ORIGINAL ARTICLE

https://doi.org/10.21708/avb.2023.18.4.12573

Dynamics of virus-neutralizing IgG antibody titres in veterinary students submitted to rabies pre-exposure prophylaxis

Dinâmica do título de anticorpos IgG neutralizantes em estudantes de veterinária submetidos à profilaxia pré-exposição da raiva

Clélia Carolina Alves[1], Walter Bertequini Nagata[2], Yuri Tani Utsunomiya[3], Marco Milanesi[4], Cáris Maroni Nunes[5], Luzia Helena Queiroz[5]

ABSTRACT: Rabies is a viral disease distributed worldwide, characterized by an acute and almost invariably fatal encephalitis. Pre-exposure prophylaxis (PrEP) is a protective measure for individuals continuously exposed to the risk of infection such as veterinary students and practitioners. The objective of this study was to evaluate the frequency with which the students perform the PrEP, to evaluate the protection they presented as well as the dynamics of the neutralizing IgG antibodies response. A retrospective study was carried out with data from the archives of the Epidemiological Surveillance Service of the Health Department of the Araçatuba Municipality, spanning the period between 2000 and 2017. A total of 2,404 records were evaluated, 86.4% of which presented protective titers against rabies (≥0.5 IU/mL). The records comprised 875 individuals who performed the titration of neutralizing antibodies (IgG). Adhesion of the undergraduate students to PrEP in the year they were admitted was 85%. The mixed linear model used showed a mean annual decrease of the neutralizing antibody titre of 15.1 ± 4.3% (p=3.86 x 10-4) after the last dose of vaccine. There was no significant difference of the antibody titres between individuals who received one, two or three doses of vaccine (P>0.05), nor between males and females. Although PrEP had good adhesion by the incoming students and was efficient in generating neutralizing antibodies against rabies, titres decreased over time, reinforcing the need of performing antibody titration at least every two years to check for the necessity of a booster vaccination.

KEYWORDS: *Lyssavirus*; antibodies titration; vaccination; public health.

RESUMO: A raiva é uma doença viral de distribuição mundial caracterizada por encefalite aguda e quase que invariavelmente fatal. A profilaxia pré-exposição (PrPE) é indicada para a proteção de indivíduos expostos continuamente ao risco de infecção, como os estudantes e os profissionais de medicina veterinária. Objetivou- se avaliar a frequência com que os estudantes de Medicina Veterinária se submetem à PrPE, avaliar a proteção conferida, bem como a dinâmica da resposta de anticorpos neutralizantes (IgG) contra o vírus da raiva. Um estudo retrospectivo foi realizado com os dados do Serviço de Vigilância Epidemiológica da Secretaria de Saúde de Araçatuba, dos anos de 2000 a 2017. Foram avaliados 2.404 registros, compreendendo 875 indivíduos que realizaram a titulação de anticorpos (IgG) neutralizantes contra o vírus da raiva, 86,4% dos quais apresentaram título protetor (≥0,5UI/mL). A adesão à PrPE dos estudantes de graduação no ano de seu ingresso foi de 85%. O modelo linear misto utilizado revelou decréscimo médio anual do título de anticorpos neutralizantes de 15,1 ± 4,3% (p = 3,86 x 10^{-4}) após a última dose de vacina. Não houve diferença significativa no título de anticorpos entre indivíduos que receberam uma, duas ou três doses de vacina (P>0,05), nem entre os sexos. Embora a PrPE tenha tido boa adesão pelos estudantes e mostrou-se eficaz para geração de anticorpos neutralizantes contra a raiva, o título de anticorpos neutralizantes diminuiu ao longo do tempo, reforçando a necessidade da realização da titulação, pelo menos a cada dois anos, para avaliar a necessidade de reforço vacinal.

PALAVRAS-CHAVE: *Lyssavírus*, titulação de anticorpos, vacinação, saúde pública.

[1]São Paulo State University (UNESP), School of Veterinary Medicine, Araçatuba, SP, Brazil.
[2]Department of Agriculture of the State of São Paulo. Agricultural Defense Coordination.
[3]São Paulo State University (UNESP), School of Veterinary Medicine, Araçatuba, SP, Brazil – Biochemistry and Animal Molecular Biology Laboratory.
[4]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Viterbo, Italy.
[5]São Paulo State University (UNESP), School of Veterinary Medicine, Unesp, Araçatuba, SP, Brazil – Department of Animal Production and Health.
*Corresponding author: caris.nunes@unesp.br or luzia.h.queiroz@unesp.br
Received: 04/03/2024. Accepted: 07/29/2024

INTRODUCTION

Rabies is the anthropozoonosis with the highest case fatality of any conventional infectious disease that has occurred in all continents except for Antarctica (Khairullah *et al.*, 2023; Bonaparte *et al.*, 2023). The disease affects the central nervous system of mammals, resulting in about 25,000–159,000 estimated deaths annually and therefore is considered a major Public Health problem (WHO, 2018a).

Transmission of the rabies virus occurs through the contact of a susceptible mammal with the saliva of an infected animal by a bite and, more rarely, by the scratching and licking of mucous membranes or injured skin. Rabies presents almost 100% of lethality, although some isolated cases of treatment in humans have been described in the literature (Khairullah *et al.,* 2023; Manoj *et al.*, 2016; Sumirini *et al.,* 2022).

In Brazil, the National Program for Rabies Prophylaxis started in 1973, based mainly on dogs and cats mass vaccination and post-exposure treatment in humans, for which there is an established protocol in accordance with the type of exposure (Schneider, *et al.*, 2023; BRASIL, 2014).

Pre-exposure prophylaxis (PrEP) is recommended for groups at continuous risk of exposure to rabies virus such as virology and pathology laboratory professionals and technicians, animal handlers, researchers, veterinarians, zootechnicians, biologists and related professionals as well as individuals traveling to areas where canine rabies is prevalent. In addition to conferring protection against infection, in cases of subsequent exposure to the rabies virus, this strategy simplifies post-exposure treatment by reducing the number of vaccine doses and exempts the application of passive immunotherapy (WHO, 2018c; Khairullah *et al.*, 2023)

The Fuenzalida & Palacius vaccine (Fuenzalida; Palacius, 1995) was widely used for human and animal's vaccination around the word and, in Brazil, it was used for pre-exposure prophylaxis and post-exposure treatment until the 2000, when it was replaced by vaccines produced in Vero cell cultures which showed high immunogenicity and low frequency of local and systemic side effects (BRASIL, 2014; Rao *et al.*, 2022).

The PrEP protocol is offered annually to undergraduate students entering the School of Veterinary Medicine at Unesp, Araçatuba, SP, Brazil, since 1993. The hypothesis that a percentage of individuals vaccinated in PrEP may not present adequate antibodies titres against rabies virus suitable for its protection and the interest in knowing how the adhesion to this strategy has been, generated the present research.

Therefore, the aim of this study was to assess the adhesion of students to PrEP and the dynamics of the virus neutralizing IgG antibody (VNA) titre against the viral glycoprotein.

MATERIAL AND METHODS

This is a dynamic cohort study since new individuals are included each year and, at the same time, the majority of those initially recruited was kept for at least five years, the regular period to graduating in Veterinary Medicine.

PrEP is offered annually to the incoming undergraduate students at the School of Veterinary Medicine of Unesp, campus Araçatuba (FVMA), in accordance with the technical standards recommended by the Brazilian Ministry of Health, including the application of three doses of 0.1 ml of inactivated virus vaccine produced in cell cultures "Purified Vero cell vaccine"-VeroRab®, given at 0, 7 and 28 days, intradermally (BRASIL, 2014). After 10-30 days of the last dose of vaccine, incoming students, as well as the entire FVMA community who have already undergone PrEP were invited to evaluate their VNA titres.

The anti-rabies IgG antibodies were tested by the Pasteur Institute of São Paulo for the detection of neutralizing antibodies against rabies virus glycoproteins by the simplified fluorescence inhibition microtest (SFIMT) (WHO, 2018b). Protective titers are those samples that result in values ≥0.5 IU/ml of serum sample, in accordance with the WHO recommendations (WHO, 2018a).

Data were obtained from the Human Anti-rabies Attention Report of the National System of Notifiable Diseases (SINAN), adapted for the information regarding the vaccination in a pre-exposure protocol, as well as the Pasteur Institute of São Paulo records VNA titres results.

We included in the database only the records of individuals from the FVMA community who had received at least one dose of vaccine in PrEP protocol during the period from 2000 to 2017. Thus, 2,404 records (in average 133 per year) were analyzed, which represent 875 individuals with potential exposure to rabies virus, referring to undergraduate students (n=726), postgraduate students (n=82) and FVMA personnel (n=67).

Of the total number of subjects, 34.5% (302/875) did not have a complete vaccination record and this was particularly seen on the postgraduate students as well as on the FVMA personnel records.

The antibody titration data were analyzed by adjusting the following linear mixed model in R v3.4.4 (R Development Core Team, 2018)

$$Yijkl = M + GENi + VACj + REFk + INDl + \sum XnBn + eijkl$$

in which Yijkl is the natural logarithm of the observed titre of sex i, vaccination group j, vaccine booster group k and individual l; GENi is the fixed effect of sex i; VACj is the fixed effect of vaccination group j; REFk is the fixed effect of vaccine booster group k; INDl is the random effect of individual l; Xn is the value of covariable n; Bn is the fixed regression coefficient of covariable n; and eijkl is the residual value. The vaccination groups were composed of observations that presented a vaccine schedule with one (n=41), two (n=170) or three doses (n=1576) of the vaccine. Similarly, for the booster groups, observations were divided between those receiving (n=364) or not (n=1423),

booster vaccination. Covariables included age, days elapsed after the first dose of the vaccine and days elapsed after the last dose of the vaccine or booster. Individual and residual random effects were treated as independently and normally distributed. A 5% significance level was adopted.

This research was approved by the National Research Ethics Committee (CAAE: 669670717.0.0000.5420) in compliance with the guidelines and regulatory requirements for human research.

RESULTS

The highest frequency of which the individual performed serological evaluation was two times (Table 1), with a subsequent decrease in this practice. Of the total number of records, 86.4% (2,078/2,404) presented protective VNA titres (≥0.5 IU) against the rabies virus.

Considering only the data of the undergraduate students in the year of their enrollment in FMVA, PrEP against rabies had 85% adhesion (629/740), and 72.2% completed the PrEP protocol (3 doses of vaccine) in the first year of the course, 82.8% (521/629) underwent subsequent serological evaluation (titration) and 97.9% (510/521) presented VNA (IgG) titers considered protective against rabies virus. Among these, 10.2% (52/510) of the students presented protective VNA titers, even if they had not completed the vaccination protocol.

For the analysis of the anti-rabies humoral immune response (VNA IgG), we considered all the undergraduate students records that i) had registered at least the first dose of vaccine in PrEP protocol, regardless of whether they had received it in the year of their entry or later, and ii) who underwent titration (n=1787). Thus, 628 records of the undergraduate students were analyzed, 67.8% (426/628) of which were of females and 32.2% (202/628) were of males, with 86.9% (546/628) of the total receiving three vaccine doses (Table 2).

Of the undergraduate students who composed this database, 20.9% performed antibody titration only once and 24.4% performed it 3 times (Table 2). A marked reduction in the frequency with which the student performs their titration from the 4th time onwards was observed.

The analysis of the results carried out with the aid of the linear mixed model showed that among the covariables, age, days elapsed after the first dose of the vaccine and days elapsed after the last dose of the vaccine or booster, only the latter had a significant effect on the observed titre, resulting in an annual mean decrease of 15.1 ± 4.3% ($p=3.86 \times 10^{-4}$). To illustrate the dynamics of the VNA titres after PrEP over time, results from eight individuals are shown in Figure 1.

There were no significant differences in titration between men and women (Figure 2A), or among individuals who received one, two or three doses of the vaccine ($p>0.05$). However, suggestive differences ($p<0.10$) were observed between these groups, showing a titre increase at each extra dose of the vaccine (Figure 2B). Similarly, individuals receiving booster vaccination had a significant mean titration increment (49.8 ± 14.2%, $p=0.002$) compared to those who did not receive a booster (Figure 2C). However, this result may have been influenced by bias, since not all booster doses are routinely recorded on the SINAN database.

DISCUSSION

The protective VNA titres observed in this study was high, either while evaluating the total titration records of the FMVA community or when considering only the undergraduate students who completed the full PrEP protocol. These results confirm,

Table 1. Records related to the pre-exposure prophylaxis for rabies carried out in the community of the School of Veterinary Medicine at Unesp, Araçatuba, SP, Brazil, from 2000 to 2017.

Variable	(n)	(%)
Records	2,404	
protective titre	2,078	86.4
non protective titre	326	13.6
Sex	875	
female	561	64.1
male	314	35.9
Number of titration	875	
1	211	24.1
2	234	25.7
3	180	20.6
4	149	17.0
5	66	7.6
>5	35	4.0

Table 2. Distribution of undergraduate students at the School of Veterinary Medicine at Unesp, Araçatuba, SP, Brazil, from 2000 to 2017, according to the pre-exposure prophylaxis and titration of neutralizing antibodies against rabies virus

Variable	(n)	(%)
Students with at least 1 vaccination	628	
female	426	67.8
male	202	32.2
PrEP		
complete	546	86.9
incomplete	97	15.1
Number of titration		
1	131	20.9
2	141	22.4
3	153	24.4
4	129	20.5
5	55	8.8
>5	19	3.0

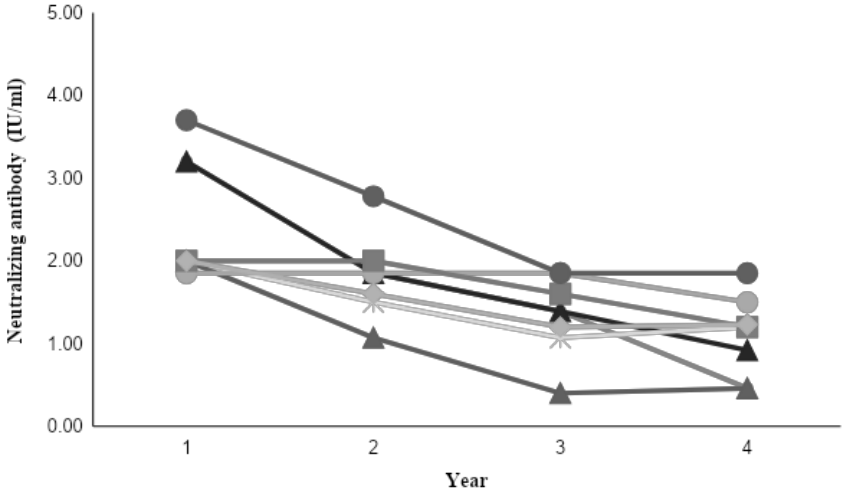

Figure 1. Dynamics of rabies virus neutralizing antibody titres over time from eight undergraduate students at the School of Veterinary Medicine at Unesp, Araçatuba, SP, Brazil, submitted to the pre-exposure prophylaxis protocol.

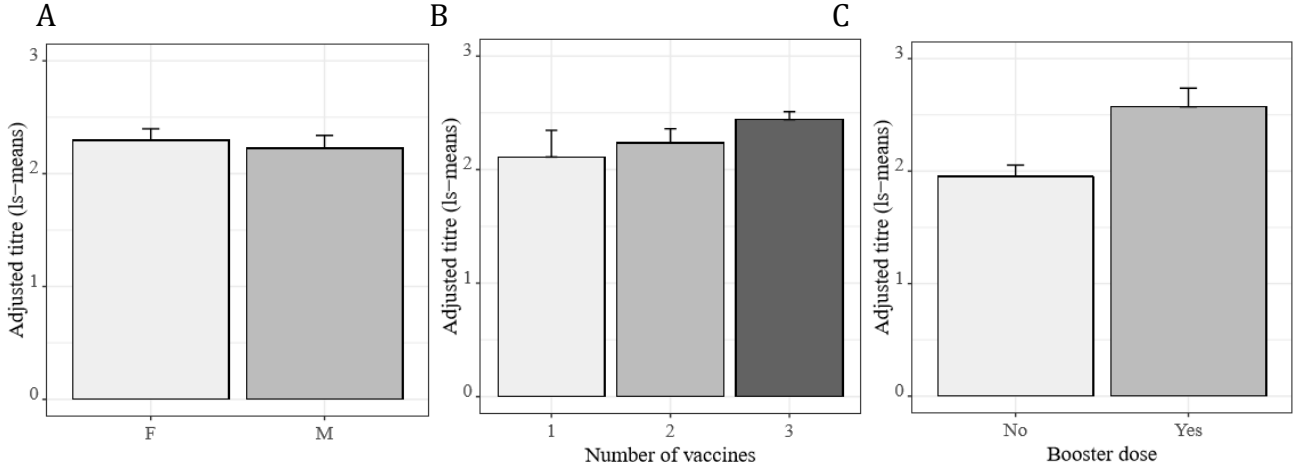

Figure 2. Distribution of undergraduate students from the School of Veterinary Medicine at Unesp, Araçatuba, SP, Brazil, from 2000 to 2017, according to titration and sex (A), number of doses of vaccine (B) and vaccine booster (C).

once again, the antigenicity of the used vaccine as well as the PrEP strategy that aims to induce the production of high levels of neutralizing IgG antibodies against the rabies virus (Warrel, 2012; Briggs, Moore, 2021). Cunha and his colleagues (Cunha *et al.,* 2010) also observed a high percentage of protective titres (96.7%) in subjects evaluated 10 days after the last dose of vaccine, although they have evaluated fewer individuals submitted to intradermal PrEP. However, a minor percentage of protective titres (29.6%) was observed in a study carried out with 102 individuals who have occupational exposure at the Veterinary Hospital of the Federal University of Mato Grosso, Cuiabá, MT, Brazil and had received PrEP (Nociti *et al.*, 2003). In addition to the lower number of individuals evaluated, the time elapsed between the last dose of vaccine and the titre of neutralizing antibodies against rabies were not mentioned in that study, which may have influenced the result. In the present study such evaluation was performed between 10 and 30 days after the last dose of vaccine. Furthermore, it is known that about 3% of healthy individuals may have lower antibody levels even after a booster dose due to individual and/or genetic characteristics (Warrel, 2012).

The fact that a small percentage (10%) of the students showed satisfactory protective titres without completing the PrEP protocol corroborates the results of the study carried out by Nociti *et al.* (2003) where 7.8% of the individuals that reported never having been immunized against rabies virus presented VNA titres above 0.5 IU/ml. In addition to being in permanent contact with animals, that is, with potential exposure to rabies virus, this result may be due to a lack of information about the vaccination history of these individuals, a fact that may also have occurred in our research. In fact, this was one of our study limitations since the information on the vaccination booster is not always registered in SINAN's database, particularly when the individual receives such boosters in the local Health Units, whose records we did not have access to. Joining all health information, including vaccinations, in

a single database would be of great value facilitating this and other assessments as well as planning control strategies.

In our study, sex was not a variable that significantly influenced the titre of rabies VNA, as already observed by Mota *et al.* (2016), by Lim and Barkham (2010) and by Cunha *et al.* (2010). Nevertheless, Mansfield *et al.* (2016) observed significantly higher neutralizing antibody titres in women than in men by a retrospective study of 270 persons at occupational risk. Similar results were observed by Guo *et al.* (2023) in Australian bat careers with female presenting antibody titres 1,42 times higher than male.

The model used in our study to evaluate the dynamics of neutralizing antibodies against rabies virus revealed a mean annual decrease in antibody titre of 15.1 ± 4.3% after the last dose of vaccine. Although there is variation in titration values due to variations in the titres of work virus and standard serum in the SFIMT (WHO, 2018b) other authors have also observed VNA decrease over time. A study by Lim and Barkham (2010) in individuals with occupational exposure in Singapore revealed antibody titres lower than 0.5 IU/mL in 39.4% of them after one year of complete vaccination. Likewise, the study by Cunha *et al.* (2010) showed that only 20.7% of the persons evaluated had adequate antibody titres after 180 days of complete PrEP. Mansfield *et al.* (2016) evaluated antibody titres over 20 years in laboratory technicians under occupational risk and observed a decay rate of 37% per 2-fold change in time after the initial PrEP vaccination protocol. Guo *et al.* (2023) observed a proportion of 29.8% antibody-negative individuals, 1–2 years of interval between the PrEP and the serology test.

Considering the costs involved in the evaluation of the neutralizing antibody titer as well as the WHO recommendation for the titration of individuals with a permanent risk of exposure to the rabies virus, we suggest that such evaluation could be performed every two years during the time of the undergraduating course (for example at the first, third and fifth years) for those veterinary students whose PrEP accompaniment is done as presented here.

The admission of students entering rabies PrEP in this study was high (85%) possibly because it was offered in a single day, on the college's own premises, without the need for the student to go to another place to be vaccinated, besides the fact that they received previous information about the importance of this practice. This hypothesis is reinforced by the results of the study carried out with users of the Unified Health System of the State of Rio Grande do Sul, Brazil, where they observed that PrEP was performed only by 11.8% of the students with occupational risk, a percentage estimated according to the number of public vacancies available for the Biology, Veterinary Medicine, and Animal Science courses of that state (Mota *et al.*, 2016).

The three doses protocol of PrEP vaccines is quite effective; however, in order to decrease the duration and number of vaccine doses some studies have evaluated other PrEP protocols. In general, intradermal, and intramuscular routes have been evaluated, with the protocol being performed within 7 days, with one or more doses of vaccine applied at one or more sites (Rao *et al.*, 2022; WHO, 2018a, 2018c). Kessels *et al.* (2016) carried out a systematic review of nine studies on pre-exposure prophylaxis developed in an accelerated manner with Vero cells culture vaccine in and observed adequate antibody titer for up to one year after vaccination. This review was conducted to analyze the safety and immunogenicity of pre-exposure rabies prophylaxis and recommended when PEP is limited or delayed (Shneider *et al.*, 2023). Pijper *et al.* (2018) conducted a cohort study of volunteers submitted to two doses of Vero cells vaccine by intradermal route and observed adequate serum conversion after 15-21 days of the first dose in 99.3% of the volunteers. Based on such evidence WHO has recommended the application of two doses of vaccine at two sites on days 0 and 7, intradermally, or at one point on the same days, intramuscularly, for PrEP (WHO, 2018c). Since in our study the students were vaccinated only by the intradermal route it was not our objective to compare other application routes, a very frequent kind of evaluation done in other studies. In general, intramuscular application although easier, requires a greater amount of vaccine resulting in a more expensive PrEP. The intradermal route, however, requires trained human resources, but uses one-tenth of the dose used intramuscularly and it has been preferred when there are trained personnel (Warrel, 2012).

In fact, given the change in the epidemiological scenario of urban rabies in the last decade (2010-2020) the Brazilian Ministry of Health started to recommend the application of two doses of vaccine on days 0 and 7at two sites, by intradermal route (0,1ml by site) or at one site by intramuscular route (1ml) for PrEP (BRASIL, 2022). This recommendation, in addition to saving on rabies vaccine, should contribute to the increase in individuals who complete the pre-exposure prophylaxis protocol, given the fact that is only 1 week long.

Other studies should be done to follow the individuals submitted to the new PrEP protocol to check the dynamic of the VNA and the need of VNA titration along time.

CONCLUSIONS

We conclude that the great majority of the incoming students of the Veterinary Medicine course adhere to PrEP which proved to be effective for generating protective neutralizing-antibodies titres against rabies glycoprotein, and these titres decrease over time, reinforcing the need of VNA titration at least every two years to check for the necessity of a booster vaccination.

ACKNOWLEDGEMENTS

To the Epidemiological Surveillance Service of the Municipal Health Secretariat of Araçatuba for data concession; to Capes for the MSc scholarship to Clélia Carolina Alves; to the Laboratory of Biochemistry and Animal Molecular Biology of FVMA at Unesp, for the logistical support; to all the people who somehow contributed to this work.

REFERENCES

BONAPARTE, S.C. Evaluation of country infrastructure as an indirect measure of dog-mediated human rabies deaths. **Frontiers of Veterinry Science,** v. 10, n. 1, p: 114-543, 2023.

BRASIL. Ministério da Saúde. **Normas técnicas de profilaxia da raiva humana**. Brasília: Ministério da Saúde. 2014. Available from: http:/portalarquivos.saude.gov.br/images/pdf/2015/outubro/19/Normas-tecnicas-profilaxia-raiva.pdf.

BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. NOTA TÉCNICA Nº 8/2022-CGZV/DEIDT/SVS/MS. **Informa sobre atualizações no Protocolo de Profilaxia pré, pós e reexposição da raiva humana no Brasil**. 2022. Available from: https:/www.gov.br/saude/pt-br/assuntos/saude-de-a-a-z/r/raiva/imagens/nota-tecnica-n-8_2022-cgzv_deidt_svs_ms.pdf.

BRIGGS, D. J.; MOORE, S. M. The route of administration of rabies vaccines: comparing the data. **Viruses**, v. 13, n. 7, p. 12-52, 2021. doi: 10.3390/v13071252.

CUNHA, R. S. *et al.* Equivalence between pre-exposure schemes for human rabies and evaluation of the need for serological monitoring. **Revista de Saúde Pública,** v. 44, p. 1-7. 2010.

FUENZALIDA, E.; PALACIUS, R. Un método para la preparación de la vacuna antirrábica. Boletin del **Instituto Bacteriologico de Chile**, v. 8, p. 3-10, 1995.

GUO, Y. *et al.*. Immune response after rabies pre-exposure prophylaxis and a booster dose in Australian bat careers. **Zoonoses and Public Health**. v. 70, n. 6, p. 465-472. 2023. doi: 10.1111/zph.13048.

KESSELS, J. A. et al. **Pre-exposure rabies prophylaxis: a systematic review**. Geneva: World Health Organization. 2016. Available from: http:/www.who.int/bulletin/volumes/95/3/16-173039/en/.

KHAIRULLAH, A. R. *et al.* 2023. Tracking lethal threat: in-depth review of rabies. **Open Veterinary Journal,** v. 13, n. 11, p. 1385–1399. DOI: 10.5455/OVJ.2023.v13.i11.1.

LIM, P. L.; BARKHAM, T. M. S. Serologic response to rabies pre-exposure vaccination in persons with potential occupational exposure in Singapore. **International Journal of Infectious Diseases.** 2010 (14): e511-3. 2010.

MANOJ, S. *et al.* Recovery from rabies, a universally fatal disease. **Military Medical Research**, v.3, n.21, 2016. DOI 10.1186/s40779-016-0089-y. Available from: https:/mmrjournal.biomedcentral.com/articles/10.1186/s40779-016-0089-y.

MANSFIELD, K. L. *et al.* Rabies pre-exposure prophylaxis elicits long-lasting immunity in humans. **Vaccine**, v. 34, n. 48, p.5959-5967. 2016.

MOTA, R. S. S. *et al.* Perfil da profilaxia antirrábica humana pré-exposição no estado do Rio Grande do Sul, 2007-2014. **Epidemiologia e Serviços de Saúde**, v. 25, n. 3, p. 511-58, 2016.

NOCITI, D. L. P. *et al.* Anticorpos contra o vírus rábico em seres humanos com atividades no Hospital Veterinário da Universidade Federal de Mato Grosso. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 36, n. 3, p. 355-358, 2003.

PIJPER, C. A. *et al.*. Rabies intradermal pre-exposure prophylaxis immunizations: an observational cohort study. **Travel Medicine and Infectious Diseases**, v. 22, p. 36-39, 2018.

R DEVELOPMENT CORE TEAM. A Language and Environment for Statistical Computing. **R Foundation**, 2018. Available from: https:/www. r- project. org/.

RAO, A.K. et al, Use of a modified preexposure prophylaxis vaccination schedule to prevent human rabies: recommendations of the advisory committee on immunization practices—United States, 2022. MMWR. **Morbility and Mortality Weekly Report**, v. 71, n. 18, p. 619– 627, 2022.

SCHNEIDER, M.C. *et al.* Fifty Years of the National Rabies Control Program in Brazil under the One Health Perspective. **Pathogens**, v. 12, n. 11, p. 1342, 2023. doi: 10.3390/pathogens12111342.

SUMIRINI *et al.* The rare case of a human rabies survivor & the comparative study of all documented human rabies survivors till 2021. **Journal of Neuroinfectious Diseases,** v. 13, p. 372. 2022.

WARREL, M. J. Current rabies vaccines and prophylaxis schedules: preventing rabies before and after exposure. **Travel Medicine and Infectious Diseases**, v. 10, n. 1, p-. 1-15, 2012. doi: 10.1016/j.tmaid.2011.12.005.

WHO - World Health Organization. **Expert Consultation on Rabies**. Geneva: World Health Organization. 2018a. Available from: http:/apps.who.int/iris/bitstream/10665/85346/1/9789240690943_eng.pdf.

WHO - World Health Organization. .A simplified fluorescence inhibition microtest for the determination of rabies virus neutralizing antibodies. In: **Laboratory techniques in rabies** Fifth edition Geneva: World Health Organization. 2018b; Available from: https:/iris.who.int/bitstream/handle/10665/310836/9789241515153-eng.pdf?sequence=1&isAllowed=y.

WHO - World Health Organization. **Rabies vaccines: WHO position paper**. Geneva: World Health Organization. 2018c. Available from: http:/www.who.int/wer/2018/wer9316/en/.