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Research article

Influencing factors and prediction of neutralizing antibodies in post-exposure rabies vaccine recipients

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ABSTRACT

To assess the factors that influencing the persistence of virus neutralizing antibody (VNA), and to establish prediction models to provide the appropriate timing for booster administration, a cohort of post-exposure rabies vaccine recipients was investigated. The VNA determined records from 2019 to 2023 and interrelated factors were analyzed, including gender, age, rabies immunoglobulin (RIG) administration, vaccine products, vaccination schedule, and vaccination intervals etc. The geometric mean of VNA titre within 1 month after primary vaccination with 2-1-1 schedule was statistically higher than that with 5-dose course ($P = 0.031$). The interaction between exposure and vaccination schedule was observed on primary vaccination, which showed that a decrease of 19.74 % (95 % CI: 5.99%–64.95 %, $P = 0.008$) of VNA titre among vaccinee with 5-dose and exposure III. Individuals with RIG administration produced lower VNA titres than those without RIG administration ($P = 0.001$). Vaccine products (Chengda, $P = 0.015$; human diploid cell, $P = 0.026$) and re-exposed time ($P = 0.000$) exhibited independent effects following booster vaccination. Based on the prediction model, the 99 % individual prediction intervals (IPI) of VNA titres were established at 3, 6, 12 and 18 months for the 12 characteristic populations respectively. The cases of VNA below 0.5 IU/ml first appeared at 6 months in group D of primary vaccinations and at 10 years in group F of boosters. We conclude that for primary vaccination 2-1-1 schedule is more efficient than 5-dose; the use of residual rabies immunoglobulin for distal intramuscular injection isn't recommended. The 99 % IPI of VNA titres could provide the appropriate timing for booster vaccination.

1. Introduction

Rabies is a zoonotic disease caused by infection with rabies virus (RABV), characterized by specific symptoms such as anemophobia, hydrophobia, pharyngeal muscle spasms, and progressive paralysis. It currently holds the highest fatality rate among infectious diseases worldwide, with a 100 % case fatality rate, once clinical symptoms are observed [1–3]. Nearly 99 % of human rabies cases occur in developing countries, mainly due to the low economic development, general lack of rabies prevention awareness, and extremely low animal vaccination rates [4,5]. In recent years, rabies has been one of the top 3 causes of death from infectious diseases in China. The incidence of rabies in China is second only to India [6].

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Although rabies is almost invariably fatal, it can be 100 % effectively prevented by post-exposure prophylaxis (PEP) when administered immediately and appropriately after exposure [7]. The relatively long incubation period of rabies (usually 1–3 months) provides a favorable window for PEP [8,9]. PEP is administered promptly following exposure to rabies, and consists of timely and rigorous wound care, administration of rabies immunoglobulin (RIG) in severe exposures, and a series of injections of rabies vaccines [10]. Rabies vaccination confers complete protection against RABV infection that is due to the presence of virus neutralizing antibody (VNA) targeted against the RABV glycoprotein [11]. VNA plays important roles in preventing the invasion of RABV into peripheral nerves at the site of exposure and subsequent transport to the brain [12]. The WHO Expert Advisory Committee on Rabies believes that individuals are only protected when their neutralizing antibody levels are equal to or above 0.5IU/ml; if the level is found to be below 0.5IU/ml, booster immunization should be given until the protective level is reached [13]. PEP for entire populationsveryone develops sufficient resistance to the rabies virus after complete vaccination. Therefore, assessment of the key factors influencing human VNA titres after post-exposure rabies vaccination is crucial for effective prevention and control measures.

In this study, human immunity was investigated in a cohort of subjects received post-exposure rabies vaccination, to assess the independent effects and interactions of factors that may influence VNA titre. Factors including gender, age at vaccination, animal, exposure site, type of contact, RIG administration, vaccine products, vaccination schedule, and vaccination intervals were investigated. Of particular interest was an establishment of forecast models for VNA titre decline prediction, which can provide an insight into the recommended time for booster administration to maintain antibody protection in vaccinated subjects with different characteristics.

2. Methods

2.1. Sample population

Data were derived from a sample population of post-exposure rabies vaccine recipients who were tested for VNA titre in Hangzhou Hospital for the Prevention and Treatment of Occupational Disease from 2019 to 2023. Based on the voluntary principle, the detection time and frequency of VNA titre was random for all subjects. Blood samples were tested for VNA titre using the accredited and validated rapid fluorescent focus inhibition test (RFFIT) recommended by WHO [14]. A retrospective cohort study was conducted. A total of 1123 records were collected, of which 40 records were eliminated due to duplicates, incompletions, or logical errors. Therefore, 1083 records including 908 subjects were finally available for assessment. Records of individual factors were unknown or missing in some cases. Of the 908 subjects, 101 had two or more VNA detection records at different vaccination intervals. The earliest detection time was the day of vaccination completion, and the latest detection time was 218.72 months (about 18 years) since vaccination completion.

In this study, 386 subjects completed rabies vaccination for the first time (referred to as primary vaccination), and 522 subjects had ever received full-course vaccination of rabies vaccine and were re-immunized after re-exposure (referred to as booster vaccination). Current PEP regimens administered by the departments of outpatient in China, are based on the recommendations of the "Guidelines for the Prevention and Disposal of Rabies Exposure (2009 Edition)" [15]. For exposed persons who have never been vaccinated against rabies, rabies vaccination should be administrated as soon as possible and following the 5-dose course: one injection per day on days 0 (injection day), 3, 7, 14 and 28. For re-exposed persons who have been completely vaccinated against rabies, the vaccination schedules are as follows: (i) when re-exposed within 6 months, there is no need for re-immunization; (ii) when re-exposed within 6 months to 1 year, additional doses should be administered on days 0 and 3; (iii) when re-exposed within 1–3 years, additional doses should be administered on days 0, 3 and 7; (iv) when re-exposed over 3 years, a full vaccination schedule should be administered. Since 2016, according to the recommendations of the Technical Guidelines for Human Rabies Prevention and Control (2016) formulated by Chinese Center for Disease Control and Prevention [16], the 2-1-1 schedule has been implemented as another full vaccination schedule in addition to the 5-dose course. The 2-1-1 schedule implies bilateral application of the vaccine on day 0, with subsequent single doses on days 7 and 21. Of course, the guidelines stipulate that the 2-1-1 schedule only applies to rabies vaccine products that have been approved for use with the 2-1-1 schedule in China.

2.2. Data analysis

The value of VNA titre was normalized by converted into $\log_3$ (titre) to make the data conform to normal distribution and meet the requirements of the statistical analysis method [17]. The geometric mean of VNA titre was calculated and performed antilogarithmic conversion to facilitate the presentation of reported results. The $\log_3$ (titre) was taken as the dependent variable to compare the different characteristics between primary vaccination and booster vaccination with Univariate Analysis. These characteristics included gender (female/male), age (<30/30+), vaccination place (the local city/outside the local city, local province/outside the local province), animal (cat/dog/rat/bat/others), origin of animal (domestic animal/homeless or wild animal), exposure site (lower limbs/head and neck/upper limbs), type of contact (I/II/III), vaccine products (Ningbo RongAn/Liaoning Chengda/human diploid cell rabies vaccine, HDCV/others), and RIG administration (yes/no). Through this analysis, the effect of each factor on the VNA titre can be observed. After adjusting the effect of factors with statistical significance ($P < 0.05$), the independent effect and interaction of factors were further analyzed.

2.3. Statistical analysis

A multiple regression model of $\log_3$ (time) was then established with the statistically significant variables ($P < 0.05$). Statistical

prediction was performed using the regression model, in which the independent variables were substituted into the regression model and the individual values of the dependent variables were estimated. Given the value of the independent variable x_p, the corresponding predicted value of individual y also has a fluctuation range, and its standard deviation $S_{y \mid x_p}$ is:

$$S_{y \mid x_p} = S_{y \bullet x} \sqrt{1 + \frac{1}{n} + (x_p - \hat{x})^2 \Big/ l_{xx}}$$

So when $x = x_p$, then two-sided (1-α) prediction intervals for y-values is:

$$\hat{y}_p \pm t_{\alpha/2,\nu} S_{y \mid x_p}$$

The individual prediction intervals (IPI) of the dependent variable indicate that 100 individuals sampled randomly at a fixed x, there will be 100 × (1-α) individuals whose y-values fall within the range found on average [18]. IPI of VNA titres was used to evaluate the appropriate time for booster vaccination of populations with different characteristics. The independent variables in the model with different levels of value represent different characteristics of the population. For example, vaccination type = 0 indicates the primary vaccination group, and the value 1 indicates the booster vaccination group. In the established prediction model, the values of each independent variable are combined to form a prediction group with different characteristics. Then the model estimation of the dependent variables of each prediction group can obtain IPI of the VNA titer at a specific time. The lower limit of IPI reflects the lowest level of VNA titers in the prediction group at a specific time. If the lower limit of IPI is below 0.5 IU/ml at a specific time, it indicates that the prediction group needs booster immunization at the specific time. In our research, 99 % IPI was calculated for VNA titres over time (3, 6, 12 months for primary vaccinations and 5, 10, 20 years for booster vaccinations). It should be noted that 101 of the 908 subjects had repeated VNA detection records. Considering that the repeated detection records did not conform to the independence assumption of the linear regression model, only the first detection records were selected into the model fitting. Independent variables were entered into the model in Stepwise way. *Wald* χ^2 test was used for variable screening with $\alpha = 0.05$. SPSS16.0 software was used for statistical analysis.

3. Results

3.1. Baseline characteristics

Of the 908 subjects assessed, 582 were male (64.10 %) and 326 were female (35.90 %). At the first detection records, a total of 483 subjects were aged under 30 years (53.19 %), and 425 subjects were aged 30 years or above. The majority of these subjects came from

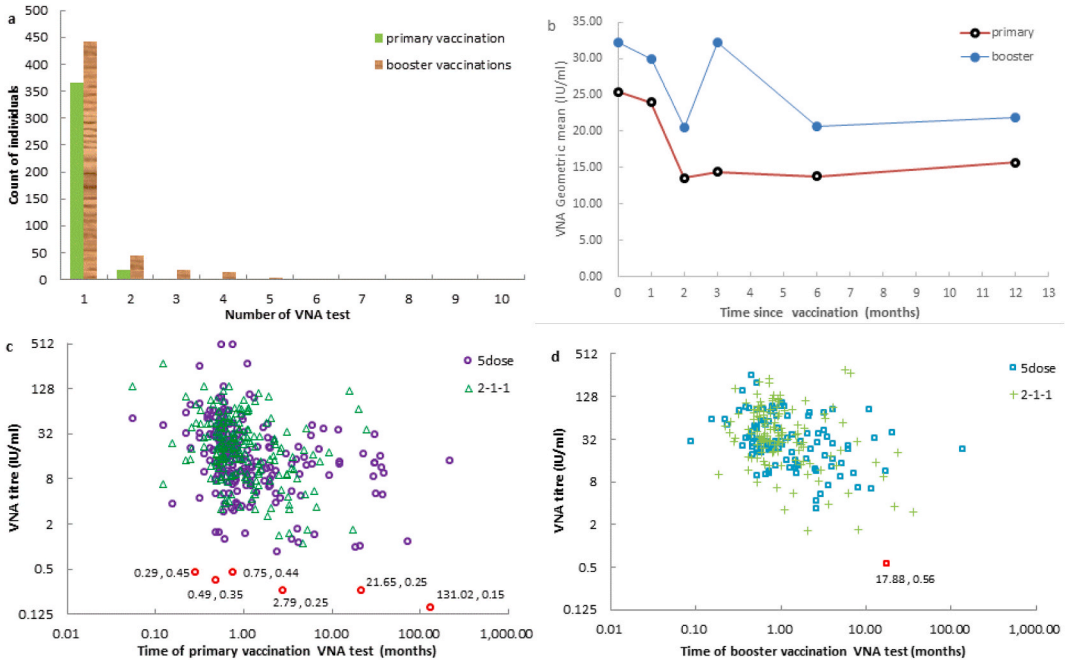

Fig. 1. (a) Distribution of the number of VNA test for individuals with primary vaccination and booster vaccination, and (b) the profile of VNA titres over time for repeated detection individuals, and (c) VNA titres (IU/ml) over time following primary vaccination with 5-dose course and 2-1-1 schedule, and (d) VNA titres (IU/ml) over time following booster vaccination with 5-dose course and 2-1-1 schedule, those who were re-exposed after more than 3 years.

the local city (35.46 %) or the provinces that outside the local province (39.10 %). Primary vaccination data was available for 386 subjects (42.51 %), whilst booster vaccination data was available for 522 subjects (57.49 %). There were 51 VNA repeated detection records in the 24 subjects administrated primary vaccinations, and 225 repeated records in the 77 subjects administrated boosters. The number of VNA detection tests for individuals with primary vaccination and booster vaccination is shown in Fig. 1a. The Maximum number of VNA tests per individual was 6 for primary vaccination, and 9 for booster vaccination. The majority of individuals received only one VNA test (94.56 % and 84.67 % for primary vaccinations and boosters, respectively), with a mean detection time of 1.40 months (95 % *CI*: 1.29–1.51 months).

The main animals causing human injuries were dogs (64.65 %), followed by cats (29.52 %). Most of the animals were domesticated animals (56.06 %), and homeless/wild animals accounted for 18.72 %. The most common injury sites were upper limbs (50.66 %) and lower limbs (44.60 %). Individuals with category I + II exposure accounted for 71.26 %. Two top vaccine products injected were Chengda vaccine (61.12 %) and HDCV (18.28 %). Only 14.87 % of the subjects had administrated with RIG. There was a similar vaccination rate for 2-1-1 schedule and 5-dose course in both primary vaccination (46.63 % vs. 53.37 %) and booster vaccination (24.52 % vs. 20.69 %). Among the rabies re-exposed subjects, most were re-exposed for more than 3 years (45.21 %), followed by re-exposure for 6 months to 1 year (39.27 %).

3.2. VNA response following primary vaccination

The geometric mean of VNA of 386 individuals with primary vaccination was 16.69 IU/ml (95%*CI*: 14.82–18.80 IU/ml). The longest period of VNA detection since complete primary vaccination was 218.72 months (about 18 years), with a mean of 1.07 months (95%*CI*: 0.96–1.19 months). For the 24 individuals with repeated detection records since primary vaccination, the shortest and longest follow-up time were 4 days and 38.49 months, respectively. And the minimum and maximum values for VNA titre were 0.85 IU/ml and 118.45 IU/ml, respectively. The profile of VNA titres over time for those individuals is shown in Fig. 1b (red line), which demonstrates that VNA titres peaked in the first month since vaccination, then declined rapidly in the second month, and remained stable until the 12th month.

A summary of VNA titres following primary vaccinations is shown in Fig. 1c. The horizontal and vertical axes have been set to logarithmic scale, to display the VNA titres at all time points. Regardless of the 2-1-1 schedule or the 5-dose course, the majority of the data points were distributed in the region near the first month (VNA titres = 32 IU/ml). This indicates that a high enough VNA titres occurred in less than 1 month since full primary vaccination (2-1-1 schedule: $P_5 = 3.18$ IU/ml, $P_{95} = 91.58$ IU/ml; 5-dose course: $P_5 = 1.19$ IU/ml, $P_{95} = 87.25$ IU/ml). However, there are six data points (red circles in Fig. 1c) distributed below 0.5 IU/ml, which occurred at 0.29, 0.49, 0.75, 2.79, 21.65, and 131.02 months since full vaccination, respectively. Among them, 3 cases occurred within 1 month and were all administrated with the 5-dose course. The VNA titres (IU/ml) following primary vaccination within 1 month were used as the approximate value of immediate titre to compare the serum VNA positive rate (VNA titre $\geq$0.5 IU/ml) of the two vaccination schedules [19]. Of the 180 individuals vaccinated with 2-1-1 schedule, 118 detected their VNA titres within 1 month since primary vaccination completed, and the VNA positive rate reached to 100 % (118/118). While, of the 206 individuals vaccinated with 5-dose course, 140 had VNA detection records within 1 month after full primary vaccination, and their VNA positive rate was 97.86 % (137/140). Fisher's Exact Test showed that there was no significant difference observed in VNA positive rates between the two vaccination schedules ($P = 0.253$). However, the geometric mean of VNA detected within 1 month following primary vaccination with 2-1-1 schedule was statistically higher than that with 5-dose course (25.21 IU/ml vs. 18.96 IU/ml, $P = 0.031$).

In addition, rabies antibody response following primary vaccination significantly differed in the factors including the origin of animal, type of contact, and RIG administration (see Table 1). Individuals injured by homeless/wild animals developed significantly higher VNA titres than those injured by domesticated animals ($P = 0.031$). Individuals suffering from category III exposure had lower VNA titres than category I + II ($P = 0.000$). And individuals administrated with RIG produced lower VNA titres than those without RIG administration ($P = 0.001$). After adjusting these factors, only the origin of animal was still statistically significant ($P = 0.045$), which indicates that the effects of these factors on VNA titres do not exist independently, but interact with other factors. Compared to category I + II exposure, individuals suffering from category III exposure had a significantly VNA titres decrease of 19.74 % (95%*CI*: 5.99%–64.95 %, $P = 0.008$) and 22.15 % (95%*CI*: 4.31%–113.87 %, $P = 0.071$) for the 5-dose course (Supplementary Fig. a) and RIG administration (Supplementary Fig. b), respectively.

3.3. VNA response following booster vaccination

The mean of VNA titres in 522 subjects administrated booster vaccination was 29.16 IU/ml (95%*CI*: 26.91–31.59 IU/ml), higher than that in subjects with primary vaccination (16.69 IU/ml, 95%*CI*: 14.82–18.80 IU/ml) ($P = 0.000$). The longest VNA detection time since booster vaccination was 133.42 months (about 11 years), with a mean of 1.64 months (95%*CI:* 1.48–1.82 months). For 77 subjects with repeated VNA detection following booster vaccination, the shortest follow-up time was 0.12 months (about 4 days), and the longest follow-up time was 93.15 months (about 7+years). The detected VNA titres for these subjects were the lowest at 1.66 IU/ml and the highest at 371.99 IU/ml. The geometric mean of VNA titre (IU/ml) over time of these individuals is shown in Fig. 1b (blue line). Unlike the primary vaccination, there were two peaks of VNA titres following booster vaccination, occurring within the first month and in the third month, respectively. Although the rabies antibody response following booster vaccination was higher in each period than the primary vaccination, the rapid decline trend in the second month post-vaccination was consistent (the line segment from the first month to the second month was almost parallel). After reaching its peak in the third month, the VNA titres declined slowly over the next three months and then stabilized since the 6th month until the 12th month. In 191 subjects re-exposed for more than 3 years, the

Table 1
Geometric mean of VNA titre following primary vaccination in subjects with different characteristics.

Variable	Level	N	Geometric mean (95 % *CI*)	Fold effect (95 % *CI*)	*P*-value	Adjusted fold effect[a] (n = 280)	*P*-value
All		386	16.69 (14.82, 18.80)				
Gender	male	229	17.36 (14.99, 20.11)	Baseline			
	female	157	15.76 (12.90, 19.25)	−0.09 (-0.31, 0.13)	0.432		
Age	<30	214	17.99 (15.34, 21.10)	Baseline			
	30+	172	15.21 (12.73, 18.17)	−0.15 (-0.37, 0.06)	0.167		
Animal	cat	94	20.20 (15.89, 25.69)	Baseline			
	dog	268	15.32 (13.29, 17.66)	−0.25 (-0.51, 0.00)	0.052		
	rat	13	27.14 (14.22, 51.80)	0.27 (-0.36, 0.90)	0.401		
Origin of animal	Domesticated animal	223	15.77 (13.43, 18.51)	Baseline		Baseline	
	Homeless/wild animal	70	22.62 (16.99, 30.12)	0.33 (0.30, 0.63)	**0.031**	0.31 (0.01, 0.62)	**0.045**
Exposure site	Lower limbs	172	18.55 (15.53, 22.15)	Baseline			
	head and neck	13	13.71 (7.19, 26.15)	−0.28 (-0.89, 0.33)	0.375		
	upper limbs	196	15.66 (13.26, 18.49)	−0.15 (-0.38, 0.07)	0.172		
type of contact	I + II	259	19.42 (16.83, 22.42)	Baseline		Baseline	
	III	112	11.98 (9.63, 14.90)	−0.44 (-0.68, −0.20)	**0.000**	−0.34 (-0.73, 0.05)	0.089
Vaccine product	RongAn	20	13.76 (8.20, 23.09)	Baseline			
	Chengda	235	17.96 (15.44, 20.89)	0.24 (-0.25, 0.73)	0.332		
	HDCV	79	16.35 (12.61, 21.22)	0.16 (-0.37, 0.69)	0.558		
	Others	18	13.53 (7.84, 23.35)	−0.02 (-0.70, 0.67)	0.965		
Vaccine schedule	2-1-1 schedule	180	19.39 (16.31, 23.06)	Baseline		Baseline	
	5-dose course	206	14.64 (12.46, 17.21)	−0.26 (-0.47,-0.04)	**0.020**	−0.18 (-0.44, 0.09)	0.188
Vaccination agency	Outside local Province	151	15.49 (12.82, 18.72)	Baseline			
	Local city	132	17.10 (13.96, 20.94)	0.09 (-0.16, 0.34)	0.484		
	Outside local city- local province	102	18.50 (14.70, 23.30)	0.16 (-0.11, 0.43)	0.242		
RIG administration	No	276	19.00 (16.53, 21.83)	Baseline		Baseline	
	yes	102	11.85 (9.42, 14.90)	−0.43 (-0.67, −0.19)	**0.001**	−0.16 (-0.57, 0.25)	0.432

[a] Adjusted for type of contact, RIG administration, origin of animal, and vaccination schedule.

distribution of their antibody responses following booster vaccination was observed similar to primary vaccination (Fig. 1d). The horizontal and vertical axes have been set to logarithmic scale, to display the VNA titres at all time points. The lowest VNA titre following booster vaccination was 0.56 IU/ml (approaching to the cutoff value of 0.5 IU/ml), which was detected in the 17.88 months (about 1.5 years) since the 5-dose course vaccination completed (data point indicated by the red box in Fig. 1d). While for the 2-1-1 schedule, the lowest VNA titre was 1.66 IU/ml detected in the 2.05 months (62 days), and no data points below or near 0.5 IU/ml were found during the longest follow-up time of 35.95 months (about 3 years). There were 70 out of 109 individuals administrated with 2-1-1 schedule and 46 out of 82 individuals administrated with 5-dose course that had VNA detection records within 1 month since booster vaccination. And no significant difference was observed in the geometric mean of VNA titres between the two vaccination schedules (2-1-1 schedule: 50.07 IU/ml, 5-dose course: 43.24 IU/ml, $P = 0.299$).

Rabies antibody response following booster vaccination was significantly influenced by vaccine products and re-exposure time (Table 2). There was evidence of differences by vaccine products due to the higher VNA titre observed for the subjects who received Chengda vaccine ($P = 0.021$) and HDCV vaccine ($P = 0.042$) when compared to those vaccinated with other vaccine products. Additionally, the VNA titre following booster vaccination in individuals re-exposed for less than 1 year was observed statistically lower than those re-exposed for more than 3 years ($P = 0.000$). After calculating the adjusted fold effect, factors of Chengda vaccine ($P = 0.015$), HDCV vaccine ($P = 0.026$) and re-exposed for less than 1 year ($P = 0.000$) still showed statistically significant, which indicates that these factors had independent effects on the VNA titres following booster vaccination.

3.4. Prediction of VNA titres over time of individuals with different characteristics

Based on the above analysis, variables of vaccine product (Chengda vaccine/HDCV/other products), re-exposure time (<3 years/3+ years), vaccination type (primary vaccination/booster vaccination), RIG administration × type of contact interaction item, vaccine schedule × type of contact interaction item, and $\log_3$ time were used as explanatory variables for $\log_3$ titre prediction modeling ($F = 36.79$, $P = 0.000$). Finally, explanatory variables left in the model were vaccination type, re-exposure time, Chengda vaccine, RIG administration × type of contact interaction item, and $\log_3$ time. The regression coefficients after controlling for the influence of other variables and percentage change (95%CI) compared to reference variable were shown in Table 3 top.

Different levels of 3 variables (re-exposure time, Chengda vaccine, RIG administration × type of contact interaction item) were combined into 8 groups, including primary vaccination 4 groups (A, B, C, D), and booster 4 groups (E, F, G, H) (Table 3 under, "+" for "yes", "-" for "no"). It should be noted that RIG is actually only used in primary vaccination and not in booster vaccination. The 99 % individual prediction intervals (IPI) of VNA titres for A-D groups at 3, 6, 12 months, and for E-H groups at 5, 10, 20 years after vaccination were calculated and plotted in Fig. 2. For primary vaccination, the earliest occurrence of VNA titre below 0.5 IU/ml was at 6 months after vaccination for groups D (99 % IPI: 0.46–72.85 IU/ml) (Fig. 2a). Actually, the lower limit of 99 % IPI (0.57–89.33 IU/ml) for group D at 3 months after vaccination was close to 0.5 IU/ml. Group D indicates the primary vaccinations with RIG administration, category III exposure and other (non-Chengda) vaccine. While for booster vaccination, the VNA titre below 0.5 IU/ml occurred earliest at 10 years after vaccination for groups F (99 % IPI: 0.47–76.00 IU/ml) (Fig. 2b). Group F indicates the booster vaccinations with re-exposed <3 years and 2–3 doses of other (non-Chengda) vaccine. These results provide a reference of the appropriate timing for booster administration to maintain an effective protection (VNA titre ≥0.5 IU/ml) in outpatient PEP practice, by prediction of antibody response according to Fig. 2 following classification of patients based on Table 3.

4. Discussion

In this retrospective cohort study, the average time for VNA detection after vaccination was 1.40 months, with the longest detection time of 18 years. There were 6 out of 386 subjects that developed VNA titres below 0.5 IU/ml after primary vaccination, and 3 cases occurring within 1 month after full vaccination. Excluding the factor of titre decreasing over time, this result suggests that these 3 cases may have failed primary vaccinations (VNA titre<0.5 IU/ml), which was further confirmed by the trend results of antibody response observed in subjects with repeated detection records after primary vaccination. According the trend results, the VNA titres peaked within one month after vaccination and then decreased rapidly. However, the VNA titres of these 3 cases were detected lower than 0.5 IU/ml within one month after full vaccination. Thus, the original immunization strategy needs to be re-evaluated and adjusted for the primary vaccination failure individuals.

The results demonstrated that the interaction of type of contact × vaccination schedule had an effect on rabies antibody response following primary vaccination. The vaccinated subjects administrated with the 5-dose course after category III exposure exhibited a significant VNA titre decrease of 19.74 % ($P = 0.008$). Within one month after vaccination, the VNA positive rate of the 2-1-1 schedule (100 %, 118/118) was not significantly different from that of the 5-dose course (97.86 %, 137/140) ($P = 0.253$), but the geometric mean of VNA titres showed a significant difference between the two vaccination schedules (2-1-1 schedule: 25.21 IU/ml vs. 5-dose course: 18.96 IU/ml, $P = 0.031$). Moreover, the 6 cases with VNA titre <0.5 IU/ml were all administrated with 5-dose course of primary vaccination. These results above reveal that the efficiency of antibody response generated by the first two doses of the 2-1-1 schedule is higher than that generated by the first one dose of 5-dose course [20]. Therefore, for those who were vaccinated with 5-dose course but failed their primary vaccination, it is recommended to administration with the 2-1-1 schedule, especially for those who exposed to category III.

In addition, the present study found that the interaction of type of contact × RIG administration had an effect on rabies antibody response following primary vaccination. By modeling and controlling for other factors, the VNA titres of category III exposed individuals with RIG administration decreased by 39.67 % ($P = 0.000$), which may be related to the remote intramuscular injection of

Table 2
Geometric mean of VNA titre following booster vaccination in subjects with different characteristics.

Variable	Level	N	Geometric mean (95 % *CI*)	Fold effect (95 % *CI*)	*P*-value	Adjusted fold effect[a] (n = 455)	*P*-value
All		522	29.16 (26.91, 31.59)				
Gender	male	353	27.80 (25.23, 30.64)	Baseline			
	female	169	32.21 (27.99, 37.08)	0.13 (-0.02, 0.29)	0.091		
Age	<30	269	31.19 (27.90, 34.87)	Baseline			
	30+	253	27.15 (24.20, 30.45)	−0.13 (-0.27, 0.02)	0.089		
Animal	Cat	174	28.18 (24.51, 32.40)	Baseline			
	Dog	319	31.19 (26.63, 32.72)	0.04 (-0.12, 0.20)	0.600		
	Rat	10	29.52 (22.54, 92.13)	0.33 (-0.22, 0.87)	0.240		
	Bat	6	40.32 (17.17, 77.10)	0.23 (-0.46, 0.93)	0.511		
Origin of animal	Domesticated animal	286	28.98 (25.93, 32.38)	Baseline			
	Homeless/wild animal	100	25.47 (21.11, 30.73)	−0.12 (-0.32, 0.08)	0.246		
Exposure site	Lower limbs	233	29.01 (25.72, 32.71)	Baseline			
	head and neck	19	32.87 (21.58, 50.06)	0.11 (-0.29, 0.51)	0.575		
	upper limbs	264	29.29 (26.16, 32.79)	0.01 (-0.14, 0.16)	0.908		
Type of contact	I + II	397	29.98 (27.33, 32.88)	Baseline			
	III	105	28.40 (23.73, 33.99)	−0.05 (-0.23, 0.14)	0.599		
Vaccine product	RongAn	28	20.28 (14.42, 28.53)	Baseline		Baseline	
	Chengda	320	30.88 (27.91, 34.16)	0.38 (0.06, 0.71)	**0.021**	0.39 (0.08, 0.71)	**0.015**
	HDCV	87	30.48 (25.12, 36.99)	0.37 (0.01, 0.73)	**0.042**	0.40 (0.05, 0.75)	**0.026**
	Others	21	22.23 (14.99, 32.96)	0.08 (-0.39, 0.56)	0.730		
Re-exposure time	>3 year	191	34.78 (30.54, 39.62)	Baseline		Baseline	
	<1 year	223	23.66 (20.97, 26.69)	−0.35 (-0.51, −0.19)	**0.000**	−0.38 (-0.55, −0.21)	**0.000**
	1–3 year	108	32.87 (27.65, 39.09)	−0.05 (-0.25, 0.15)	0.609		
Vaccination agency	Outside local Province	204	28.38 (24.97, 32.26)	Baseline			
	Local city	190	27.59 (24.17, 31.51)	−0.03 (-0.19, 0.14)	0.764		
	Outside local city, local province	127	33.30 (28.31, 39.16)	0.15 (-0.04, 0.33)	0.130		
RIG administration	No	482	29.14 (26.81, 31.67)	Baseline			
	yes	33	32.09 (23.34, 44.13)	0.09 (-0.21, 0.39)	0.564		

[a] Adjusted for vaccine product and re-exposure time.

Table 3
The prediction model of VNA titres for individuals with PEP regimens.

Variable	Level	*B* (95%*CI*)	*P*-value	Percentage change (95%CI) compared to ref.
Intercept		2.55 (2.41, 2.69)	0.000	/
Vaccination type	Booster vaccination (primary vaccination as ref.)	0.38 (0.24, 0.53)	0.000	52 % (30–79 %)
Re-exposure time	3+ years (<3 years as ref.)	0.29 (0.12, 0.46)	0.001	38 % (14–66 %)
Vaccine product	Chengda vaccine (others as ref.)	0.15 (0.02, 0.29)	0.023	18 % (2–38 %)
RIG administration × type of contact	Yes × III (no/yes × I + II as ref.)	−0.46 (-0.66, −0.26)	0.000	−40 % (-52 to −25 %)
Log_3 time (months)		−0.30 (-0.36, −0.24)	0.000	−28 % (-33 to −23 %)

	N	Primary				Booster			
		A	B	C	D	E	F	G	H
		188	45	87	29	196	89	110	39
Re-exposure time	<3 years					+	+	-	-
	3+ years					-	-	+	+
Vaccine product	Chengda vaccine	+	+	-	-	+	-	+	-
	Others	-	-	+	+	-	+	-	+
RIG administration × type of contact[a]	No × I/II/III or yes × I/II	+	-	+	-	+	+	+	+
	Yes × III	-	+	-	+	-	-	-	-

[a] RIG administration × type of contact, there is only No × I/II/III for booster.

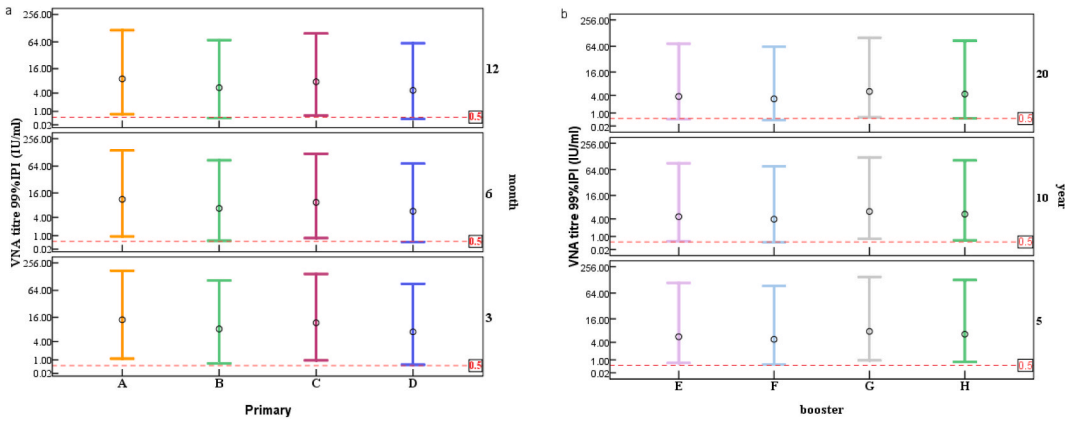

Fig. 2. The 99 % IPI values of VNA titres (IU/ml) over time (3 months, 6 months, 12 months after vaccination) for primary vaccinations (a) and (5 years, 10 years, 20 years after vaccination) for boosters (b). The red dashed line represents a level of VNA titre = 0.5 IU/ml; A–H: groups with different characteristics (See Table 3).

remaining RIG in PEP regimens [16]. Due to the inability of human immunity in producing sufficient VNA to resist the rabies virus within 7 days after primary vaccination, it is necessary to administer exogenous immune agents to quickly neutralize the virus around the wound to reduce the risk of disease. This operation has been internationally recognized, but there is controversy over that whether the remaining RIG needs to be further injected into the distal muscle after infiltration injection. In the latest version of the "Guidelines for the Prevention and Disposal of Rabies Exposure (2023 Edition)" [21] in China, it is still required to inject the remaining RIG into the muscles far away from the vaccine injection site. However, some experts at home and abroad no longer recommend remote intramuscular injection of the remaining RIG [22–24]. In the 2019 position paper on rabies vaccines, the use of residual RIG for intramuscular injection is not recommended by WHO as its benefits are very limited [1]. The results of this study support the recommendation not to use residual RIG for distal intramuscular injection.

The results also showed that origin of animal had an independent effect on rabies antibody response following primary vaccination. Subjects who were exposed to homeless/wild animals developed a higher VNA titres than those exposed to domesticated animals ($P = 0.045$). This may be related to the increased attention paid by individuals after exposure to homeless/wild animals, leading to a series of positive psychological-physiological reactions and the implementation of favorable measures. However, whether it is more attributed to certain pathological and physiological reactions produced by human exposure to homeless/wild animals remains to be analyzed. Currently, there is rare literature focusing on the impact of the origin of animal on the VNA titres of rabies vaccinated subjects.

Because of the existence of human immune memory, the antibody response following booster vaccination was observed to be

stronger than primary vaccination. After adjusting for other factors, the VNA levels following booster vaccination significantly increased by 51.81 % when compared to primary vaccination ($P = 0.000$). Moreover, rabies antibody levels maintaining effective protection post-booster vaccination were significantly longer than primary vaccination. The earliest occurrence time of VNA titre below 0.5 IU/ml was at 10 years post-booster vaccination, and was at 6 months post-primary vaccination. The trends of VNA levels in 77 subjects with repeated VNA detection records after booster vaccination revealed that the peaks of VNA titres appeared in both the first month and the third month since vaccination. This phenomenon may be related to the comprehensive immune reaction after the re-immune response, but the specific mechanism remains to be further studied.

Based on the influencing factors of vaccination type, re-exposure time, vaccine product, and RIG administration × type of contact interaction, the vaccinated individuals were divided into 8 groups with different characteristics, including 4 groups for primary vaccinations and 4 groups for boosters. And the 99 % IPI of VNA titres for these groups over time were calculated. Due to the 100 % fatality rate of rabies, the lower limit of 99 % IPI was used as the reference value for recommended re-immunization in the present study, to reduce the risk of rabies in the exposed populations. According to the model, less than 1 % of the individuals in group D had VNA titres of <0.5 IU/ml at 6 months after primary vaccination. Group D was characterized by RIG administration and category III exposure with non-Chengda vaccine, and the lower limit of 99 % IPI (0.57–89.33 IU/ml) at the 3 months for the VNA titre was approaching 0.5 IU/ml. Thus, for group D populations, the booster immunization is recommended if re-exposure occurs at 3 months after vaccination. This is in line with the latest requirements of our country's paper "Guidelines for the Prevention and Disposal of Rabies Exposure (2023 Edition)" [21], that is, persons who are re-exposed 3 months or more after the full vaccination should be given one dose of rabies vaccine at 0 and 3 days, respectively. This requirement is a revision of the previous paper "Guidelines for the Prevention and Disposal of Rabies Exposure (2009 Edition)" [15], which states that the re-exposed persons generally do not need to be re-immunized within 6 months. The results of the present study confirm the necessary for such revision. This study also found that the concentration of VNA in serum decreased proportionally over time. The serum VNA titres decreased by 28.08 % every 3 months after vaccination ($P = 0.000$). Based on the 99 % IPI values predicted for antibody concentrations following vaccination, it is possible to assess the appropriate timing for booster administration, particularly in subjects who are frequently exposed to rabies risk, such as animal breeders, to ensure an effective antibody protection [25].

5. Conclusion

The 2-1-1 schedule is more effective than 5-dose course in primary vaccination, so it is recommended to administration with the 2-1-1 schedule for those who were vaccinated with 5-dose course but failed their primary vaccination, especially for those who exposed to category III. We found that the use of RIG in primary vaccination reduced the VNA titres of category III exposed individuals, thus this study support the recommendation not to use residual RIG for distal intramuscular injection. Rabies antibody levels maintaining effective protection post-booster vaccination were significantly longer than primary vaccination. Based on the 99 % IPI values predicted for antibody concentrations following vaccination, it is possible to assess the appropriate timing for booster administration, particularly in subjects who are frequently exposed to rabies risk, to ensure an effective antibody protection. Certainly, the model was still predictive and needed be clarified and confirmed in practice.

Ethical statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

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CRediT authorship contribution statement

Chunping Huang: Writing – original draft, Formal analysis, Data curation. **Ling Zhang:** Writing – review & editing, Methodology, Investigation. **Xiaoyue Shan:** Writing – review & editing, Methodology, Investigation. **Siwei Tan:** Methodology, Investigation. **Haipeng Ye:** Project administration, Methodology, Investigation. **Chengjian Cao:** Supervision, Conceptualization. **Lei Zhang:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35673.

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