PEP vaccination
10	Quiambao 2009	One-year surveillance study in subjects with exposure to a confirmed rabid animal and who received PEP
11	Quiambao 2008	Follow-up study for patients having received pERIG at the time of a potential rabies exposure

The following data were then extracted from these publications in order to build tables:

- Type of study
- Information on the vaccine: tradename (or manufacturer), volume, potency
- Type of vaccination: route and schedule
- Information on the subjects: number of subjects vaccinated, number of cat. III exposure and number of subjects with exposure to confirmed rabid animal, country
- Survival: duration of follow-up and percentage of survivors

The quality of the 11 publications was also assessed as presented in Table 4 below.

Table 4. GRADE Table (efficacy)

Quality assessment							Summary
No. of studies	**Design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	
2	randomized	non serious	no	no	non serious	-	⊕⊕⊕◯ MODERATE
4	prospective	non serious	no	no	non serious	-	⊕⊕⊕◯ MODERATE
5	observational	serious	no	no	serious	-	⊕⊕◯◯ LOW

Studies on rabies vaccine efficacy are based on observations of survival in vaccinated subjects as obviously, placebo-controlled studies on this fatal disease are ethically unacceptable. Normally, uncontrolled efficacy studies achieve only very low quality of evidence, but this quality assessment was based on the assumption that survival data are acceptable for evaluating rabies vaccine efficacy.

Evidence overview

A. Immunogenicity according to vaccine potency

What is the potency of current rabies vaccines and what is their immunogenicity when used in an ID regimen (0.1ml per injection)?

The present review included 38 studies that assessed the immunogenicity of rabies vaccines administered by ID route over the past 20 years. The potency of vaccines used in these trials ranged from 0.03 to 2.32 IU per ID dose, when reported. A first analysis focused on the percentage subjects reaching the RVNA threshold of 0.5 IU/mL on day 14. Such study outcome was disclosed for 53 study

groups, corresponding to a total of 2349 subjects. This population consisted of subjects from 2 years of age, healthy volunteers as well as patients with category II or III animal bite/exposure and HIV patients. In this series, a PEP vaccination schedule was most often studied.

As illustrated in Table 5, when considering the RVNA threshold of 0.5 IU/mL, virtually all vaccines with a potency per ID dose of at least 0.25 IU were found to induce adequate levels of immune response by day 14 after first vaccination. In the 2014 study of Miranda, 15/56 healthy subjects did not reach the 0.5 IU threshold. In this pre-exposure prophylaxis (PrEP) trial however, by day 14, subjects had only received a single ID dose of the vaccine. The other exception was for one subject in the report by Quiambao (2005), who presented with a neutralization titer of 0.4 IU/mL after initiation of ID PEP. This subject had been bitten by a proven rabid dog but found to survive. Finally, in the group of subjects that received PrEP in the 2001 Tantawichien trial, 3 HIV patients with lymphocyte count < 200 cells per µL had an antibody titer lower than the 0.5 IU threshold following PEP with a 4-4-4-0-2-2 regimen.

Table 5. Percentage subjects reaching the 0.5 IU/mL neutralizing antibody threshold on day 14 following ID vaccination

N subjects	Type of subjects	RIG	age	vaccine tradename	potency per ID dose	schedule	% subjects ≥ 0.5 IU/mL titer day 14	source
27	cat. II animal bites/exposures	NO	5-77 yrs	Rabivax-S	0.496 or 0.424 IU	2-2-2-0-2	100	Bose 2016
27	cat. III animal bites/exposures	HRIG	5-77 yrs	Rabivax-S	0.496 or 0.424 IU	2-2-2-0-2	100	Bose 2016
45	cat. II/III animal bites/exposures	ERIG for cat. III	18-54 yrs	Rabipur	0.69 or 0.75 IU	4-4-4-0-0	100	Naranaya 2015
44	cat. II/III animal bites/exposures	ERIG for cat. III	18-54 yrs	Verorab	1.4 IU	4-4-4-0-0	100	Naranaya 2015
43	cat. II/III animal bites/exposures	ERIG for cat. III	18-55 yrs	Vaxirab-N	0.704 IU	2-2-2-0-2	100	Ravish 2014
43	cat. II/III animal bites/exposures	ERIG for cat. III	18-55 yrs	Rabipur	≥ 0.25 IU	2-2-2-0-2	100	Ravish 2014
35	healthy	ERIG	19-60 yrs	Speeda	0.64 IU	2-2-2-0-2	100	Tantawichien 2014
33	healthy	ERIG	19-60 yrs	TRCS Speeda	0.64 IU	2-2-2-0-2	100	Tantawichien 2014
31	healthy	ERIG	19-60 yrs	Verorab	1.06 IU	2-2-2-0-2	100	Tantawichien 2014
36	healthy	NO	18-55 yrs	Vaxirab-N	0.704 IU	2-2-2-0-2	100	Ashwath Naranaya 2014
56	healthy	NO	7-65 yrs	Speeda	0.5 IU	1-0-1-0-1	73	Miranda 2014
56	healthy	NO	7-65 yrs	Speeda	0.5 IU	2-2-2-0-2	100	Miranda 2014
62	healthy	NO	7-65 yrs	Rabipur	≥ 0.25 IU	2-2-2-0-2	100	Miranda 2014
38	healthy	NO	adults	Rabipur	≥ 0.25 IU	4-4-4-0-0	100	Sudarshan 2012
40	healthy	NO	adults	Verorab	≥ 0.5 IU	4-4-4-0-0	100	Sudarshan 2012
10	cat. II animal bites/exposures	NO	N.R.	N.R. Chiron PCEC	1.023 IU	2-2-2-0-2	100	Saraya 2010
45	healthy	NO	19-58 yrs	Verorab	0.96 IU	4-4-4-0-0	100	Shantavasinkul 2010
45	healthy	ERIG IM	21-57 yrs	Verorab	0.96 IU	4-4-4-0-0	100	Shantavasinkul 2010
41	cat. III animal bites	ERIG	19-54 yrs	Verorab	0.96 IU	2-2-2-0-1-1	100	Shantavasinkul 2010
73	healthy	NO	5-50 yrs	Abhayrab	1.11 IU	2-0-2-0-2	100	Magpantay 2010
76	healthy	NO	5-50 yrs	Abhayrab	1.11 IU	2-2-2-0-2	100	Magpantay 2010
10	healthy	NO	18-50 yrs	Verorab	≥ 0.5 IU	1-0-1-0-1	100	Laurent 2010
10	healthy	NO	18-50 yrs	Verorab	≥ 0.5 IU	1-0-1-0-1	100	Laurent 2010
10	healthy	NO	18-50 yrs	Verorab	≥ 0.5 IU	1-0-1-0-1	100	Laurent 2010
10	healthy	NO	18-50 yrs	Verorab	≥ 0.5 IU	1-0-1-0-1	100	Laurent 2010
9	HIV with CD4 count ≤200 cells/µl	NO	21-55 yrs	Rabipur	0.905 IU	8-8-8-8-8	100	Sirikwin 2009
18	HIV with CD4 count >200 cells/µl	NO	21-55 yrs	Rabipur	0.905 IU	8-8-8-8-8	100	Sirikwin 2009
20	healthy	NO	21-33 yrs	N.R. (PCEC Kaketsuken)	≥ 0.25 IU	2-0-2-0-2	100	Shiota 2008
55	healthy	NO	18-50 yrs	Verorab	1.06 or 1.68 IU	4-0-2-0-1-1	100	Warrell 2008

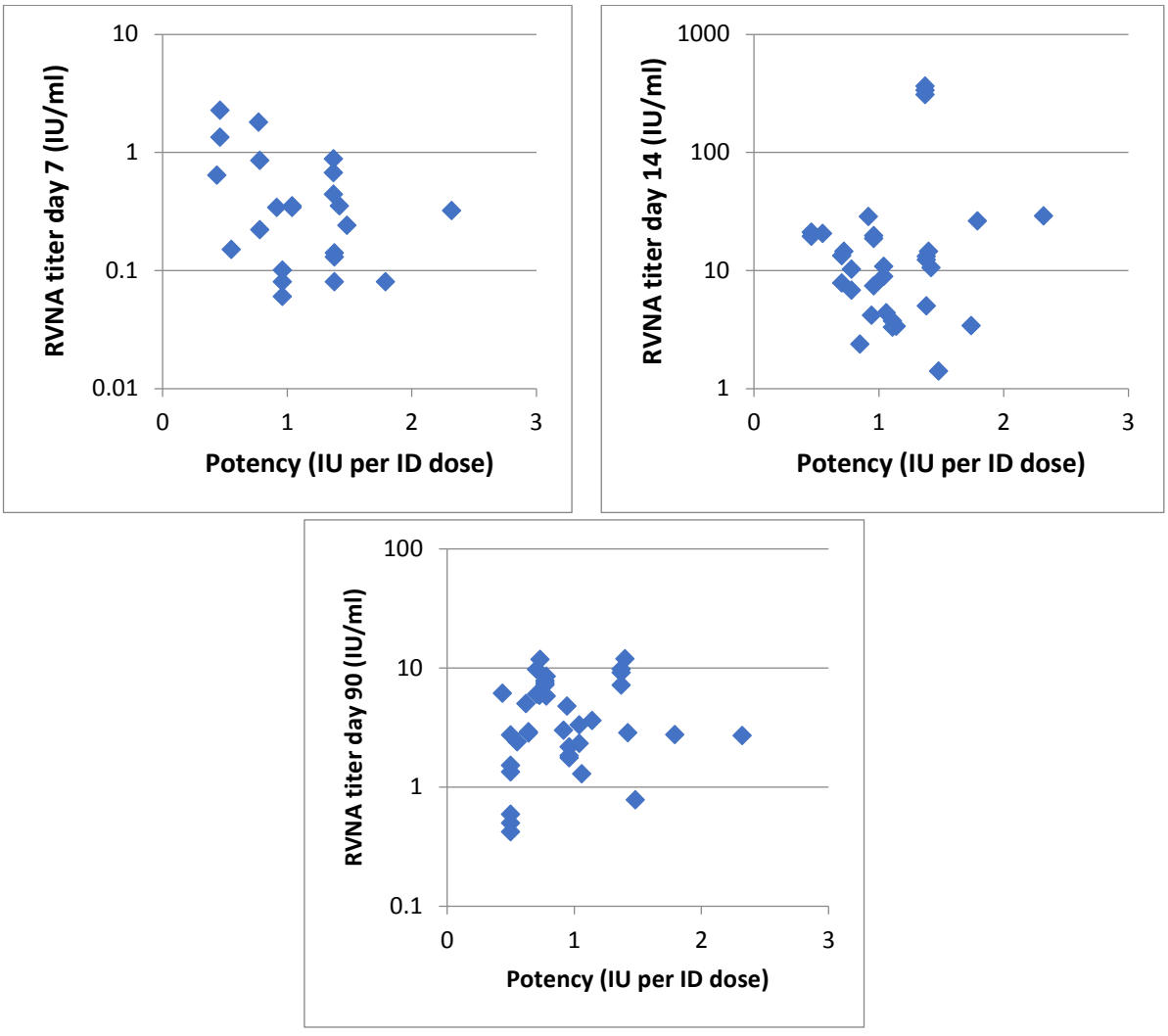
N subjects	Type of subjects	RIG	age	vaccine tradename	potency per ID dose	schedule	% subjects ≥ 0.5 IU/mL titer day 14	source
60	healthy	NO	18-50 yrs	Verorab	1.06 or 1.68 IU	8-0-4-0-1-1	100	Warrell 2008
58	healthy	NO	18-50 yrs	Verorab	1.06 or 1.68 IU	2-2-2-0-1-1	100	Warrell 2008
55	cat. III animal bites	ERIG	5-60 yrs	Rabipur	N.R.	2-2-2-0-1-1	100	Madhudusana 2006
50	cat. III animal bites	ERIG	5-60 yrs	Verorab	N.R.	2-2-2-0-1-1	100	Madhudusana 2006
86	healthy	NO	18-60 yrs	Rabipur	0.55 IU	4-0-2-0-1-1	100	Ambrozaitis 2006
87	healthy	NO	18-60 yrs	Verorab	1.79 IU	4-0-2-0-1-1	100	Ambrozaitis 2006
105	cat. III animal bites	HRIG	N.R.	N.R. Aventis CPRV	1.04 IU	2-2-2-0-1-1	100	Khawplod 2006
104	cat. III animal bites	HRIG	N.R.	Verorab	1.42 IU	2-2-2-0-1-1	100	Khawplod 2006
107	cat. III animal bites	HRIG	N.R.	N.R. Aventis CPRV	1.04 IU	2-2-2-0-2	100	Khawplod 2006
45	healthy	NO	≥ 18 yrs	Rabipur	0.943 IU	2-2-2-2-2	100	Sudarshan 2005
32	healthy	HRIG	18-40 yrs	Rabipur	0.51 IU	2-2-2-0-1-1	100	Beran 2005
32	healthy	HRIG	18-40 yrs	Rabipur	0.25 IU	2-2-2-0-1-1	100	Beran 2005
30	healthy	HRIG	18-40 yrs	Rabipur	0.13 IU	2-2-2-0-1-1	97	Beran 2005
30	healthy	HRIG	18-40 yrs	Rabipur	0.06 IU	2-2-2-0-1-1	97	Beran 2005
32	healthy	HRIG	18-40 yrs	Rabipur	0.03 IU	2-2-2-0-1-1	100	Beran 2005
113	cat. III animal bites/exposures confirmed rabid	ERIG or HRIG	5-60 yrs	Rabipur	≥ 0.25 IU	2-2-2-0-1-1	99*	Quiambao 2005
22	healthy	NO	N.R. (young adults)	Verorab	1.38 IU	8-0-4-0-0	100	Khawplod 2002a
22	healthy	NO	N.R. (young adults)	Verorab	1.38 IU	4-4-4-0-0	100	Khawplod 2002a
22	healthy	NO	N.R. (young adults)	Verorab	1.38 IU	2-2-2-0-0	100	Khawplod 2002a
10	HIV with cat. II/III animal bites/exposures	HRIG for cat. III	7-43 yrs	N.R. Mérieux PVRV	1.48 IU	4-4-4-0-2-2	70	Tantawichien 2001
43	cat. I animal bites/exposures	NO	N.R. (adults)	N.R. PCEC	0.78 IU	2-2-2-0-1-1	100	Madhusudana 2001
39	cat. I animal bites/exposures	NO	N.R. (adults)	N.R. PCEC	0.78 IU	8-0-4-0-1-1	100	Madhusudana 2001
58	cat. II/III animal bites/exposures	HRIG as needed	2-73 yrs	N.R. Chiron Behring PCEC	0.916 IU	2-2-2-0-1-1	100	Briggs 2000
59	cat. II/III animal bites/exposures	HRIG as needed	4-78 yrs	N.R. Aventis PVRV	2.32 IU	2-2-2-0-1-1	100	Briggs 2000

* day 30 result for subjects with missing day 14 data

What is the relationship between potency and immunogenicity upon ID vaccination?

To illustrate the relationship between potency and immunogenicity, scatter plots were built (Figures 2, 3 and 4). Data from all study groups with both vaccine potency and RVNA geometric mean titer disclosed were used. From the 38 papers identified by the literature search on immunogenicity, 25 pairs of such data were found when considering day 7 immunogenicity data, 32 for day 14 data and 39 for day 90 data. The data sets corresponded to vaccines with potency per ID dose ranging from 0.435 to 2.32 IU (day 7), 0.46 to 2.32 IU (day 14) or 0.435 to 2.32 (day 90).

Figures 2, 3 and 4. Distribution of RVNA geometric mean titer on day 7, 14 and 90 according to potency per ID dose



No evidence of a relationship between potency and immunogenicity was observed. However, a serious limitation to this type of analysis related to the low number of datasets available, and the fact that numerous parameters, in addition to vaccine potency, are expected to affect immune responses. This set of data was obtained from subjects aged 2 to 78 years, healthy or HIV infected, using different type of vaccine, different schedules, with or without co-administration of RIG… etc.

Data generated in a same clinical trial are less affected by such limitation. Unfortunately, only few studies compared vaccines with different potency, and none of them compared different lots of a same vaccine tradename. The data published by Narayana (2015), Ambrosaitis (2006), Khawplod (2002b) and Briggs (2000) are presented in Tables 6 to 9. Vaccines with 2- to 3-fold difference in potency were not clearly associated with a difference in immune response.

Table 6. Immunogenicity of 2 vaccines with different potency administered by ID route (Narayana 2015)

N subjects	type of subjects	RIG	age	vaccine tradename	potency per ID dose	schedule	RVNA GMT day 14 (95% CI)	RVNA GMT day 90 (95% CI)
45	cat. II/III animal bites/exposures	ERIG for cat. III	18-54 yrs	Rabipur	0.69 or 0.75 IU	4-4-4-0-0	14.50 (13.50-15.57)	11.78 (11.27-12.31)
44	cat. II/III animal bites/exposures	ERIG for cat. III	18-54 yrs	Verorab	1.4 IU	4-4-4-0-0	14.43 (13.41-15.53)	11.93 (11.47-12.40)

Table 7. Immunogenicity of 2 vaccines with different potency administered by ID route (Ambrosaitis 2006)

N subjects	type of subjects	RIG	age	vaccine tradename	potency per ID dose	schedule	RVNA GMT day 14	RVNA GMT day 90
86	healthy	NO	18-60 yrs	Rabipur	0.55 IU	4-0-2-0-1-1	20.5	2.39
87	healthy	NO	18-60 yrs	Verorab	1.79 IU	4-0-2-0-1-1	26.1	2.75

Table 8. Immunogenicity of 2 vaccines with different potency administered by ID route (Khawplod 2002b)

N subjects	type of subjects	RIG	age	vaccine	potency per ID dose	schedule	RVNA GMT day 14
39	healthy	NO	N.R.	Chiron PCEC	0.85 IU	1-0-1-0-1	2.37
42	healthy	NO	N.R.	Aventis PVRV	1.74 IU	1-0-1-0-1	3.40

Table 9. Immunogenicity of 2 vaccines with different potency administered by ID route (Briggs 2000)

N subjects	type of subjects	RIG	age	vaccine tradename	potency per ID dose	schedule	RVNA GMT day 14	RVNA GMT day 90
58	cat. II/III animal bites/exposures	HRIG as needed	2-73 yrs	Rabipur	0.916 IU	2-2-2-0-1-1	28.5	3.0
59	cat. II/III animal bites/exposures	HRIG as needed	4-78 yrs	Verorab	2.32 IU	2-2-2-0-1-1	28.9	2.7

Of note, the relationship between antigen dose and immune response can also be assessed by analyzing the different vaccination regimens employed. A few studies compared indeed schedules of immunization with a doubled number of intradermal injections on the same day, resulting in the administration of doubled doses of a same vaccine. Table 10 provides an overview of these data. All three trials included in this analysis were conducted in healthy subjects.

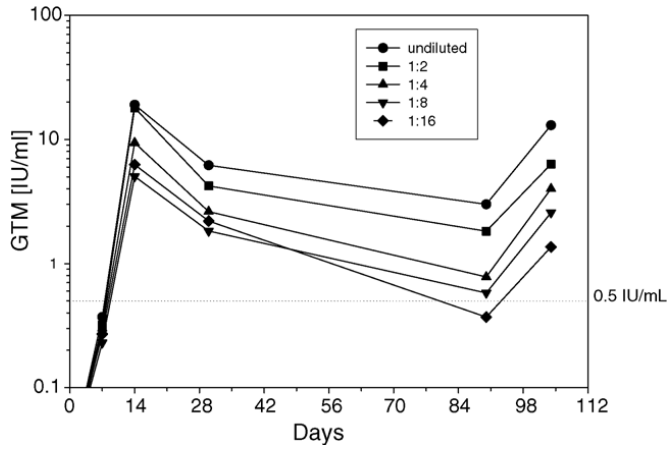
Table 10. Immunogenicity of a same vaccine administered according to different ID regimens

N subjects	age	vaccine tradename	potency per ID dose	schedule	RVNA titer day 14 (95% CI)	ratio titer high/low dose day 14	RVNA titer day 28	ratio titer high/low dose day 28	RVNA titer day 90 (95% CI)	ratio titer high/low dose day 90	source
55	18-50 yrs	Verorab	1.06 or 1.68 IU	4-0-2-0-1-1	334.66 (278.68-401.79)		-		7.18 (5.63-9.15)		Warrell 2008
60	18-50 yrs	Verorab	1.06 or 1.68 IU	8-0-4-0-1-1	308.11 (248.66-381.77)	**0,92**	-	**-**	9.75 (7.46-12.76)	**1,36**	
22	N.R. (young adults)	Verorab	1.38 IU	4-4-4-0-0	12.24		-		-		Khawplod 2002a
22	N.R. (young adults)	Verorab	1.38 IU	2-2-2-0-0	5.00	**2,45**	-	**-**	-	**-**	
29	N.R. (students)	N.R. Behring PCEC	0.5 IU	1-0-1-0-1	-		0.47		0.5		Jaijaroensup 1999
27	N.R. (students)	N.R. Behring PCEC	0.5 IU	1-0-1-0-1	-		0.37		0.42		
27	N.R. (students)	N.R. Behring PCEC	0.5 IU	2-0-2-0-2	-		1.98		1.34		
27	N.R. (students)	N.R. Behring PCEC	0.5 IU	2-0-2-0-2	-	**-**	1.90	**4,62**	1.52	**3,11**	

Warrell (2008) did not detect any increase in immunogenicity when the number of ID doses administered increased from 4 to 8 on day 0 and 2 to 4 on day 7, which may at least in part be explained by the very high antibody titers seen in this trial. By contrast, the studies reported by Khawplod (2002,PEP schedule) and Jaijaroensup (1999, PrEP schedule) suggested an increased immune response when doubling the number of ID doses. Unfortunately, the relevance of these data to the question whether the required minimum vaccine potency should be revised is questionable: additional ID doses are indeed administered at different body sites and thereby modify the immune response (e.g. additional lymph nodes involved) as compared to the situation where more antigen is injected at the same site.

The study by Beran et al. (2005) was designed in a more adequate manner to evaluate the relationship between potency and immune response. It showed that, when a vaccine with an initial potency of 5.06 IU/ml was diluted up to 16-fold, the immunogenicity of the two-site ID PEP regimen was proportional to the antigen dose (Figure 5). The study was conducted in a population of healthy adult subjects who also received HRIG, as a means to simulate treatment of category III patients and take into account possible interference with response to vaccination. Day 14 RVNA geometric mean titers were found well above the 0.5 IU/mL threshold even with the most diluted vaccines, but as depicted in Table 5 above, one subject in each of the 2 groups receiving the 0.13 or 0.06 IU vaccine dose (1/4 and 1/8 dilutions) did not reach this threshold.

Figure 5. Geometric Mean Titers of neutralizing antibodies following ID administration of vaccine dilutions (Beran 2005)



How does the immunogenicity of rabies vaccines administered by ID route compare to IM route?

RVNA titers measured in a same trial after vaccination with the same vaccine by ID or IM route were compared. Such data were found in 16 of the 38 publications resulting from the immunogenicity search. The corresponding trials were conducted in populations of subjects of different age and different health status. This analysis aimed at assessing the suitability of rabies vaccines administered by ID route in a variety of clinical settings. Of note, as clinical trials used vaccines characterized by a volume of either 0.5 or 1 mL, comparisons between ID and IM routes presented in this section

involved ID antigen doses of either 1/5 or 1/10 of the IM dose. Most data on the comparison between ID and IM route were obtained from healthy subjects vaccinated according to a PrEP schedule. Table 11 below presents the selection of studies in populations of healthy adult subjects where the same PrEP vaccination schedule was applied to the ID and IM route. Interestingly, in this setting the ID route was consistently found less immunogenic than the IM route.

Table 11. Immunogenicity of rabies vaccines administered to healthy subjects by ID and IM route (same PrEP schedule)

N subjects	age	vaccine tradename or manufacturer and type	potency per IM dose	potency per ID dose	route	schedule	RVNA titer day 14	Ratio ID/IM day 14	RVNA titer day 28	Ratio ID/IM day 28	RVNA titer day 90 (95% CI)	Ratio ID/IM day 90	source
31	18-23 yrs	Speeda	6.2 IU ?	-	IM	1-0-1-0-1	-	-	-		14.23 (10.49-19.30) (day 42)		Tantawichien 2014
32	18-23 yrs	Speeda	6.2 IU ?	0.62 IU ?	ID	1-0-1-0-1	-		-		5.02 (3.79-6.65) (day 42)	**0,35**	
17	18-50 yrs	N.R. SIIL PVRV	4.35 IU	-	IM	1-0-1-0-1	-		-		7.82 (6.31-9.67) (day 42)		Kulkarni 2013
20	18-50 yrs	N.R. SIIL PVRV	4.35 IU	0.435 IU	ID	1-0-1-0-1	-	-	-		6.12 (5.06-7.39) (day 42)	**0,78**	
10	18-50 yrs	Verorab	≥ 2.5 IU	-	IM	1-0-1-0-1	15.94		49.94 (day 21)		56.29 (day 49)		Laurent 2010
10	18-50 yrs	Verorab	≥ 2.5 IU	≥ 0.5 IU	ID (device1)	1-0-1-0-1	9.82	**0,62**	26.36 (day 21)	**0,53**	28.65 (day 49)	**0,51**	
10	18-50 yrs	Verorab	≥ 2.5 IU	≥ 0.5 IU	ID (device2)	1-0-1-0-1	11.65	**0,73**	35.62 (day 21)	**0,71**	45.11 (day 49)	**0,80**	
10	18-50 yrs	Verorab	≥ 2.5 IU	≥ 0.5 IU	ID (device3)	1-0-1-0-1	5.92	**0,37**	15.13 (day 21)	**0,30**	31.50 (day 49)	**0,56**	
10	18-50 yrs	Verorab	≥ 2.5 IU	≥ 0.5 IU	ID (device4)	1-0-1-0-1	15.76	**0,99**	23.93 (day 21)	**0,51**	38.13 (day 49)	**0,64**	
73	≥ 18 yrs	Verorab	≥ 2.5 IU	-	IM	1-0-1-0-1	-		-		1.20		Cunha 2010
76	≥ 18 yrs	Verorab	≥ 2.5 IU	≥ 0.5 IU	ID	1-0-1-0-1	-	-	-	-	0.76	**0,63**	
37	N.R.	N.R. Chiron PCEC	8.5 IU	-	IM	1-0-1-0-1	5.29		-		-		
39	N.R.	N.R. Chiron PCEC	8.5 IU	0.85 IU	ID	1-0-1-0-1	2.37	**0,45**	-		-		
44	N.R.	N.R. Aventis PVRV	8.7 IU	-	IM	1-0-1-0-1	7.08		-		-		

N subjects	age	vaccine tradename or manufacturer and type	potency per IM dose	potency per ID dose	route	schedule	RVNA titer day 14	Ratio ID/IM day 14	RVNA titer day 28	Ratio ID/IM day 28	RVNA titer day 90 (95% CI)	Ratio ID/IM day 90	source
42	N.R.	N.R. Aventis PVRV	8.7 IU	1.74 IU	ID	1-0-1-0-1	3.40	**0,48**	-	**-**	-	**-**	Khawplod 2002b
28	N.R. (students)	N.R. Behring PCEC	5 IU	-	IM	1-0-1-0-1	-		1.86		2.07		
29	N.R. (students)	N.R. Behring PCEC	5 IU	0.5 IU	ID	1-0-1-0-1	-		0.47		0.5		Jaijaroensup 1999
27	N.R. (students)	N.R. Behring PCEC	5 IU	0.5 IU	ID	1-0-1-0-1	-	**-**	0.37	**0,23**	0.42	**0,22**	

A similar conclusion could be drawn from the comparison of ID and IM vaccination routes in healthy infants and toddlers (Table 12). While antibody titers well above the 5 IU/mL threshold were obtained by day 90 after ID vaccination, in the 2 studies reporting this type of comparison, the IM route was found to induce higher RVNA titers.**Table 12.** Immunogenicity of rabies vaccines administered to healthy infants or toddlers by ID and IM route (same PrEP schedule)

N subjects	age	vaccine tradename or manufacturer and type	potency per IM dose	potency per ID dose	route	schedule	RVNA titer day 90 (95% CI)	Ratio ID/IM day 90	source
44	12-18 mo.	Rabipur	7.25 IU	-	IM	1-0-1-0-1	22 (15-31) (day 49)		
44	12-18 mo.	Rabipur	7.25 IU	0.725 IU	ID	1-0-1-0-1	5.9 (4.2-8.2) (day 49)	**0,27**	Pengsaa 2009
44	12-18 mo.	Rabipur	7.25 IU	0.725 IU	ID	1-0-0-0-1	5.9 (4.1-8.5) (day 49)	**0,27**	
117	2-4 mo.	Verorab	3.5 or 6.4 or 12.0 IU	-	IM	3 injections at 2, 3 & 4 mo. of age	30.6 (27.9-33.7) (day 105)		Lang 1999

116	2-4 mo.	Verorab	3.5 or 6.4 or 12.0 IU	0.7 or 1.28 or 2.4 IU	ID	2 injections at 2 & 4 mo. of age	12.0 (10.5-13.6)	**0,39**	

Alternative ID PrEP schedules are also described, using 2-site ID injections. Table 13 presents the comparison of these ID schedules to the standard IM PrEp regimen. Unfortunately, only a limited set of data is available to support this comparison.

Table 13. Immunogenicity of rabies vaccines administered to healthy subjects by ID and IM route (2-site ID PrEP schedule)

N subjects	age	vaccine tradename or manufacturer and type	potency per IM dose	potency per ID dose	route	schedule	RVNA titer day 28	Ratio ID/IM day 28	RVNA titer day 90	Ratio ID/IM day 90	source
16	18-24 yrs	Verorab	4.8 IU	-	IM	1-0-1-0-1	6.74 (day 35)		-		Wongsaroj 2013
39	18-24 yrs	Verorab	4.8 IU	0.96 IU	ID	2-0-0-0-2	4.51 (day 35)	**0,67**	-	**-**	
17	18-45 yrs	Rabipur	10.23 IU	-	IM	1-0-0-0-0	1.58		-		Khawplod 2012
16	18-45 yrs	Rabipur	10.23 IU	-	IM	1-0-0-0-0	1.50		-		
17	18-45 yrs	Rabipur	10.23 IU	1.023 IU	ID	1-0-1-0-1	4.22		-		
19	18-45 yrs	Rabipur	10.23 IU	1.023 IU	ID	1-0-1-0-1	4.37	**2,79**	-	**-**	
16	18-45 yrs	Rabipur	10.23 IU	1.023 IU	ID	2-0-0-0-0	1.07		-		
24	18-45 yrs	Rabipur	10.23 IU	1.023 IU	ID	2-0-0-0-0	0.94	**0,65**	-	**-**	
28	N.R. (students)	N.R. Behring PCEC	5 IU	-	IM	1-0-1-0-1	1.86		2.07		Jaijaroensup 1999
27	N.R. (students)	N.R. Behring PCEC	5 IU	0.5 IU	ID	2-0-2-0-2	1.98		1.34		
27	N.R. (students)	N.R. Behring PCEC	5 IU	0.5 IU	ID	2-0-2-0-2	1.90	**1,04**	1.52	**0,69**	

Table 14 presents data generated in populations of patients seeking PEP after category II or III exposure. These studies compared the standard IM PEP regimen to the 2-site ID PEP. Data from children from 2 years of age as well as adults were reported. The study by Briggs (2000) excluded subjects under anti-malarial drug treatment, while the other two studies did not have such exclusion criteria. Subjects received concomitant HRIG treatment, as per WHO

recommendations, when they presented with category III exposure. In this clinical setting, the ID route was found to induce as high RVNA titers as the IM route.

Table 14. Immunogenicity of rabies vaccines administered to patients with cat II or III exposure by ID and IM route (WHO recommended PEP schedules)

type of subjects	N subjects	RIG	anti-malarial drug = excl. criteria	age	vaccine tradename or manufacturer and type	potency per IM dose	potency per ID dose	route	schedule	RVNA titer day 14 (95% CI)	Ratio ID/IM day 14	RVNA titer day 28 (95% CI)	Ratio ID/IM day 28	RVNA titer day 90	Ratio ID/IM day 90	source
cat. II animal bites/exposures	27	NO	NO	5-77 yrs	Rabivax-S	4.96 or 4.24 IU	-	IM	1-1-1-1-1	20.57 (17.03-24.84)		30.22 (27.43-33.30)		-		Bose 2016
	27	NO	NO	5-77 yrs	Rabivax-S	4.96 or 4.24 IU	0.496 or 0.424 IU	ID	2-2-2-0-2	20.99 (17.45-25.24)	**1,02**	27.93 (24.51-31.82)	**0,92**	-	**-**	
cat. III animal bites/exposures	27	HRIG	NO	5-77 yrs	Rabivax-S	4.96 or 4.24 IU	-	IM	1-1-1-1-1	16.47 (13.39-20.26)		22.50 (18.75-27.00)		-		Bose 2016
	27	HRIG	NO	5-77 yrs	Rabivax-S	4.96 or 4.24 IU	0.496 or 0.424 IU	ID	2-2-2-0-2	19.46 (16.03-23.62)	**1,18**	23.20 (18.89-28.49)	**1,03**	-	**-**	
cat. II animal bites/exposures	10	NO	NO	N.R.	N.R. Chiron PCEC	10.23 IU ?	-	IM	1-1-1-1-1	6.37		-		-		Saraya 2010
	10	NO	NO	N.R.	N.R. Chiron PCEC	10.23 IU ?	1.023 IU ?	ID	2-2-2-0-2	9.48	**1,49**	-	**-**	-	**-**	
cat. II/III animal bites/exposures	37	HRIG as needed	YES	5-66 yrs	N.R. Chiron Behring PCEC	9.16 IU	-	IM	1-1-1-1-1-1	12.3		18.5		4.7		Briggs 2000
	58	HRIG as needed	YES	2-73 yrs	N.R. Chiron Behring PCEC	9.16 IU	0.916 IU	ID	2-2-2-0-1-1	28.5	**2,32**	10.9	**0,59**	3.0	**0,64**	

Finally, a group of 3 studies was identified, which evaluated alternative PEP schedules in healthy subjects (Table 15). Except for the trial reported by Sudarshan (2005) where the ID route was found less immunogenic than the IM Essen regimen, these analyses supported the high level immunogenicity of the vaccines administered by ID route.

Table 15. Immunogenicity of rabies vaccines administered to healthy volunteers by ID and IM route (alternative PEP schedules)

N subjects	age	vaccine tradename or manufacturer and type	potency per IM dose	potency per ID dose	route	schedule	RVNA titer day 14 (95% CI)	Ratio ID/IM day 14	RVNA titer day 28 (95% CI)	Ratio ID/IM day 28	RVNA titer day 90 (95% CI)	Ratio ID/IM day 90	source
56	18-50 yrs	Verorab	5.3 or 8.4 IU	-	IM	1-1-1-1-1-0	228.45 (161.99-322.18)		-		6.21 (4.86-7.95)		Warrell 2008
55	18-50 yrs	Verorab	5.3 or 8.4 IU	1.06 or 1.68 IU	ID	4-0-2-0-1-1	334.66 (278.68-401.79)	**1,46**	-	-	7.18 (5.63-9.15)	**1,16**	
60	18-50 yrs	Verorab	5.3 or 8.4 IU	1.06 or 1.68 IU	ID	8-0-4-0-1-1	308.11 (248.66-381.77)	**1,35**	-	-	9.75 (7.46-12.76)	**1,57**	
58	18-50 yrs	Verorab	5.3 or 8.4 IU	1.06 or 1.68 IU	ID	2-2-2-0-1-1	363.66 (299.16-442.08)	**1,59**	-	-	9.14 (6.86-12.20)	**1,40**	
46	≥ 18 yrs	Rabipur	9.43 IU	-	IM	1-1-1-1-1	6.89 (6.33-7.49)		11.53 (10.82-12.27		6.99 (6.37-7.67)		Sudarshan 2005
45	≥ 18 yrs	Rabipur	9.43 IU	0.943 IU	ID	2-2-2-2-2	4.17 (3.69-4.71)	**0,61**	7.60 (6.93-8.33)	**0,66**	4.79 (4.26-5.38)	**0,69**	
23	N.R. (young adults)	Verorab	6.9 IU	-	IM	1-1-1-1-0	3.81		-		-		Khawplod 2002a
22	N.R. (young adults)	Verorab	6.9 IU	1.38 IU	ID	8-0-4-0-0	13.09	**3,44**	-	-	-	-	
22	N.R. (young adults)	Verorab	6.9 IU	1.38 IU	ID	4-4-4-0-0	12.24	**3,39**	-	-	-	-	
22	N.R. (young adults)	Verorab	6.9 IU	1.38 IU	ID	2-2-2-0-0	5.00	**1,31**	-	-	-	-	

B. Recent observations in terms of efficacy / effectiveness

Table 16 below provides an overview of recent data on patient survival after rabies post-exposure prophylaxis by intradermal route. Most available reports provided information on subjects who had not been previously vaccinated against rabies, and were therefore prescribed a full ID PEP regimen. A single study was identified where PEP was administered as a single dose to previously immunized patients (Table 17). In total, follow-up data corresponding to

more than 36 000 patients who received ID PEP were described in this set of 11 publications. Unfortunately, in most cases, the fate of the animal and its rabid status remained unknown. Approximately one third of the patients were classified as category III exposure.

Table 16. Patient survival after rabies post-exposure prophylaxis by intradermal route

type of study	vaccine tradename	potency per IM dose	route	ID schedule	N subjects	N rabid exposure confirmed	duration of follow-up	survival (%)	country	Source
observational	N.R.	N.R.	ID	2-2-2-0-2	2188	N.R.	N.R.	100%	Pakistan	Salahuddin 2016
prospective	Vaxirab-N	0.718 IU	ID	2-2-2-0-2	269	N.R.	9 mo.	100%	India	Barthi 2016
observational	N.R. (mostly Sanofi)	N.R.	ID	N.R.	20610	227	6 mo.	100%	Cambodia	Tarantola 2015
randomised	Rabipur	0.69 or 0.75 IU	ID	4-4-4-0-0	45	N.R.	1 yr	100%	India	Narayana 2015
randomised	Verorab	1.4 IU	ID	4-4-4-0-0	44	N.R.	1 yr	100%	India	Narayana 2015
randomised	Vaxirab-N	0.704 IU	ID	2-2-2-0-2	43	N.R.	1 yr	100%	India	Ravish 2014
randomised	Rabipur	≥ 0.25 IU	ID	2-2-2-0-2	43	N.R.	1 yr	100%	India	Ravish 2014
prospective	N.R.	N.R.	ID&IM	2-2-2-0-2	47	N.R.	6 mo.	100%	India	Ravish 2014b
observational	N.R.	N.R.	ID	2-2-2-0-2	43	N.R.	6 mo.	100%	India	Ravish 2014b
prospective	Verorab	N.R.	ID	2-2-2-0-1-1	123	N.R.	up to 1 yr	100%	India	Satapathy 2011
prospective	Abhayrab	≥ 0.25 IU	ID	2-2-2-0-2	1494	N.R.	100 d.	100%	India	Behera 2011
observational	N.R.	N.R.	ID(&IM)	N.R.	193	193	1 yr	99,48%	Philippines	Quiambao 2009
observational	N.R.	N.R.	ID	N.R.	6609	120	35 d. - 29 mo.	99,97%	Philippines	Quiambao 2008

Table 17. Patient survival after short rabies post-exposure prophylaxis by intradermal route (in previously vaccinated subjects)

type of study	vaccine tradename	potency per IM dose	route	ID schedule	N subjects	N subjects with cat III bite / exposure	N rabid exposure confirmed	duration of follow-up	survival (%)	country	Source
observational	N.R. (PVRV or PCEC)	N.R.	ID	4-0-0-0-0	5116	3335	253	N.R.	100%	Thailand	Shantavasinkul 2010b

Survival was close to 100%. Only 3 treatment failures were reported in this set of data:

- a healthy 6-year-old boy bitten by a dog on the upper lip. Wound care with soap and water. Two days later, infiltration of pERIG into the wound with the rest administered IM in the anterolateral part of the thigh, and first dose (0.1 mL) of Verorab ID at two sites. Second and third doses of rabies vaccine administered IM on treatment days 3 and 7. One month after bite, presented with fever, loss of appetite and malaise. Then developed seizures. Several coughing episodes, became cyanotic and died (Quiambao 2009)
- a 4-year-old malnourished girl attacked by a dog. Multiple deep lesions on the shoulder, back and nape. Immediate treatment with wound washing, wound infiltration with diluted pERIG and IM injection of the remaining volume, and rabies vaccine by ID route. Wounds sutured because of their severity and continued bleeding. Subsequent doses of rabies vaccine ID as scheduled. On day 24, however, hospitalized with signs and symptoms of rabies and died 55 days post-exposure (Quiambao 2008)
- an 8-year-old boy bitten by a dog with a cat. III single laceration of the right eyelid. Received pERIG, partly infiltrated around the wound with the remaining volume administered IM in the buttocks and a first dose of rabies vaccine (0.1 mL ID in both deltoids). It was not documented whether wound cleaning was performed. One month later, moderate fever. Five days later, restlessness, irritability and increased salivation. During consultation, hydrophobia and aerophobia could be elicited. The history revealed the boy had not received any further doses of rabies vaccine. Boy died at home. (Quiambao 2008)

PART 2: FROM EVIDENCE TO RECOMMENDATIONS

Summary of evidence

1. Immunogenicity of rabies vaccines administered by ID route

a. Proportion of subjects reaching the 0.05 IU/ml RVNA threshold on day 14

Table 18 summarizes data from Table 5 by classifying the percentage subjects reaching the RVNA 0.5 IU/mL titer threshold on day 14 according to vaccine potency, type of population and vaccination regimen. The data suggest lower antibody responses among healthy subjects receiving PrEP and HIV patients receiving PEP.

Table 18. Percentage subjects with RVNA titer ≥ 0.05 IU/ml according to vaccine potency and ID vaccination schedule (day 14 data)

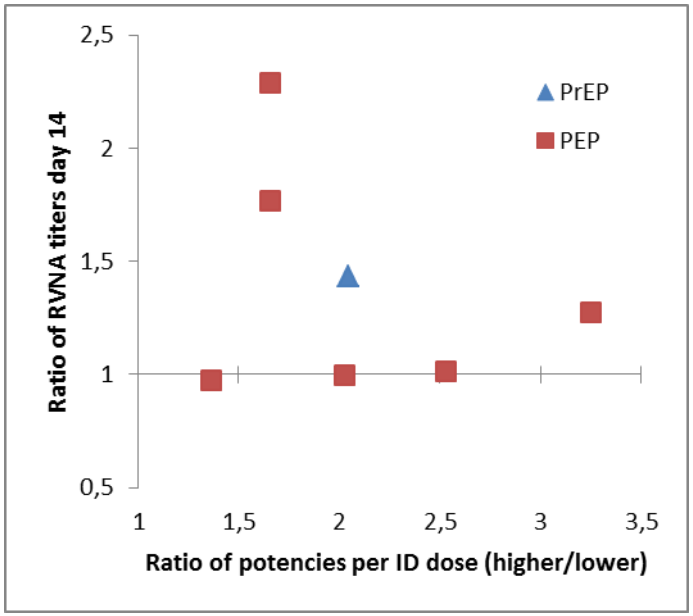
	Population (N subjects)	PrPEP	PEP
vaccines with potency ≥0.25 IU per ID dose*	healthy (N = 1207)	92,06%	100%
	animal bite/exposure (N = 1013)	-	99,90%
	HIV (N = 37)	-	91,89%
vaccines with potency <0.25 IU per ID dose	healthy (N = 92)	-	97,83%

** data available for vaccines with potency from 0.424 IU per ID dose*

b. Relationship between potency and immunogenicity by ID route

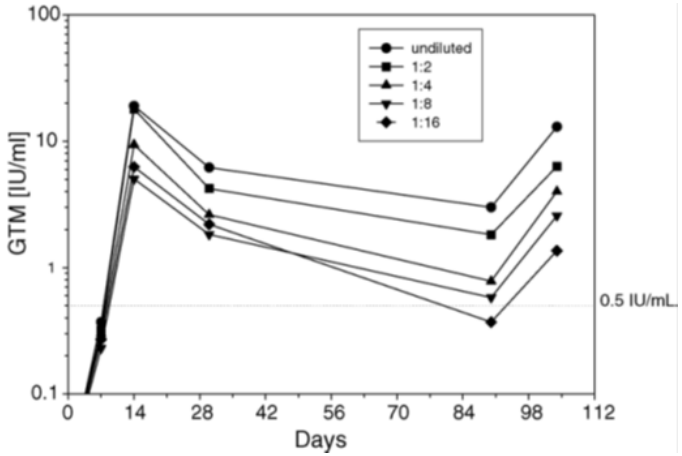
Figure 6 summarizes data presented in Tables 6 to 9, corresponding to situations where 2 vaccines with different potency per ID dose were compared, in a same trial, same population, and with same vaccination regimen. Results of 2 additional studies not presented in the tables (Khawplod 2006, Tantawichien 2014) were also included, where vaccines with a potency of a less than 2-fold difference were compared. Overall, in this set of data, the vaccines tested had a potency ranging from 0.55 to 2.32 IU per ID dose. No evidence of a relationship between potency and immunogenicity was detected.

Figure 6 Distribution of ratios of RVNA titers measured on day 14 vs. ratios of vaccine potency per ID dose injected



By contrast, the Beran study, where dilutions of a same vaccine down to 0.03 IU per ID dose were studied in a same trial (PEP regimen), concluded in a dose-response relationship.

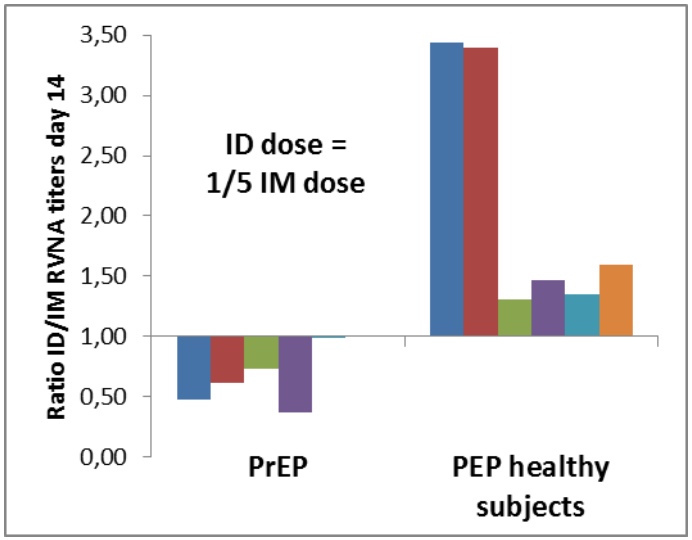
Figure 5. Geometric Mean Titers of neutralizing antibodies following ID administration of vaccine dilutions (Beran 2005)

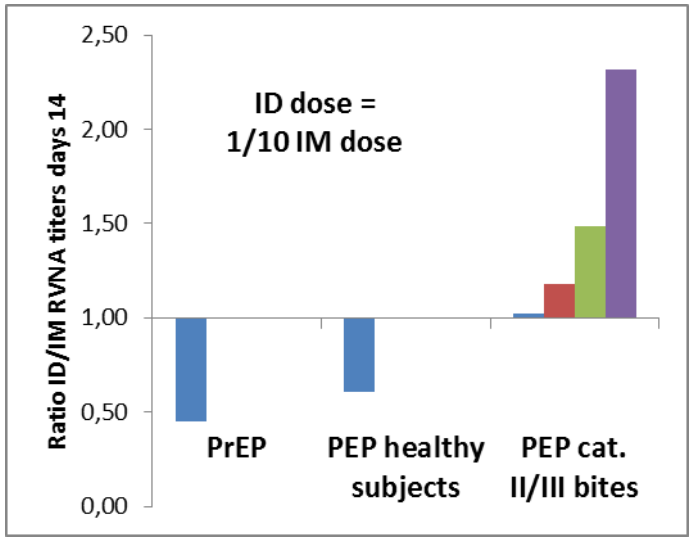


c. Comparison between ID and IM routes of vaccination

Figure 7 and 8 summarize data presented in Tables 11, 14 and 15, showing the ratio of antibody titers on day 14 when a same vaccine was tested in a same trial, same population, by ID or IM route. Figures 7 and 8 compiled data obtained with vaccine ID doses corresponding to respectively 1/5th and 1/10th of an IM dose. When compared to the IM vaccination regimen, the ID route was found more immunogenic in PEP schedules than PrEP schedules.

Figures 7 and 8 Ratio of ID/IM RVNA geometric mean titers on day 14 for ID doses corresponding respectively to 1/5th and 1/10th of the IM dose





d. Discussion

Overall, the immunogenicity data compiled in the present review indicate a very good immunogenicity of current rabies vaccines administered by intradermal route. In many cases, the potency of the vaccines tested was not reported, but data corresponding to ID vaccine doses from 0.424 IU were reported, except for the trial by Beran (2005) using vaccine dilutions down to 0.03 IU.

Optimal immunogenicity was observed for vaccination with an ID **PEP** schedule (2-site vaccination regimen). Both healthy subjects and patients presenting with animal bite or exposure developed RVNA titers reaching the 0.5 IU/mL threshold by day 14 post immunization, and these immune responses triggered by ID vaccination were at least as high as those induced by IM route.

The relevance of these data to the ultimate target population receiving PEP vaccination can be considered as very high. Subjects from 2 to 78 years of age were included in the corresponding trials. A significant proportion of results (1394/4224 subjects) were obtained in patients seeking vaccination after animal bite/exposure, in countries with a high incidence of rabies. Some clinical trials excluded subjects with specific underlying conditions likely to decrease immune responses (e.g. anti-malarial drug treatment was reported as an exclusion criteria in 13 out of the 38 immunogenicity studies included in the present review), but there were also studies indicating that co-administration of RIG did not affect immune responses to ID vaccination (Bose 2016, Madhudusana 2002).

Only 2 studies evaluating rabies PEP in HIV patients were identified. Vaccination was associated with suboptimal immunogenicity (34/37 subjects reaching the 0.5 IU/mL threshold on day 14) in this target population. This finding was not unexpected in view of the impairment of the immune system observed in the course of HIV infections, especially in patients with low CD4 counts. In addition failure to achieve the recommended level of neutralizing antibodies in this type of population had also been reported following IM PEP regimens (see for instance Toovey 2007).

In the present review, the set of studies investigating the immunogenicity of current rabies vaccines used in an ID **PrEP** regimen suggested lower antibody titers with the ID route when compared to standard IM vaccination. The same observation was made irrespective of the age of vaccinated subjects. Nevertheless, the relevance of this finding should be interpreted in light of the objectives of PrEP. Unlike PEP regimens, which aim at inducing high levels of neutralizing antibodies as soon as possible after exposure to a rabid animal, PrEP schedules target long lasting priming of the immune system against rabies, so that a short course of vaccination at the time of exposure will suffice to stimulate an anamnestic effect and rapidly induce adequate levels of antibodies. The present review focused on the analysis of immune responses in subjects who were naïve to rabies antigens, as situations of priming provide more discriminating conditions to compare the immunogenicity of different vaccines or vaccination regimens. However, a variety of additional studies demonstrated successful booster vaccination with a short PEP regimen in subjects previously primed by ID PrEP. For instance,

- Kamoltham (2011) reported the outcome of a randomized trial where 703 Thai schoolchildren received short ID PEP vaccination (day 0, day 3) at 1, 3 or 5 years after ID PrEP. All children developed adequate RVNA titers above 0.5 IU/mL within 14 days after booster vaccination.
- Sudarshan (2006) found similar immunogenicity of ID PEP in 10 subjects primed by IM route to that of IM PEP in 10 subjects primed by ID route (RVNA geometric mean titer on day 14 of respectively 8.84 [95% CI 7.58-10.30] and 9.17 [7.84-10.70]).
- In the pediatric study of Pengsaa (2009), 200 healthy children aged 12-18 months received either ID PrEP followed by ID PEP one year later or IM PrEP followed by IM PEP. By 7 days post-booster, strong anamnestic responses were seen with both routes of vaccination while the RVNA geometric mean titer reached 25 (95% CI 16 –38) IU/mL in the ID group, a significantly lower titer than that found in the IM group (190, 121–299).
- Tantawichien (2014) compared the immunogenicity of a short ID PEP administered 1 year after ID or IM PrEP (32 and 31 subjects respectively). Very high RVNA titers were measured in both groups at 14 days after PEP (11.93 [95% CI 8.95-16.66] vs. 45.99 [28.27–74.82]).

In conclusion, current vaccines administered by ID PrEP appear less immunogenic than when given by IM route, but the clinical relevance of this finding has not been confirmed.

2. Efficacy of rabies vaccines administered by ID route

The present review identified follow-up data for a total of more than 36 000 patients who received PEP by ID route. Confirmation that vaccine recipients had been bitten by a rabid dog was provided in 793 cases. Only 3 cases of treatment failure were reported. Therefore the data support the very high level of efficacy of current rabies vaccines administered by ID route and confirm the findings of earlier studies (reviewed by Giesen 2005).

Unfortunately, there was no information available as to the potency of the vaccines used in the 3 patients who did not survive. One of these treatment failures was identified in a child who received an incomplete vaccination course and for whom wound cleaning had not been confirmed. More detailed analyses would also have been required to assess whether suboptimal wound management was involved in the 2 other cases of failure.

Reports of vaccination failures are not frequently published in scientific journals, but investigations into these cases show that for the majority of patients there was an omission of at least one of the essential steps of PEP (wound treatment, administration of RIG and complete course of vaccine) (Giesen 2005). True prophylaxis failure is rare, but has been described following IM PEP regimens (see for instance Tinsa 2015). Consequently, the data of the present review do not cast doubt on the efficacy of current rabies vaccines administered by ID route.

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Evidence Profile: New Vaccines

Question: What potential new vaccines for rabies are in the pipeline that may be more cost-effective (e.g. easier storage, longer shelf-life, etc.) and still retain vaccine safety and efficacy?

Background:
The WHO Collaborating Centre for Neurovirology and the WHO Collaborating Centre for Reference and Research on Rabies, conducted a landscape analysis of new vaccine candidates and their stages of advancement. The assessment and the overview on the potential of new vaccines for improvements for programmatic experiences of countries(Table 1) are based on available evidence on clinical trials and expert opinion. The present landscape analysis was validated by the 3rd WHO Expert Consultation on Rabies, held 26-28 April 2017 in Bangkok.

New evidence:

1) Vaccines in clinical testing

Phase I (not recruiting) RNActive rabies vaccine (CureVac Ag)
Based on 3 doses of an mRNA encoding the rabies virus glycoprotein. Preclinical testing in mice and pigs using intradermal immunization given twice. VNA titers and protection similar to that achieved with Rabipur.
Cost effectiveness unknown. Stability may be an issue as mRNA although it can be lyophilized will be very sensitive to degradation once it is reconstitute and kept at room temperature

Phase I (completed), PIKA vaccine (Yisheng Biopharma)
Rabipur + Polyinosinic-Polycytidylic Acid Based Adjuvant.
An accelerated reduced vaccine dose (2IU rather than 5.9IU) 2-2-1 regimen (2 doses on day 0, 2 on day 3, 1 on day7) achieved in human volunteers a more rapid seroconversion rate. In experimental animals, the vaccine protected 80% of animals if given post-exposure compared to 20% protection in the traditional vaccine group.
Probably cost-effective as it reduces numbers and doses of vaccine. Can be freeze-dried.

Phase II, Rabies G protein nanoparticle vaccine (CBL Biological)
Technical information on this vaccine is scarce. Based on other vaccines in their pipeline I assume this is a baculovirus-derived glycoprotein that spontaneously form micelles (nanoparticles).
Preclinical results: a 3-dose regimen induces higher rates of seroconversion compared to Rabipur.
Good immunogenicity profile, cost effectiveness will depend on scalability of the rather cumbersome purification method. In addition, the breadth of the neutralizing antibody responses has not yet been analyzed.

2) Vaccines scheduled for clinical testing

E1-deleted adenovirus vector of chimpanzee-origin expressing rabies virus glycoprotein
(Oxford/Wistar)
Not suited for post-exposure vaccination. Excellent immunogenicity, sustained immune responses, good memory formation. Similar vaccines that have been tested clinically were well tolerated. Has the potential to be a cost-effective one dose pre-exposure vaccine. Cold chain independent storage is possible. Can be given i.m.

Deactivated rabies virus virions as novel vaccine platform
(NIAID, IDDR, TJU) .
We develop a heat-stable, safe, and immunogenic tetravalent vaccine containing four inactivated rabies virus chimeras expressing the glycoproteins of Ebola Zaire, Ebola Sudan, Marburg and Lassa viruses and a potent, clinically tested adjuvant (TLR-4 agonist).
Cold chain independent, i.m. inoculations. Two inoculations needed.

3) Pre-clinical vaccines

Adjuvants

Advantage: Would reduce vaccine cost by reducing dose per injection and/or numbers of injections needed

Disadvantage: Pre-clinical results are poorly predictive. Reactogenicity likely to increase.

- **Flagellin** (Salmonella typhimurium) – clinical experience, may cause multiple organ damage
- **Saponin** (leaves of Quillaja brasiliensis) - clinical experience, problems with production, quality control, stability and toxicity
- **ISCOMATRIX** - clinical experience, well tolerated mainly local reactions and occasionally Flu-like symptoms
- **Monophospho lipid A** - routinely used in some HepB and HPV vaccines
- **Isatis indigotic root polysaccharides** - no clinical experience to early to say if this product from a traditional Chinese Medicine can meet production and purification challenges
- **Water-in-oil-in-water emulsions** – no human experience
- **Uridine 5'triphosphate** – no human experience

Genetically modified rabies virus

Advantages: suited for pre-exposure

Disadvantages: Production issues, regulatory issues

- **Attenuated RABV (replication-competent)**
  Advantages: Highly immunogenic, attenuated even in the immunocompromised host. Relatively stable
  Disadvantages: Unlikely approval for humans, cold-chain needed

- **Attenuated RABV (replication-deficient)**
  Advantages: Highly immunogenic, single shoot vaccine
  Disadvantages: Production requires trans-complementing cell lines, modest titers

Adjuvanted genetically modified rabies virus

- **GM-CFS or** flagellin – increases the immunogenicity of the parent virus, work both i.m. and orally
- **ICAM-1** – accelerated and enhances antibody responses
- **IL-21** – increases transiently antibody responses
- **IL-6** - accelerates and increases antibody responses compared to the parent virus
- **IL-7** – prolongs antibody responses
- **CXCL13** – increases antibody responses

Protein vaccines

Advantages – excellent safety profiles

Disadvantages – Generation of sufficient quantities of pure, correctly folded and glycosylated rabies virus glycoprotein remains a challenge.

- **Mammalian expression system:** HEK 293 cells **(**transfected with a lentivirus), BHK-21 cells, COS cells, NA cells: Glycosylation varies depending on the cell type and culture conditions, which affects the proteins immunogenicity. Production cost is likely to be high; regulators may have concerns about the mode of production (e.g., lentivirus) and inter-batch glycosylation variability.

- **Plant-derived protein vaccines:** Maize, tomato plants, tobacco plants, spinach (fusion protein with alpha mosaic virus coat protein), tomato hairy roots (Rabies glycoprotein-ricin toxin B chain fusion protein) immunogenic, low cost production but purification issues
- **Insect cell expression system** – very efficient expression system
- **Drosophila melanogaster Schneider 2 cells** – immunogenic in mice

Peptide vaccines
Advantages: None
Disadvantages: Poorly immunogenic, restricted responses unlikely to have the breadth needed for protection against the wide variety of rabies virus isolates.

- **Branched lipopeptide vaccine** – induces a CD8+ T cell response which to the best of my knowledge does not protect against rabies virus

- **Lipopeptide vaccine adjuvanted with TLR-7** – efficacious in mice

- **Multi-epitope based vaccine coated with canine gp69** – efficacy unimpressive

Genetic vaccines
Advantages: Cost-effective, in general safe
Disadvantages: Unsuited for post-exposure treatment.

Viral or bacterial vectors

- **Pseudotyped recombinant baculovirus:** Expresses rabies glycoprotein on the surface and encodes the protein – this vector may be suited for pre-exposure
- **Parainfluenza virus 5:** in very early pre-clinical development. It's a live virus, which does not cause disease in humans but kennel cough in dogs. Many humans are immune to this virus, which is shed by vaccinated dogs. The effect of pre-existing immunity has not been examined. Will require a cold chain
- **E1-deleted adenovirus of human serotype 5**: highly immunogenic; not recommended for use in humans which commonly have high titers of neutralizing antibodies to the vaccine carrier
- **Salmonella** – expressing a rabies virus glycoprotein – heat-labile enterotoxin B subunit of E. coli. Could be used orally. Likely to be highly reactogenic in humans
- **Recombinant parapoxvirus (ORFV):** ORFV infections have been observed in humans, which pose safety risks.
- **Newcastle disease virus:** Highly immunogenic, has been used for cancer treatment where it was shown to be well tolerated.
- **Poxviruses**
- Vaccinia virus recombinants: used for immunization of wildlife but are too reactogenic for use in humans.
- **MVA based vectors**: safer but less immunogenic.
- **ORFV based vectors**: the virus can infect humans and cause lesions

DNA vaccines
Advantages: cost-effective, safe, can be used repeatedly, require no cold-chain, can be used by different routes (ID, IM, SC)**.**
Disadvantages: Not highly immunogenic, this could be address by adding adjuvants, which will increase cost and reactogenicity, or by using electroporation, which could be unduly cumbersome in developing countries.

4) Outlook

Stages of adjuvant development for rabies

In clinical trials

PIKA vaccine: Rabipur + Polyinosinic-Polycytidylic Acid Based Adjuvant - allows for reduction in vaccine dose (2 IU instead of ~6) and accelerated vaccination (2 doses on day 0, 2 on day 3, 1 on day 7).

Stages of vaccine development for rabies

In clinical trials:

- **RNActive rabies vaccine:** Based on 3 doses of an mRNA encoding the rabies virus glycoprotein.
- **Phase II, Rabies G protein nanoparticle vaccine:** Based on 3 doses of a baculovirus-derived glycoprotein that spontaneously form micelles (nanoparticles).
- ***Scheduled for clinical testing:***
- **E1-deleted adenovirus vector of chimpanzee-origin expressing rabies virus glycoprotein** (Oxford/Wistar)

The planned trial will test a one dose regimen followed by a late boost with 2 doses of a conventional vaccine to assess recall responses. Clinical results are expected to become available by late 2020. The trial includes an arm that tests a new method to enhance thermostability at ambient temperatures. Discussions are underway with partners for larger scale clinical trials. It is expected depending on the efficacious dose that the vaccine may eventually be made available for <$1. Thermostabilization, a long-term goal, may increase the overall cost.

Table 1: Summary overview on potential of new vaccines

Novel vaccines have the potential to simplify delivery and increase affordability of PEP and PrEP. New vaccines are in different phases of trials, and some are being reviewed by national and international regulatory bodies.

Vaccine type/group	Suitable for PEP	Suitable for PREP	Administration mode (ID, IM, SC, other)	Safety	Efficacy, imunogenicity	Cost-effectiveness	Potential for delivery (cold chain, community delivery, CTC, etc.)	Other
Current vaccines								
Cell culture vaccines CCEEV:								
- Purified chicken embryo vaccine PCEC	yes	yes	IM/ID	proven	Low immunogenic requiring multiple doses to achieve protective titers	No	lyophilized	3-5 doses
- Purified verso cell vaccine PVRV	yes	yes	IM/ID	proven	Low immunogenic requiring multiple doses to achieve protective	No	lyophilized	3-5 doses

					titers			
- Human diploid cell vaccine HDCV	yes	yes	IM/ID	proven	Low immunogenic requiring multiple doses to achieve protective titers	No	lyophilized	3-5 doses
Duck embryo vaccines	yes	yes	IM/ID	proven	Low immunogenic requiring multiple doses to achieve protective titers	No	lyophilized	3-5 doses
Nerve tissue vaccines NTV	These vaccines induce more-severe adverse reactions and are less immunogenic than CCEEVs. It is therefore imperative that production and use of nerve-tissue vaccines be discontinued and replaced with CCEEVs.							
New vaccines								
Attenuated rabies vaccines	Yes	yes	IM/ID/oral	depends	High	yes	variable	1 dose
Deactivated, genetically modified rabies virus	yes	yes	IM/ID	high	High	maybe	lyophilized	2 doses
Protein vaccines	yes	yes	IM/ID	high	to be determined	No	yes	3 doses
Peptide	no	no			Low			

vaccines								
DNA/RNA vaccines	no	yes	IM/ID	high	Low	maybe	Yes	3-5 doses
Adenoviral vectors	no	yes	IM/potentially oral	high	High	yes	Yes	1 dose
Other viral vectors	no	yes	IM/potentially oral	variable	Variable	maybe	variable	1 dose
Bacterial vectors	no	yes	IM/potentially oral	depends	Low	no	no	1 dose