A 1-week intradermal dose-sparing regimen for rabies post-exposure prophylaxis (RESIST-2): an observational cohort study

Tineke Cantaert, Laurence Borand*, Lauriane Kergoat, Chanthy Leng, Sivlin Ung, Sotheary In, Yiksing Peng, Chandara Phoeun, Chanthy Hing, Chun Navy Taing, Manil Saman, Sivuth Ong, Channa Mey, Rithy Choeung, Sowath Ly, Philippe Dussart, Hervé Bourhy*, Arnaud Tarantola**

Summary

Background The international health authorities are backing an effort to eliminate canine-mediated rabies in humans by 2030. This effort will require improving access to adequate and timely rabies post-exposure prophylaxis as compliance is low with WHO-recommended regimens (given in four to five visits over 1 month). Access could be substantially improved by an abridged regimen to reduce doses, direct and indirect costs, and improve vaccine equity by better sharing of available vaccine. We aimed to compare rabies virus neutralising antibody titres before and after the fourth visit to determine whether that session was needed or the current regimen could be abridged.

Methods In this observational cohort study, we measured rabies virus neutralising antibody titres using rapid fluorescent focus inhibition tests in 116 people bitten by dogs with laboratory-confirmed rabies and 20 control individuals. Percentages of circulating plasmablasts were determined by flow cytometry. All individuals had been referred to the rabies prevention clinic at Institut Pasteur in Cambodia and received two intradermal injections of post-exposure prophylaxis on days 0, 3, 7, and 28 (Thai Red Cross regimen) with or without equine rabies immunoglobulin, as per 2010 WHO recommendations.

Findings All individuals had rabies virus neutralising antibody titres considered protective (≥0·5 IU/mL) and plasmablast activation on day 28 before the last injection. The median rabies virus neutralising antibody concentration in the group of individuals bitten by rabies virus-positive dogs was 1·08 IU/mL (IQR 0·37–3·09) on day 7, 26·86 (22·68–49·50) on day 28, and 26·74 (11·78–49·06) on day 42. No significant differences were observed in titres between days 28 and 42, after titres reached a plateau. These titres were reached notwithstanding equine rabies immunoglobulin use, age, sex, nutrition status as indicated by upper-arm circumference in children or BMI in adults, or dog infection status. Titres or plasmablast percentages did not increase between the day of the last injection and 2 weeks later. All patients were alive 1 year after post-exposure prophylaxis.

Interpretation The fourth vaccine session on day 28 provides no additional benefit. Rabies post-exposure prophylaxis can be abridged to a two-dose, three-session, 1 week regimen to improve post-exposure prophylaxis coverage and equity at no risk to patients.

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*Contributed equally

Immunology Unit (T Cantaert PhD, S Ung MS), **Epidemiology and Public Health Unit** (L Borand PhD, C Leng MD, S In MD, Y Peng MD, C Phoeun RN, C Hing RN, C N Taing MD, M Saman MD, S Ly PhD, A Tarantola PhD), **and Virology Unit** (S Ong MD, C Mey MD, R Choeung PharmD, P Dussart PhD), **Institut Pasteur du Cambodge, Institut Pasteur International Network, Phnom Penh, Cambodia; and Lyssavirus Epidemiology and Neuropathology, WHO Collaborating Centre for Reference and Research on Rabies, Institut Pasteur, Paris, France** (L Kergoat MS, H Bourhy PhD)

Correspondence to: Dr Arnaud Tarantola, Epidemiology and Public Health Unit, Institut Pasteur du Cambodge, Phnom Penh 12201, Cambodia
arnaud.tarantola@pasteur.fr

Introduction

Rabies virus is a neurovirulent virus from the Lyssavirus genus of the Rhabdoviridae family causing acute myeloencephalitis. Most human rabies cases occur in Africa and Asia, following a dog bite.[1] Rabies is overwhelmingly fatal but highly preventable by post-exposure prophylaxis.[2] It was estimated in 2015 that at least 3·3 billion people in endemic countries were potentially vulnerable to rabies, which causes an estimated 60 000 deaths worldwide every year.[3] High cost of vaccines, complexity of post-exposure prophylaxis protocols, and poor access to health care contribute to mortality.

Until 2018, WHO recommended post-exposure prophylaxis schedules requiring up to five visits over the course of 1 month.[3] This recommendation included the dose-sparing, highly effective Thai Red Cross post-exposure prophylaxis protocol, which entails two injections at two different sites of 0·1 mL intradermal post-exposure prophylaxis administered in four sessions over a period of 28 days.[3] A rabies virus neutralising antibody concentration of 0·5 IU/mL or more at 14 days after immunisation is considered a proxy for protection in vaccine efficacy studies.[4] Abridged and dose-sparing vaccination regimens increase accessibility and reduce both direct and indirect costs, especially in resource-constrained countries where rabies virus prevalence is highest. Several efficacy studies evaluated abridged regimens in healthy adult volunteers[5] or individuals bitten by dogs,[6] using either four-site intradermal or intramuscular regimens, and showed them to be safe and immunogenic. However, rabies virus can develop immunoevasive strategies, potentially leading to an

Research in context

Evidence before this study

The international health authorities are backing an effort to eliminate canine-mediated rabies in humans by 2030. This goal might be achieved in the long-term through mass vaccination of dogs, but lives will be spared in the short-term by improving access to adequate and timely post-exposure prophylaxis. The effective intradermal, four-session, 1-month post-exposure prophylaxis Thai Red Cross regimen has been used successfully for decades. However, access would be improved by an abridged regimen, reducing doses and direct and indirect costs and improving vaccine equity through better sharing of the available vaccine. A previous systematic review by Kessels and colleagues of studies published between January, 2007, and June, 2017, identified seven published studies on post-exposure prophylaxis for rabies, all of which suggested that post-exposure prophylaxis schedules could be abridged. The conclusion of this systematic review was supported by a retrospective clinical study of an abridged regimen in Cambodia. Although the power of the study was limited, no significant difference was found in survival in patients who received three verses four or more sessions (with or without rabies immunoglobin) after a bite from a confirmed rabid or suspected rabid but untested dog.

Added value of this study

Our post-exposure prophylaxis effectiveness study done in Cambodia showed that neutralising antibody titres in rabies virus-exposed and unexposed patients were similar immediately before the fourth session (on day 28) and 2 weeks after the fourth session (on day 42). This finding was shown in patients of both sexes, all ages, and varied nutritional status. All patients were alive at 1 year.

Implications of all the available evidence

The fourth session of the Thai Red Cross post-exposure prophylaxis regimen on day 28 provides no additional benefit. Therefore, rabies post-exposure prophylaxis can be abridged to a three-session (on days 0, 3, and 7), 1-week Institut Pasteur du Cambodge regimen to improve vaccine coverage at no risk to patients. These findings contributed to the change in 2018 WHO recommendations for rabies post-exposure prophylaxis.

altered humoral immune response to the vaccine in the presence of the virus.[7] In addition, immune responses to vaccination might be affected by demographic factors, such as age, sex, ethnicity, and nutritional status.[8,9]

Therefore, we aimed to assess the immunogenicity of an abridged, 1-week, dose-sparing post-exposure prophylaxis protocol (intradermal 2-2-2-0) compared with the established 1-month Thai Red Cross post-exposure prophylaxis protocol (intradermal 2-2-2-2) in a cohort of Cambodian people bitten by either rabies virus-positive or rabies virus-negative dogs.

Methods

Study design and participants

The Rabies Elimination Support through Integrative Science and salvage Therapy-2 (RESIST-2) study (an integrated Pasteur research programme to help identify tools to support the fight against rabies) was a prospective, self-controlled, longitudinal study in patients who were self-referred to the Institut Pasteur du Cambodge vaccination centre after being bitten by a dog, had the biting dog's head (for rabies antigen detection to establish exposure status), and declared no previous vaccination against rabies. Written informed consent was obtained from patients (or legal representatives for patients aged ≤18 years) before prospective inclusion. Patients of all ages were eligible to participate in the study. The WHO-recommended, 1-month Thai Red Cross post-exposure prophylaxis protocol used routinely at Institut Pasteur du Cambodge was used in all participants during the course of this study. Participants included in this study were recontacted after 12 months for follow-up. A questionnaire for post-bite rabies risk analysis, which is collected routinely at the Institut Pasteur du Cambodge rabies centre, was completed. Additional characteristics, including sociodemographic, body-mass index (BMI), and mid upper-arm circumference (as a proxy for nutritional status), and clinical, epidemiological, biological, and immunological data, were collected during individual patient follow-up. Exposure to rabies virus was confirmed by analysis of brain tissue from the biting dog. This study was approved by Cambodia's National Ethics Committee (046 NEHCR).

Post-exposure prophylaxis

After lavage and antisepsis (povidone-iodine), we used the Thai Red Cross regimen of two 0·1 mL intradermal doses of post-exposure prophylaxis administered on day 0, day 3, day 7, and day 28 to the deltoid area as per recommendations[2] using insulin syringes. Post-exposure prophylaxis consisted of Vero cell-based rabies vaccine (Verorab; Sanofi, Lyons, France) that was reconstituted locally, refrigerated, and used within 4 h. Patients with confirmed rabies virus exposure also received highly purified equine rabies immunoglobulin at the same time as the first doses of the vaccine (day 0; Favirab; Sanofi, Lyons, France). A weight-adjusted dose (40 IU/kg bodyweight) of equine rabies immunoglobulin was carefully instilled in all wounds using a syringe, and any remaining dose was injected intramuscularly at a distance from the vaccination site, as per recommendations.[2] As needle size could affect immune response,[10] participants all received post-exposure prophylaxis with the same-size single-use needles and insulin syringes.[11]

Sample collection and processing

Two whole-blood samples were collected from participants before vaccinations on day 0, day 7, day 28, and day 42 (figure 1) using dry tubes and heparin tubes. Serum was isolated by centrifugation and stored at –80°C until analysis. Peripheral blood mononuclear cells were isolated by Ficoll-Paque (GE Healthcare, Chicago, IL, USA) density gradient centrifugation and washed twice in phosphate-buffered saline supplemented with bovine serum albumin. Cell viability was analysed by tryptan blue staining.

Vaccination is followed by an expansion of antigen-specific antibody-secreting cells in peripheral blood.[12] To analyse the presence of circulating plasmablasts, cells were counted and stained with antibodies (all Biolegend, San Diego, CA, USA): CD19-FITC (clone HIB19), CD27 APC-Cy7 (Clone O323), and CD38 APC (clone HB-7), and analysed on a BD FACSCanto II (BD Biosciences, Franklin Lakes, NJ, USA). Data were analysed with FlowJo software 9.3.2. Plasmablasts were identified by the expression of CD19 combined with high expression of CD38 and CD27.

Rabies virus neutralisation assay

Rabies virus-neutralising antibodies were detected using a modified rapid fluorescent focus inhibition test, recommended as the reference technique by WHO,[13] at the WHO rabies collaborating centre, Institut Pasteur, Paris, France. Briefly, a constant dose of cell culture-adapted rabies challenge virus, determined by a previous titration to give a percentage of cell infection between 80% and 95%, was incubated with a three-fold serial dilution of participants' sera. After incubation of the serum–virus mixtures for 1 h at 37°C in a humid atmosphere under 5% CO_2, a suspension of BHK-21 (CCL-10; ATCC; Manassas, VA, USA) cells was added. After 24 h of incubation at 37°C in a humid atmosphere under 5% CO_2, the cell monolayer was acetone-fixed and labelled with a fluoresceinated anti-rabies nucleocapsid antibody (Bio-Rad; Marnes-la-Coquette, France). Rabies virus-neutralising antibody titres in sera were calculated by comparison with a reference serum calibrated to the WHO reference serum.[14] The threshold of detection was 0·06 IU/mL. Negative values were set at 0·03 IU/mL for visualisation purposes. An antibody titre above the threshold of 0·5 IU/mL was considered a proxy for protection (a so-called protective titre).[4]

Rabies virus antigen detection in dogs

Exposure of participants to rabies virus was confirmed by technicians and investigators in the Institut Pasteur du Cambodge Virology Unit. Tissue samples extracted from the biting dogs' brains (hippocampus and medulla oblongata) underwent a standard direct fluorescent antibody test[15] using an anti-rabies nucleocapsid conjugate (Anti-Rabies Nucleocapsid Conjugate; Bio-Rad; Marnes-la-Coquette, France) and fluorescence microscopy.

Statistical analysis

We aimed to include at least 100 patients with confirmed rabies exposure and, as a control group, 20 patients with confirmed exposure to a rabies virus-negative dog. Statistical analysis was done using Prism (version 7.0). As the serological titres and immune cell counts were not

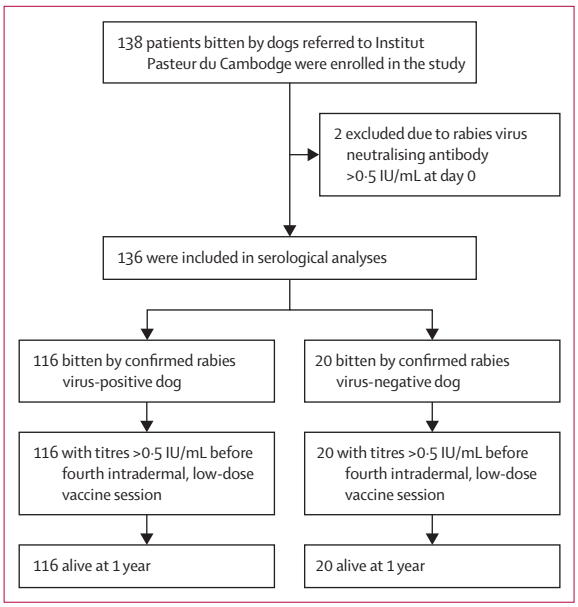

Figure 1: **Study profile**

	Unexposed group (n=20)	Exposed group (n=116)	p value
Sex	..	..	0·13
Male	7 (35%)	62 (53%)	..
Female	13 (65%)	54 (47%)	..
Age, years	11 (7–27; n=20)	17 (9–39; n=116)	0·22
Body-mass index (adults only)	NA	21·8 (20·2–24·6; n=57)	..
Mid-upper arm circumference, cm (children only)	16 (14–17; n=15)	16 (14–18; n=59)	0·65
Pregnant women	..	..	..
Yes	0	1/54 (2%)	1·00
No	13/13 (100%)	53/54 (98%)	..
Smoker	..	..	1·00
Yes	1 (5%)	10 (9%)	..
No	19 (95%)	106 (91%)	..
Recent other vaccine	..	..	0·31
No	18 (90%)	95 (82%)	..
Yes	2 (10%)	21 (18%)	..
Japanese encephalitis	0	3 (3%)	..
Meningitis	0	12 (10%)	..
Tetanus	1 (5%)	5 (4%)	..
Other	1 (5%)	1 (1%)	

Data are n (%) or median (IQR; number of participants with available data). p values were obtained by χ^2 or Fisher's exact tests or Student's *t* test or Wilcoxon rank-sum tests, where appropriate.

Table: **Demographic characteristics of patients included in the study**

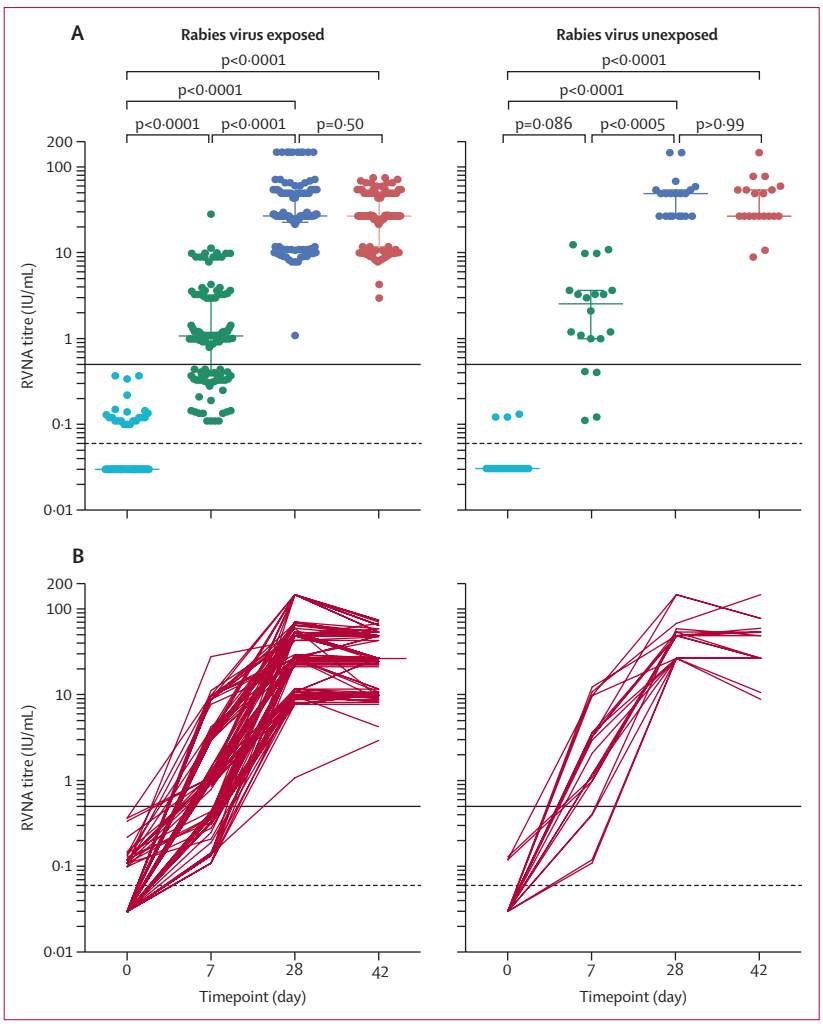

Figure 2: **Rabies virus neutralising antibody titres before and 2 weeks after the fourth post-exposure prophylaxis session in rabies-exposed and rabies-unexposed participants**

116 participants had been bitten by a rabies virus-positive dog and 20 by a rabies virus-negative dog. The black line indicates the 0·5 IU/mL antibody titre threshold considered to confer protection. The dashed line indicates the threshold of detection of the assay (0·06 IU/mL). Negative values were arbitrarily set at 0·03 IU/mL for visualisation purposes. (A) Neutralising antibody titres as determined by rapid fluorescent focus inhibition test; median and IQR are shown, with dots representing individual patients' samples. (B) Evolution of neutralising antibody titres per individual. RVNA=rabies virus neutralising antibody.

normally distributed, non-parametric tests were used. The Wilcoxon matched-pairs signed rank test was used to compare longitudinal rabies virus neutralising antibody concentrations and circulating plasmablasts within individuals between timepoints. The Mann-Whitney *U* test, Fisher's exact test, or Student's *t* test were used to compare rabies virus neutralising antibody titres or immune status between individuals who were bitten by a rabies virus-positive dog and those who were bitten by a rabies virus-negative dog and between adults and children. χ^2 test was used to compare proportions between unmatched samples and McNemar's χ^2 test was used for matched samples. To eliminate further confounding due to rabies virus status, we included only individuals who had been bitten by a rabies virus-positive dog in this analysis. Correlations between rabies virus neutralisation antibody titres and age and titres and BMI were evaluated using linear regression analysis. A statistical significance threshold of less than 0·05 was used in all computations and 95% CIs are shown for point estimates of effect size.

Role of the funding source

The sponsors of this self-funded study (the writing team) were responsible for study design, data collection, data analysis, data interpretation, and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

20 individuals bitten by dogs negative for rabies virus and 116 individuals bitten by dogs positive for rabies virus were included in the study (figure 1). The first patient was included on April 20, 2016, and the last on Feb 9, 2018. Demographic characteristics of patients are shown in the table. All had sustained a category III exposure as defined by WHO.[16] Two patients had baseline rabies virus neutralising antibodies of 0·5 IU/mL or more and were excluded from the serological study (figure 1). All patients were alive after 12 months or more (median follow-up 27·5 months; IQR 22·3–31·3; range 14·4–35·2).

The median rabies virus neutralising antibody concentration in the group of individuals bitten by rabies virus-positive dogs was 1·08 IU/mL (IQR 0·37–3·09) at day 7, 26·86 IU/mL (22·68–49·50) at day 28, and 26·74 IU/mL (11·78–49·06) at day 42. In patients bitten by rabies virus-negative dogs, median titres were 2·54 IU/mL (IQR 0·99–3·64) at day 7, 49·5 IU/mL (26·86–54·5) at day 28, and 26·86 IU/mL (26·86–54·50) at day 42 (figure 2A). Titres differed significantly in both patient groups between day 0 and day 7 and between day 7 and day 28. No differences were observed in titres between day 28 and day 42, after titres reached a plateau (figure 2). Whereas 46 of 136 (34%, 95% CI 26–42) participants did not reach protective titres by day 7, all (100%, 97–100%) participants had rabies virus neutralising antibody titres of 0·5 IU/mL or more at day 28, immediately before the last immunisation session (p<0·0001).

Although baseline rabies virus neutralising antibody titres were similar between individuals who were bitten by a rabies virus-positive dog and those who were not, all 136 participants had rabies virus neutralising antibody titres of 0·5 IU/mL or more at day 28 (figure 3). However, individuals bitten by rabies virus-positive dogs and receiving equine rabies immunoglobulin showed significantly and consistently lower rabies virus neutralising antibody titres at various timepoints than individuals bitten by rabies virus-negative dogs who did not receive equine rabies immunoglobulin (figure 3).

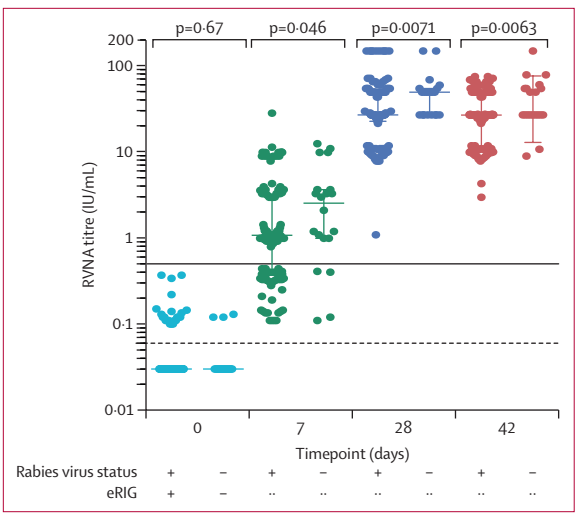

Figure 3: **Rabies neutralising antibody titres in patients exposed versus unexposed to rabies, stratified by eRIG status**
116 participants had been bitten by a rabies virus-positive dog and 20 by a rabies virus-negative dog. Median and IQR are shown, with circles representing individual patients' samples. The black line indicates the 0·5 IU/mL antibody titre threshold considered to confer protection. The dashed line indicates the threshold of detection of the assay (0·06 IU/mL). Negative values were arbitrarily set at 0·03 IU/mL for visualisation purposes. eRIG=equine rabies immunoglobulin. RVNA=rabies virus neutralising antibody.

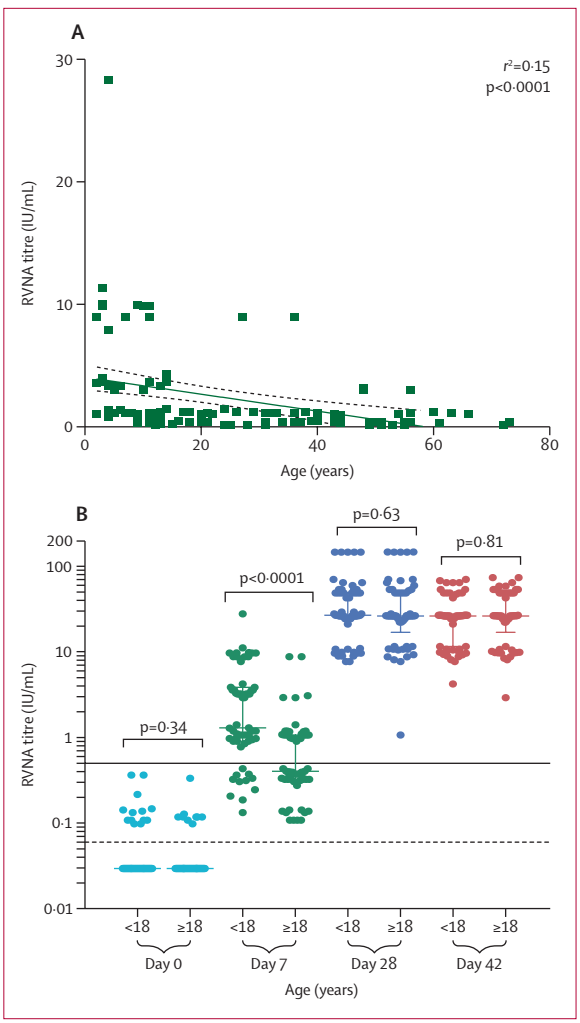

Figure 4: **Association between age and rabies neutralising antibody titres**
(A) Association between neutralising antibody titres measured at day 7 and age; the dashed line indicates 95% CIs. (B) Stratification of neutralising antibody data by age. Median and IQR are shown, with circles representing individual patient titres. The black line indicates the 0·5 IU/mL antibody titre threshold considered to confer protection. The dashed line indicates the threshold of detection of the assay (0·06 IU/mL). Negative values were arbitrarily set at 0·03 IU/mL for visualisation purposes. Only individuals bitten by dogs with confirmed rabies are included (n=116).

We assessed the effect of other demographic parameters on the development of rabies virus neutralising antibody titres. Our consecutive cohort (ie, pateints were included as they presented to the centre) included people aged 2–73 years (table). We observed a significant correlation at day 7 between age and rabies virus neutralising antibody titres, although the goodness-of-fit was low (r^2=0·13; figure 4A). This correlation was confirmed by comparing rabies virus neutralising antibody titres in adults and children: at day 7, titres reached a median of 1·32 IU/mL (IQR 0·92–3·93) in children versus 0·41 IU/mL (0·33–1·09) in adults (p<0·0001). Consequently, 48 of 59 (81%, 95% CI 69–89%) children developed protective antibody titres by day 7, compared with only 26 of 57 (46%, 33–58%) adults (p<0·0001; figure 4B), although this difference disappeared at later timepoints.

We found no significant correlation between BMI and rabies virus neutralising antibody titres in adults (appendix p 2). Similarly, no significant differences in rabies virus neutralising antibody existed after stratification by BMI status in children (data not shown). Stratifying children by nutritional status also did not show any differences in rabies virus neutralising antibody titres at various timepoints (appendix p 2). The cohort included one pregnant woman who was bitten by a rabies virus-positive dog and who developed adequate protective titres by day 28 (10·91 IU/mL), which remained stable to day 42 (9·91 IU/mL).

Plasmablast percentages increased significantly between day 0 and day 28 in patients bitten by rabies-virus positive dogs (21 days after the third immunisation session; appendix p 3). There were no differences in percentages of plasmablasts between day 28 and day 42 (appendix p 3). These data indicate that peak humoral immune activation was reached after three immunisation sessions (on day 0, day 3, and day 7), with no change following the fourth immunisation session on day 28.

See Online for appendix

Discussion

We found that all individuals who were treated with the Thai Red Cross post-exposure prophylaxis regimen at our institution in Cambodia and tested for immune responses reached rabies virus neutralising antibody titres considered protective (according to WHO guidelines)[4] by day 28. More importantly, however, we did

not observe higher rabies virus neutralising antibody titres 14 days after the fourth immunisation on day 28 than before that immunisation session. Although this observation of immunogenicity cannot replace an observation of clinical efficacy, rabies virus neutralising antibody titres did not increase further after the fourth immunisation session on day 28 and all participants were alive at 1 year.[17] Taken together, these results indicate that the Thai Red Cross post-exposure prophylaxis regimen might be abridged to a two-dose, three-session, 1-week regimen.

Abridged protocols must be evaluated to improve patient compliance, reduce direct and indirect costs, and increase equitable access to available vaccines, especially in resource-constrained countries.[16] Although a few other studies of abridged regimens for rabies post-exposure prophylaxis have been done,[5] none have assessed the dose-sparing protocol of two intradermal vaccine injections per session in children, adults, and older people with confirmed rabies virus exposure. All but two studies were done in healthy adults.[5] Narayana and colleagues[18] showed that adult patients bitten by dogs with suspected rabies developed protective rabies virus neutralising antibody titres 14 days after a 1-week, three-session protocol with four intradermal injections per session, either with chicken embryo-based or Vero-cell-based vaccines. Another study measured titres 14 days after initiation of the Thai Red Cross protocol following exposure of adults to dogs with suspected but unproven rabies, and the authors reached similar conclusions about the immunogenicity.[19]

The effect that an abridged protocol might have on persistence of rabies virus neutralising antibody titres and anamnestic response over time remains to be evaluated. Studies measuring protective rabies virus neutralising antibody titres without boosters have been done at most 1 year after abridged post-exposure prophylaxis protocols in either healthy individuals or individuals bitten by suspected rabid but untested dogs.[5,18] After vaccination, the height of the antibody response is not an established proxy for duration of protection. In any case, rabies post-exposure prophylaxis administered after a given exposure is protective for that exposure. If subsequent exposure to a rabid dog was to occur, recommended booster vaccination will cause a protective anamnestic response in the future.

Only patients bitten by rabies-confirmed dogs received equine rabies immunoglobulin in our study. We observed lower rabies virus neutralising antibody titres at all timepoints after vaccination in patients who were bitten by rabies virus-positive dogs than in those bitten by rabies virus-negative dogs. Concurrent equine rabies immunoglobulin administration is known to dampen the immune response to rabies post-exposure prophylaxis.[20] Whether rabies virus evades or suppresses vaccine-induced immune responses is unknown.[21] Other mechanisms, such as direct rabies virus-induced B-cell death or activation-induced B-cell apoptosis, might be used by the virus to impair neutralising antibody responses and should be investigated further.[22] Whichever the mechanism, all participants to our study had protective neutralising antibody titres immediately before the fourth vaccine session.

The youngest children included in our cohort were aged 2 years; at this age the risk of dog bites increases due to childrens' increasing mobility and independence. Moreover, because of their height, younger children are more likely than older children and adults to be bitten on the face, a highly innervated anatomical area that is associated with a greater risk of rabies virus transmission and infection. Evaluating the safety and immunogenicity of an abridged Thai Red Cross protocol in children is therefore crucial. At day 7, children displayed a better vaccine response than adults. These differences in titre due to age disappeared by day 28, the third vaccination session.

Overweight and obesity has been associated with impaired vaccine responses to several vaccines.[23] We found no association between rabies virus neutralising antibody titres and BMI, whether in adults or children. These data are in accordance with previous findings showing that rabies virus neutralising antibody responses were not different in patients with and without obesity who had WHO category III exposure and received equine rabies immunoglobulin and post-exposure prophylaxis.[24]

Present in small percentages in peripheral blood, plasmablasts are indicative of the total antibody-secreting cell pool in response to infection or vaccination. Although we did not investigate antigen-specific plasmablast responses in our cohort,[25] we observed higher percentages of plasmablasts at day 28 and day 42 than at day 0, suggestive of an ongoing humoral immune response to the vaccine already at day 28.

The Rabies Vaccination Centre at Institut Pasteur du Cambodge provides post-exposure prophylaxis to over 21 000 patients per year bitten by suspected rabid animals using the Thai Red Cross regimen.[26] We consecutively included individuals managed at the vaccination centre who brought the head of a suspected rabid dog for testing, for which RABV status was confirmed within a few hours. This cohort therefore reflects the real-life situation faced in low-income countries such as Cambodia, and includes individuals of various demographics, such as children, adults, older people, pregnant women, and underweight or overweight individuals of both sexes. Hence, the results of this study can be more easily generalised and extrapolated to the at-risk population worldwide.

Our study has some limitations. First, self-controlled studies can overestimate effects compared with randomised controlled trials.[27] It would have been unethical, however, to randomly assign patients to a potentially risky abridged rabies post-exposure prophylaxis regimen when an internationally endorsed four-session regimen is effective and routinely used. Furthermore, a

self-controlled study design automatically adjusts for potential confounders. Second, 14 patients chose not to participate. These patients did not differ significantly from the included patients nor from the rabies-exposed Cambodian patients who attend our rabies post-exposure prophylaxis clinic (data not shown). Third, our study participants received only the Vero-cell-based vaccine. However, all currently used, WHO-prequalified, cell-based vaccines have similar potency and provide clinically equivalent responses.[16] Fourth, our sample size of 136 patients might not have been sufficient to detect the rare event of rabies vaccine non-response. Previous studies[5,18] assessing rabies virus neutralising antibody titres with abridged protocols, however, were done either in healthy adults or in adults bitten by dogs with suspected but unproven rabies and had similar group sizes to our study.

This study is complemented by a retrospective study on clinical outcome after at least 6 months, which found no significant difference in survival among patients who received three versus four intradermal post-exposure prophylaxis sessions (with or without rabies immunoglobulin).[17] Taken together, our studies clearly show that the current four-session, 1-month, two-site intradermal regimen can be reduced to a three session, 1-week, two-site intradermal regimen at no detectable added risk to patients Based on these results from the Institut Pasteur du Cambodge, WHO endorsed the abridged regimen in their April, 2018, guidelines.[16] This so-called Institut Pasteur du Cambodge protocol is the first 1-week post-exposure prophylaxis regimen to be recommended for rabies. The Institut Pasteur du Cambodge protocol is to date the shortest and most vaccine-sparing rabies post-exposure prophylaxis protocol endorsed. Above all, it will reduce the direct costs of vaccination and indirect costs to patients such as transportation, and therefore constitutes the most cost-effective regimen for rabies post-exposure prophylaxis.[28,29]

Contributors

TC, LK, SU, and HB did the immunological experiments. LB, CL, and SL recruited patients, collected data, and monitored patient inclusion and follow-up. SI, YP, CP, CH, CNT, and MS informed patients, did the post-exposure prophylaxis, and provided the clinical monitoring data. SO, CM, RC, and PD performed virological testing in dogs. LB and SL managed the ethical board submission process. TC, LB, PD, SL, and HB interpreted the data and co-wrote and edited the manuscript. AT did the literature search, developed the research question and study design, identified funding sources, interpreted the data, and co-wrote and edited the manuscript.

Declaration of interests

HB has patents for antibodies that potentially neutralise rabies virus and other lyssaviruses and uses thereof (PCT/EP2014/003076 [Nov 18, 2014]; PCT/EP2015/002305 [July 11, 2016]), a US provisional patent application for phtazolinone derivatives for use in the treatment and prevention of rabies (62/404,435; Oct 5, 2016), and a patent for antibodies and methods for treatment of lyssavirus infection (PCT/EP2018/078751; Oct 19, 2018). AT declares that the Institut Pasteur in Cambodia received non-nominative grants from Sanofi to develop rabies prevention materials for patients bitten by dogs and World Rabies Day information campaigns for the general public. TC is funded by the Institut Pasteur International Network and the Howard Hughes Medical Institute (HHMI)/Wellcome Trust International Reseach Scholars Program. All other authors declare no competing interests.

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