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# Equivalence between pre-exposure schemes for human rabies and evaluation of the need for serological monitoring

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## ABSTRACT

**OBJECTIVE:** To evaluate the humoral immune response to the pre-exposure schedule of human rabies vaccination through intradermal and intramuscular routes, as well as the need for serological monitoring.

**METHODS:** A randomized and controlled intervention study was carried out in São Paulo, Southeastern Brazil, from 2004-2005. There were 149 volunteers, of which 127 completed the vaccination schedule (65 intradermal and 62 intramuscular) and underwent humoral immune response evaluation at ten, 90 and 180 days post-vaccination. Two outcomes were considered for comparing the two routes of administration: the geometric average of neutralizing antibody titers and the proportion of individuals with satisfactory titers ( $\geq 0.5$  IU/mL) at each evaluation point. The association of the humoral immune response with anthropometric and demographic data was analyzed through a normal distribution test and a chi-square test with a Yates correction. After completion of the vaccination schedule, the proportion of seropositive results was compared by the Kruskal Wallis test, and the average titers were compared by variance analysis. Results: the average antibody titers were higher in patients who were vaccinated intramuscularly. The percentage of volunteers with satisfactory titers ( $\geq 0.5$  IU/mL) decreased over time in both groups. However, in the group vaccinated intradermally the rate of satisfactory titers on day 180 ranged from 20% to 25%, while the intramuscular route varied from 63% to 65%. An association between the humoral immune response and the demographic and anthropometric variables was not observed.

**CONCLUSIONS:** Serology after the third dose can be considered unnecessary in unexposed patients, since 97% and 100% of volunteers respectively vaccinated by the intradermal and intramuscular route presented satisfactory antibody levels ( $\geq 0.5$  IU/mL).

**DESCRIPTORS:** Rabies Vaccines, immunology. Serology. Immunity, Humoral. Intervention Studies. Rabies, prevention & control.

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## INTRODUCTION

As part of the activities for the human rabies control program, the pre-exposure vaccination schedule is recommended for people at greater risk of contact with the rabies virus due to professional reasons (veterinarians, biologists, researchers) or people at risk of exposure in leisure activities.<sup>20</sup>

The use of this schedule can simplify prophylaxis after subsequent exposure to the virus by reducing the required number of vaccinations, thereby avoiding the use of heterologous serum or of anti-rabies immunoglobulin, which are often unavailable, especially in developing countries.<sup>2</sup> Besides these situations, the pre-exposure schedule can protect people in case of unapparent exposure to the rabies virus.<sup>20,a</sup>

The pre-exposure schedules recommended by the World Health Organization (WHO) consist of three vaccine doses administered by intradermal (ID) or intramuscular (IM) routes, on the days zero, seven and 28. In Brazil the vaccine utilized is produced in cell cultures, "Purified Vero Cell Vaccine" (PVCV), Verorab,<sup>a</sup> commercialized by the Sanofi/Pasteur laboratory and packaged in lyophilized formulation containing 0.5 mL per vial.<sup>18,20</sup>

In IM administration the recommended dose is 0.5 mL and by the ID route the dose is 0.1 mL. Vaccination guidelines recommend serological monitoring beginning the tenth day after administration of the last dose, for the verification of the humoral immune response.

Considering the regular and constant vaccine response observed in immunocompetent individuals,<sup>18</sup> it is thought that the performance of serological monitoring is unnecessary. The amount of neutralizing antibodies for the rabies virus costs an estimated R\$100.00 per test.

Besides the immune response being similar between the vaccination schedules,<sup>18</sup> the ID route uses 1/5 the dose of the IM route, which makes it more economical, mainly for large groups. This way the pre-exposure schedule can be less onerous on the public health system, especially if serological monitoring post-vaccination is stopped.

The objective of this study was to compare the humoral response of administration of anti-rabies vaccine by the ID or IM routes and evaluate the necessity of performing serological monitoring.

## METHODS

The study design was a randomized control study, carried out from May of 2005 to December of 2006.

The eligibility criteria for the participants were: professionals at risk of exposure to the rabies virus, 18 years or older, without contraindications for the use of the vaccine, who seek services for the performance of the rabies pre-exposure schedule at the Pasteur Institute in the municipality of São Paulo, Southeastern Brazil.

The volunteers were selected among veterinarians, biologists, students, researchers, municipal guards, zoonotic control workers and clients (people who annually attend the Institute to perform their pre-exposure vaccination). The exclusion criteria were: having previously had anti-rabies treatment, using antimalarial or immunosuppressive drugs or having an immunodepressive disease, factors that interfere with the immune system response.<sup>10,19</sup>

The volunteers were randomly sorted to make two groups. One group underwent the pre-exposure schedule by the IM route (n=73), with administration of 0.5 mL per vaccine dose. The other group received the pre-exposure schedule by the ID route (n=76), with administration of 0.1 mL per vaccine dose. The PVCV vaccine was utilized, with a minimum strength of 2.5 IU/mL per dose and of French origin (Sanofi/Pasteur Laboratory). It was diluted in Brazil by the Instituto Butantan, to the quantity of 0.5 mL per vial.

Two outcomes were considered for the comparison between the two routes of administration: the geometric mean neutralizing antibody titers and the proportion of individuals with satisfactory titers ( $\geq 0.5$  IU/mL) at each evaluation point. In the two groups the blood draws for the evaluation of neutralizing antibodies against rabies were performed on day zero, which is the first study day when the first vaccine dose was administered, and on days 38, 118 and 208, corresponding to ten, 90 and 180 days after the conclusion of the vaccination schedule.

The serological tests were done by rapid fluorescent focus inhibition test, (RFFIT), recommended by the World Health Organization (WHO).<sup>15</sup> The laboratory professionals, who conducted the analysis, were blinded, and only the researchers had access to the identifying information of the groups.

The calculation of the sample size for the equivalence test was based on an effect size of 5% with a test power of 80% and alpha of 10%. Ideally, for an alpha of 5% and a test power of 90%, 150 individuals would be necessary in each group. Due to operational capacity limits for performing the serological tests, it was decided to reduce the sample size to 50 individuals in each group (IM route and ID route), without harming

<sup>a</sup> Secretaria Estadual de Saúde de São Paulo. Instituto Pasteur. Profilaxia da raiva humana. 2.ed. São Paulo; 2000. (Manual técnico do Instituto Pasteur, 4).

the analysis and maintaining the test power of 80%. In order to compensate for potential losses (to dropout and follow-up), 76 volunteers were included in the ID group and 73 volunteers in the IM group.

Of the 149 volunteers who began the project, 127 completed the pre-exposure schedule. Therefore, there were 22 losses due to follow-up (15%). The losses to follow-up occurred by participant dropout (n=21) and by protocol interruption (one volunteer had a temporary, low intensity, adverse reaction related to the administration of the vaccine).

At the moment of study enrolment, or in other words on day zero, the volunteers answered a questionnaire about their health condition and filled out an individual card with identifying and anthropometric data. Also on this day, a blood sample was collected to verify the absence of neutralizing antibodies against rabies. Then the volunteers were assigned to their study group, either IM or ID route according to the results of the sorting.

On the administration date for the second and third vaccine doses, adverse reaction cards were utilized specifically for the registration of potential signs and symptoms.

The statistical analysis was performed according to intention to treat. All volunteers were included independent of having completed the planned collections at the established intervals.<sup>5</sup> Not following the recommended time intervals between doses does not affect the immunological response, just as an interruption in the vaccination schedule does not require its reinitialization.<sup>7</sup>

To compare the groups according to demographic and anthropometric variables, the analysis utilized a normal distribution test and a chi-square test with a Yates correction. For the samples from ten, 90 and 180 days after completing the schedule, the comparison of the proportion of seropositive results (neutralizing antibody titers  $\geq 0.50$  IU/mL) was done by Kruskal Wallis test and the comparison of the average titers was done by variance analysis.<sup>14</sup>

The project was approved by the Human Research Ethics Committee of the *Irmandade da Santa Casa de Misericórdia de São Paulo* (projeto nº 262/05). All the volunteers signed informed voluntary consent forms.

## RESULTS

The data presented in Table 1 show that the randomization procedure resulted in comparable groups in relation to demographic and anthropometric variables.

The serology titers did not significantly vary according to the sex, age, weight and height of the volunteers (data not shown).

The geometric mean neutralizing antibody titers were different for the two routes of administration in all dose levels, except at day zero (Table 2 and Figure).

The difference in the proportion of individuals with acceptable titers was similar between the two routes of administration at the tenth day after schedule completion. The differences were significant at days 90 and 180 after schedule completion (Table 3).

Table 4 shows that the occurrence of possible adverse events was very small, negatively affecting statistical analysis. Pain and irritation at the site of ID administration and pain, irritation and itching and the site of IM administration were mentioned as local reactions. The only systemic events registered in both groups were headache and nausea.

## DISCUSSION

Although individual factors can influence immunological response,<sup>9,13</sup> the individuals of both groups, receiving ID or IM administration, were similar in regards to age, sex, average weight and average height.

In international guidelines for the prevention of human rabies, there is no specific recommendation for the reinitiation of the vaccine schedule when the intervals between vaccine doses is not followed, since this does not significantly affect antibody levels.<sup>11</sup> Therefore, it was decided to perform an intention to treat analysis (without considering correct intervals).

From the 90<sup>th</sup> day after completing the schedule, the geometric mean titers of those vaccinated by the IM route was greater than ID route, a finding similar to those of other studies.<sup>11,12</sup>

Chaves,<sup>b</sup> in 1997, working with vaccine produced in cultures of human diploid cells (HDCV), found that there was not a significant difference in the production of neutralizing antibodies when utilizing the IM and ID route, independent of dose. Similar results were obtained by Burridge et al,<sup>3</sup> in 1982, and Briggs et al<sup>2</sup> in 1992, when utilizing the same vaccine type and administration routes. Kositprapa et al,<sup>6</sup> in 1997, observed that even though antibody titers in individuals who received the pre-exposure schedule by the ID route were inferior and less persistent than those who received the vaccine by the IM route, boosters through the ID route produced an adequate and rapid immune response.

<sup>b</sup> Chaves LB. Resposta imune humoral na imunização anti-rábica humana: comparação de títulos de anticorpos neutralizantes de isotipos de imunoglobulinas e de avidade de IgG na vacinação intramuscular e intradérmica [masters dissertation]. São Paulo: Escola Paulista de Medicina da Universidade Federal de São Paulo; 1997.

**Table 1.** Characteristics of participating volunteers according to route of administration of the anti-rabies vaccine. Municipality of São Paulo, Southeastern Brazil, 2004-2005.

Variable	Intradermal	Intramuscular	p
Sex			
Male n(%)	27 (41.5%)	18 (29.0%)	0.1408
Female n(%)	38 (58.5%)	44 (71.0%)	
Age (years)	33.2	31.8	0.3158
Weight (kg)	70.7	71.4	0.7621
Height (cm)	170.5	171.7	0.4451

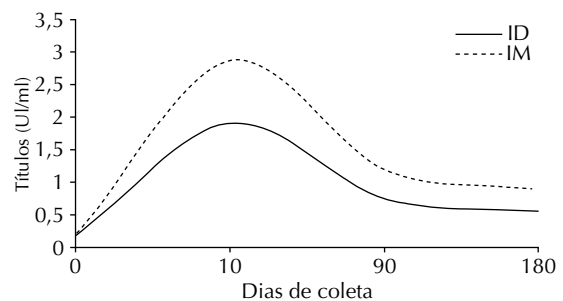
It was found that the percentage of volunteers with acceptable antibody titers decreased with time among both the ID and IM routes, but the decrease is more pronounced among volunteers who received the schedule by the ID route (Figure). In this study, at day 180 the proportion of satisfactory titers by the ID route varied from 20% to 30%, while the IM route varied from 60% to 75%.

A study by Briggs et al<sup>2</sup> shows that the persistence of antibodies in the IM schedule is more long-lived. In the evaluation of two groups of individuals, two years after having received the pre-exposure schedules by the IM and ID route, 7% of those who received IM administration had antibody titers less than 0.5 IU/mL. In the group that received ID immunization, 27% had titers less than 0.5 IU/mL.

The cost-benefit relationship of the pre-exposure schedule by the ID route supports its use by health professionals, who are under permanent control in regards to the possibility of re-exposure, and for travellers. The cost of the pre-exposure schedule with three IM doses varies from US\$ 18 to US\$34.50, and the ID vaccination varies from US\$4 to US\$7.50, according to work published by Chulasugandha et al.<sup>4</sup> In the state of São Paulo, each 0.5 mL dose of VERO vaccine costs R\$ 20.99 (US\$12.50). Therefore, vaccination by the IM route (routinely utilized), excluding expenses on personnel and other instruments, costs R\$ 71.98 (US\$ 45.00).

For pre-exposure of health professionals, for whom the identification of exposures to the virus is possible, the ID schedule should be utilized. One booster (ID or IM) is sufficient for a satisfactory immune response, indicating that memory cells persist in the immune system even without detectable rabies antibody titers in the blood.<sup>8</sup>

Since the years 2004 and 2005, there has been an important change in the epidemiological profile of rabies in Brazil and Latin America, particularly in the

**Figura.** Média geométrica de anticorpos anti-rábicos segundo a via de aplicação de vacina em esquema de pré-exposição. São Paulo, SP, 2004-2005.

Amazon Region.<sup>c</sup> In this period, human rabies began to principally be transmitted by the hematophagous or common vampire bat (*Desmodus rotundus*). The North and Northeast regions of South America have suffered a large environmental impact with human interference and a decrease in the animal population that was the main food source of these bats. The successive attacks by hematophagous bats in the Amazon Region on people, who reside in houses without barriers against these animals and with difficult access to health services, supports the adoption of mass preventative treatment for these populations. Nonetheless, this region is also a malaria endemic area, whose treatment can interfere with the immune response.<sup>2</sup>

For the routine use of the pre-exposure schedule in populations that can easily seek care in case of a new exposure, the differences observed with the use of the ID route do not appear important.

The existence of significant differences between the two groups is relevant for the planning of mass vaccinations for populations exposed to the risk of contracting rabies and with difficult regular access to health services. In Brazil, bat transmitted rabies outbreaks have been common in riverside populations of the Amazon who receive anti-malaria treatment and are not easily followed in regards to new exposures to the rabies

<sup>c</sup> Oliveira RC, Wada M, Montebello LR, Machado R, Carnieli Jr P, Castilho JG, et al. Cambios del perfil epidemiológico de la rabia en Brasil: estudios antigénicos y genéticos. In: 17. International Conference Rabies in the Américas –RITA; 2006, Brasília, Br. Rabies in the Américas.

**Table 2.** Neutralizing antibody titers (geometric means) according to route of administration and sample day. Municipality of São Paulo, Southeastern Brazil, 2004-2005.

Sample day	Intradermal (IU/mL)	Intramuscular (IU/mL)	P
0	0.1854	0.1871	0.9028
10	1.9033	2.8573	0.0019
90	0.7551	1.2040	0.0001
180	0.5508	0.8929	0.0006

virus. In this case, a pre-exposure schedule utilizing the IM route and the administration of a booster after one year appears more appropriate, as proposed by Strady et al.<sup>17</sup>

The WHO<sup>20</sup> recommends the ID or IM route for pre-exposure schedules against rabies, using vaccines produced in cell cultures. If the patient's immunological status is in doubt, after the vaccination schedule an evaluation of rabies antibody titers should be done.

In the present study three volunteers had antibody titers less than 0.5 IU/mL when evaluated after completing the schedule administered by the ID route, between the tenth and 180<sup>th</sup> day. The international literature reports lack of seroconversion rates, varying from 1.10%<sup>18</sup> to 490%.<sup>13</sup> In these cases, a probable hypothesis is technical error in vaccine administration by the ID route, since the three cases were concentrated in the same group of volunteers vaccinated by professionals who had recently received technical training in ID administration of tuberculosis vaccine. Another hypothesis is that these volunteers belong to a group considered, for unknown reasons, as poor respondents because they do not respond to certain antigenic stimulus including anti-rabies vaccination.<sup>16</sup> The evaluation performed was the humoral immune response to protein G, and the total antibodies against the other rabies virus proteins were not measured.

All individuals who received the pre-exposure schedule were vaccinated due to a risk of exposure, and

**Table 3.** Proportion of individuals with satisfactory neutralizing antibody titers and 95% confidence intervals, according to route of administration, and sample day. Municipality of São Paulo, Southeastern Brazil, 2004-2005.

Sample day	Intradermal % (95% CI)	Intramuscular % (95% CI)
0	0.0	0.0
10	96.9 (92.6;100.0)	100.0
90	48.3 (36.0;60.5)	89.5 (81.8;97.2)
180	20.7 (10.8;30.6)	63.5 (51.4;75.6)

approximately 100% of the volunteers had satisfactory titers in the serological monitoring done on the tenth day. Therefore, it is valid to consider the need to decrease the number of serological tests for rabies virus neutralizing antibodies produced after vaccination. In all Brazil, only the *Laboratório de Diagnóstico do Instituto Pasteur de São Paulo* routinely performs the serum neutralization technique in cell culture, as recommended by WHO.

This evaluation of the necessity of serology is pertinent, considering that there are guidelines<sup>a</sup> that recommend antibody titer evaluation annually for people who are involved in risk activities and biannually for individuals with high exposure, such as researchers and laboratory workers. Low frequencies of adverse events were observed for the two routes, and the most common local reactions agree with reports in the literature: pain, stiffness and erythema.<sup>18</sup>

The most frequently described systemic manifestations are: nausea, muscle pain and gastrointestinal symptoms. Briggs et al, in 2000, compared previous reports of adverse events in the PCEV and PVCV vaccines administered by the ID route. They showed that occurrence is more frequent by this route, indicating that reactions are associated more with the route of administration than with the vaccine per se.<sup>1</sup>

The impossibility of presenting results from 12 months after rabies vaccinations, due to the difficulty in

**Table 4.** Local and systemic reactions, distributed by dose received and route of administration of the rabies vaccine in the pre-exposure schedule. Municipality of São Paulo, Southeastern Brazil, 2004-2005.

Route of administration	Vaccine dose	Types of reactions					
		Local			Systemic		
		Erythema	Stiffening	Pain	Itching	Headache	Nausea
Intradermal	1 <sup>st</sup>	2	-	3	-	1	2
	2 <sup>nd</sup>	-	-	2	-	-	-
	3 <sup>rd</sup>	-	-	-	-	-	-
Intramuscular	1 <sup>st</sup>	1	1	4	2	2	1
	2 <sup>nd</sup>	-	-	1	-	-	-
	3 <sup>rd</sup>	-	-	-	-	-	-

following the group of volunteers, is among the main limitations of the study.

Prolonged studies, lasting at least five years, that evaluate the rabies antibody levels and the speed of the humoral immune response to vaccine doses in re-exposure should be incentivized. This would be particularly important in areas where the population is permanently in risk of contracting rabies, such as the Amazon Region.

In conclusion, the choice of the ID route for the administration of the rabies vaccine in pre-exposure schedules is a lower cost alternative for use in professionals and travellers, especially in developing countries. In recognized and controlled exposure situations that are rapidly reported and with access to post-exposure treatment, the fact that these individuals showed faster decrease in satisfactory titers can be considered less relevant. Serology after the third dose can be exempted in individuals with controlled exposure.

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