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Real-world evidence of rabies post-exposure prophylaxis in Serbia: Nation-wide observational study (2017–2019)

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ABSTRACT

Background: Rabies remains a deadly zoonotic disease, primarily prevalent in Eastern European countries, with a significant global burden in Asia and Africa. Post-exposure prophylaxis (PEP) is critical to prevent clinical rabies. Serbia, a country with a relatively low animal rabies incidence, has been implementing a 4-dose Essen PEP regimen for 13 years. This real-world study aimed to assess the effectiveness of the 4-dose Essen regimen, considering demographic and clinical factors, after WHO Category III exposure.

Method: The study included 601 patients who received the 4-dose Essen PEP and 79 who received an additional 5th dose.

Results: Age emerged as a critical factor influencing seroconversion rates after the 4-dose regimen, with older individuals exhibiting lower RVNA titers. Logistic regression indicated a 3.18% decrease in seroconversion odds for each added year of age. The Cox proportional hazards mixed model highlighted age-related risks, with age groups 45–60 and 75–92 at the highest risk of non-seroconversion. Human Rabies Immune Globulin (HRIG) administration was associated with lower RVNA values after the 4-dose regimen, suggesting interference with vaccine immunogenicity among people who received larger doses of HRIG.

Conclusions: This study provides valuable real-world evidence for rabies PEP in a non-homogeneous population with potential comorbidities. The results underscore the importance of optimizing PEP strategies, particularly in older individuals, and reconsidering HRIG dosing to improve seroconversion rates.

1. Introduction

Rabies is a deadly disease that can be transmitted from animals to humans. It is caused by the rabies virus (RABV) and other related viruses from the *Lyssavirus* genus, Rhabdoviridae family [1]. In Europe, animal rabies is mainly found in Poland [2], Belarus [3], Ukraine [4,5], Russia, Moldova [6] and Romania [7], with sporadic cases reported in other countries [8,9]. However, African and Asian countries face most significant concern, as they experience the majority of human rabies cases [10].

The key to preventing human rabies lies in immunizing residents and travelers visiting these rabies-endemic territories [6,11]. Travelers staying in Southeast Asia for extended periods have an increased risk of coming into contact with rabies-transmitting animals, ranging from 0.3% to 4% for each month of stay [12,13]. Even though Serbia is not considered as heavily affected by animal rabies, there is still a considerable health risk for its population, particularly for travelers returning from rabies-endemic regions [4].

To prevent clinical rabies, timely post-exposure prophylaxis (PEP) is crucial. In Serbia, this includes intensive wound washing and wound infiltration with rabies immunoglobulin (RIG) to remove and neutralize the virus after single or multiple transdermal bites or scratches and contamination of mucous membrane by licks/bites (WHO category III exposure) of any wild vertebrate. Accordingly, pre-exposure

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immunization (PrEP) against rabies is of utmost importance for persons planning to visit and/or stay in rabies-endemic territories. PEP in Serbia is initiated after WHO category III exposure in urban environment if animal was showing aggressive behavior and is untraceable or dies within 10 days after a person was attacked. Vaccination is also essential, as it induces RABV-neutralizing antibodies (RVNA) that prevent the virus from entering peripheral nerves [14]. Successful PEP (i.e., seroconversion) requires an RVNA titer of 0.5 IU/ml or higher [15]. The standard vaccination protocol, known as Essen regimen, involves one dose of vaccine each on days 0, 3, 7, 14, and 28 [16]. However, due to low compliance rates for the 5th dose (day 28) (e.g., see Ref. [17]), the World Health Organization (WHO) recommended a reduced Essen regimen that only includes the first four doses for healthy, fully immune competent individuals [18]. From 2018 WHO additionally simplified PEP, requiring only 3 visits to healthcare facility (i.e., on days 0,3,7 or 0, 7,21 for intradermal and intramuscular vaccine application, respectively) [19]. On the other hand U.S. Advisory Committee on Immunization Practices still recommend 4-dose PEP [20], pending more data to evaluate the 3 dose series endorsed by WHO.

A noninferiority clinical trial in 500 healthy adults showed that satisfactory RVNA titer can be achieved after 2-visit intradermal or intramuscular PrEP [21]. In 2018 position paper, WHO endorsed this regime, despite of the bias related to the age of study participants (all subjects were younger than 50 years) [15]. Recommendations for PrEP may be further simplified, since Jonker and Visser demonstrated that single vaccine dose can induce satisfactory RVNA titer in healthy adults [22].

While both WHO-recommended Essen regimens are considered as safe and effective, most of the data on immunization outcomes comes from studies involving healthy individuals under controlled clinical settings [23,24]. Little information exists about immunization response in other populations, such as those with comorbidities [25–31] or the elderly, whose immune response may be affected by immunosenescence [32,33]. Real-world evidence is needed to assess the effectiveness of vaccination regimes outside controlled settings, as clinical trials may have limitations and biases [34–36]. Hence, vaccination regimens require support from diverse situations that would be present in a real-world scenario, such as the ones previously reported where local patients and international travelers did not received optimal rabies PEP [11,37–40] and as a consequence inappropriate antibody response is observed [24]. The number of international travelers older than 60 years who may require PEP is rising dramatically [41], therefore there is an objective need for assessment of outcome of standard immunization protocols outside controlled clinical settings, embracing the real-world evidence data.

In Serbia, patients requiring PEP have been administered the reduced Essen regimen for the past 13 years. Patients who did not achieve an RVNA titer $\geq$0.5 IU/ml two weeks after 4th dose (day 21) received the additional vaccine doses until RVNA titer $\geq$0.5 IU/ml is achieved [42].

This study aims to assess the effectiveness of reduced (4-dose) Essen regimen in patients of different genders and ages using real-world evidence. The study focuses on the seroconversion rate in patients vaccinated after WHO category III exposure in Serbia.

2. Material and methods

2.1. Study settings

In Serbia, rabies prophylaxis is carried out through 27 Anti-Rabies Stations (ARSs) and coordinated by the Pasteur Institute Novi Sad. The official national guideline during the study period [43], prescribed PEP using Purified Vero cell Rabies Vaccine (PVRV; Verorab®, Sanofi) administered intramuscularly in 4-dose Essen regime. The vaccination schedule includes shots on days 0, 3, 7 and 14 after exposure, as per WHO category III exposure criteria. If locally produced Human Rabies Immune Globulin (HRIG; Blood Transfusion Institute of Serbia, Belgrade) was available in the ARS, it was recommended to be given along with the first vaccine dose, at a dosage of 20 IU per kg of patient body weight. HRIG was used to infiltrate possible RABV entrance points, while remaining volume (if any) was administered in gluteal region intramuscularly. All ARSs were instructed to avoid administration of HRIG and PVRV in the same extremity. If the HRIG was not locally available, only vaccine was administered according to previously described regimen (i.e. days 0, 3, 7 and 14).

Two weeks after the 4th vaccine shot, a blood sample was taken from the patient at the ARS and sent to Pasteur Institute Novi Sad for RVNA titer analysis using the Rapid Fluorescent Focus Inhibition test (RFFIT) [44]. If the RVNA titer was below 0.5 IU/ml, 4-dose PEP was considered as unsuccessful, and the ARS was instructed to administer a 5th vaccine shot. Two weeks later, another blood sample was collected from the patient at the ARS for a new RVNA titer analysis. This procedure was repeated until protective RVNA titer of $\geq$0.5 IU/ml was achieved.

The RFFIT used for quantification of RVNA is calibrated against WHO reference serum standard and undergoes routine validation through proficiency testing organized by the European reference laboratory for Rabies, The Nancy laboratory for rabies and wildlife, under the French Agency for Food, Environmental and Occupational Health Safety.

2.2. Study design

This retrospective observational study utilized medical documentation provided by ARS and/or patient themselves. It included basic demographic information (age, gender), immunization protocol data, HRIG administration data, vaccine lot number (vaccine potency), and RFFIT results (RVNA titer) obtained from the RFFIT database (Access, Microsoft Office 2013) maintained by the National Reference Laboratory for Rabies. The study focused on patients who underwent PEP between January 01, 2017 and December 31, 2019 (Fig. 1).

To be elegible for the analysis, patients needed to meet the following inclusion criteria: (i) completion of the PEP protocol (receipt of all prescribed vaccines) with RFFIT performed on their serum sample at least two weeks after the last PVRV vaccine, (ii) medical documentation provided by the local ARS upon completion of immunization, (iii) all PVRV vaccines used for PEP in one patient must be from the same LOT (having the same potency), and (iv) for cases involving HRIG administration, the applied dosage needed to be 20 IU/kg of body weight.

Data with parameters of interest were generated for all individuals who received 4 vaccines against rabies (4-dose Essen cohort). Additional records were generated for patients who did not reach an RVNA titer $\geq$0.5 IU/ml after the reduced Essen protocol, and these records formed the 5-dose Essen cohort. Both cohort's records were further analyzed and compared.

2.3. Statistical analysis

In this study, we conducted various statistical analyses using the R programming language [45] and several R-packages, namely "survival" [46], "coxme" [47], and "survminer" [48]. We began with standard descriptive summaries for all variables in the study, excluding multivariate and univariate outliers from the sample.

For determining the strongest predictors of seroconversion, we performed linear regression. Additionally, we investigated the correlation between continuous independent variables and RVNA titers after logaritmic tranformation ($Y = \log(Y)$) to understand their relationship. To identify the most influential factors for achievement of seroconversion, logistic regression was performed. Furthermore, we used individual continuous variables as dependent variables in a series of *t*-tests to assess any significant differences between patients who achieved seroconversion and those requiring extension of the PEP regimen with additional vaccine doses. For instance, we examined whether two groups with RVNA $<$0.5 IU/ml and RVNA $\geq$0.5 IU/ml after 4-dose PEP showed

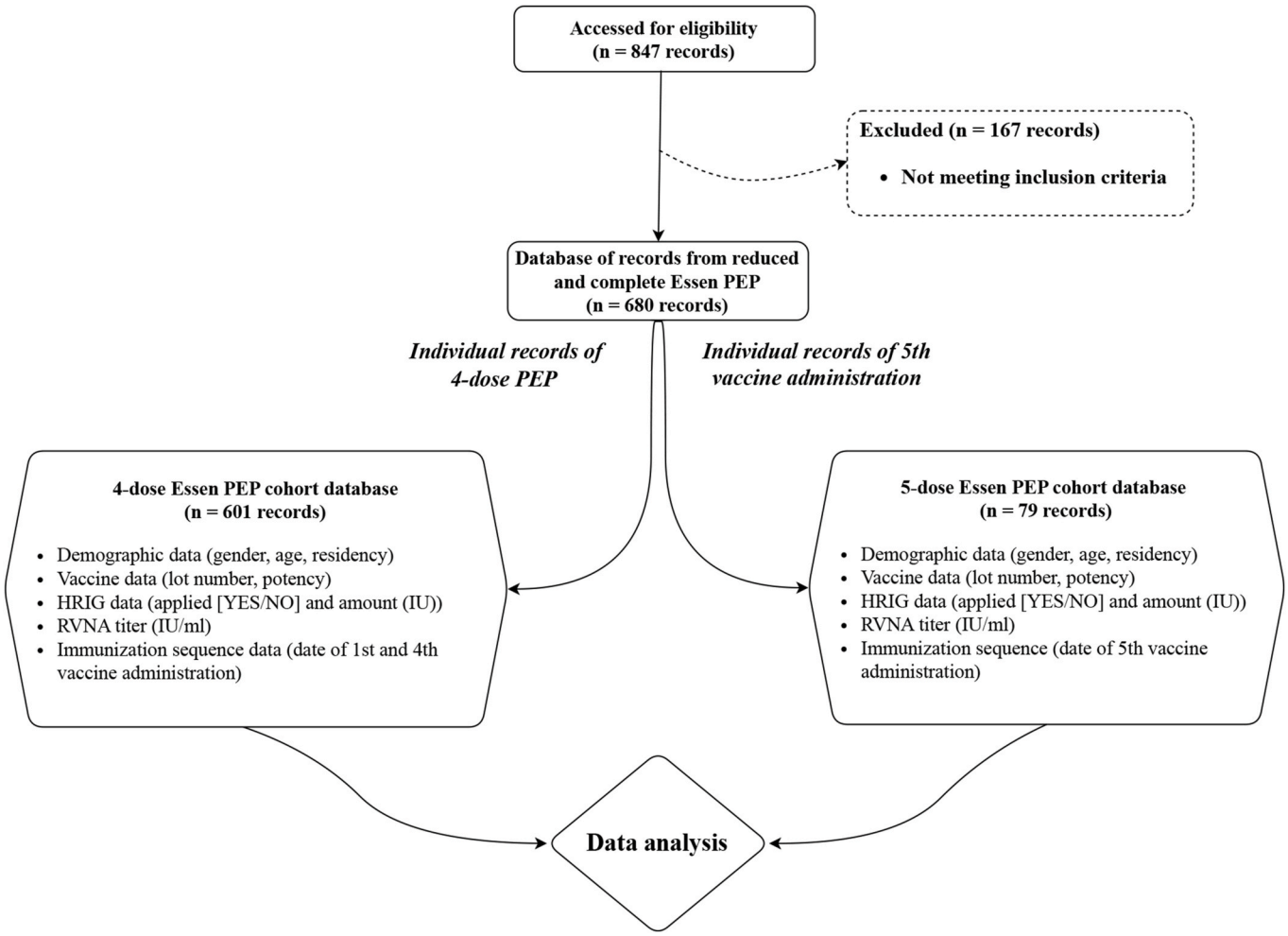


Fig. 1. Study flowchart. PEP - Post-exposure prophylaxis; HRIG - Human rabies immunoglobulin; RVNA – Rabies virus-neutralizing antibodies; IU – international units. Flowchart generated using open source software draw. io (https://app.diagrams.net/) [62].

statistically significant difference on 'Age' variable. To explore potential differences between expected and observed frequencies of the categorical variables in relation to RVNA titers (<0.5 IU/ml or ≥ 0.5 IU/ml after 4-dose PEP), we conducted a series of Chi-square tests.

For the primary objective of determining the strongest predictors of seroconversion, we employed a logistic regression model. A Cox mixed effects model, referred also as Cox proportional hazards mixed model was applied in our study to analyze the time until the event of interest (seroconversion) occurs. The examined independent variables included available demographic information (age, gender), HRIG administration, units of HRIG administered, and residency.

The model was applied to two scenarios (i) successful immunization (i.e., seroconversion): after a 4-dose Essen PEP (scenario I) (ii) and successful immunization (i.e., seroconversion), after an extended (5-dose) Essen PEP (scenario II). To visualize the duration until seroconversion occurred, Kaplan-Meier curves were constructed. Graphs were generated using GraphPad Prism 9 and RStudio softwares.

3. Results

3.1. Characteristics of study participants

In this study, from 847 patients, total 601 subjects were included, generating a total of 680 records (i.e., 601 records 4-dose Essen PEP and 79 records of 5th vaccine administration) (Fig. 1). Patients who were excluded from the study were (i) immunized with different vaccine lots, (ii) did not finish immunization in Serbia, and/or (iii) did not report for serum sampling after 4-dose Essen PEP was finished. All patients included in this study underwent 4-dose Essen PEP ($n = 601$), while only those who failed to achieve seroconversion (i.e., RVNA titer <0.5 IU/ml) had an additional record of 5th dose administration ($n = 79$). Females constituted the majority in both 4-dose Essen cohort (325/601; 54.07%) and the group requiring a 5th vaccine (47/79; 59.49%). The mean age of patients who underwent reduced Essen PEP was 43.88 years (95% CI 42.1–45.6), while the mean age of patients requiring additional vaccine dose was 55.3 years (95% CI: 51.8–58.8).

3.2. Demographic factors associated with 4-dose and 5-dose essen PEP outcome

Immunization success rates were assessed after administering 4-dose and 5-dose Essen PEP, resulting in 84.5% (508/601) and 97.46% (77/79) success rates, respectively. Only two patients required six anti-rabies vaccines for achieving a minimum RVNA titer of ≥0.5 IU/ml. These patients were males of 84 and 33 years old and had recently undergone chemotherapy before PEP initiation.

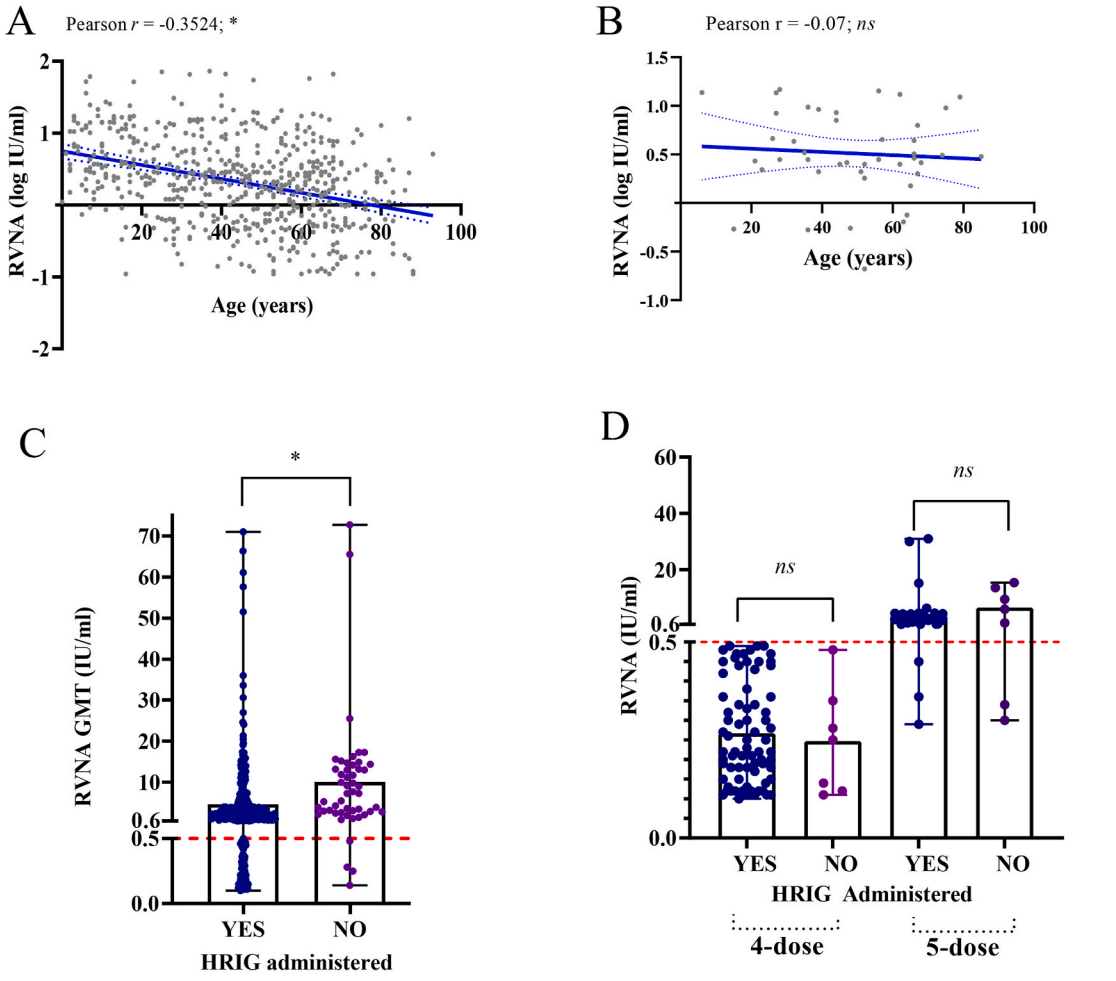
There were no significant differences in seroconversion frequency between male or female subjects who underwent either 4-dose or 5-dose Essen PEP ($\chi 2(1) = 0.027$, $p > 0.05$, and $\chi 2(1) = 0.11$, $p > 0.05$, respectively).

The patients who achieved seroconversion after 4-dose and 5-dose Essen PEP had a mean age of 46.88 (95% CI: 42.13–45.63) and 54.75

years (95% CI: 50.71–58.8), respectively. Age was found to be a factor related to immunization success after 4-dose Essen PEP (Mann-Whitney, U = 31,762, $p < 0.05$) but not after receiving a 5th dose (Mann-Whitney, U = 158.5, $p > 0.05$). A weak, yet significant negative correlation between age and RVNA titer was observed in the 4-dose Essen group ($r = -0.3524$, $p < 0.05$) (Fig. 2A), while no significant correlation was found in the in 5-dose Essen group ($r = -0.146$, $p > 0.05$) (Fig. 2B).

Logistic regression analysis revealed that age is a risk factor for unsuccessful immunization with 4-dose Essen, with odds of seroconversion decreasing by 3.18% for each additional year of age ($\beta = 0.031395$; SE = 0.005973).

Furthermore, the Cox proportional hazards mixed model showed that age influences immunization success. Specifically, age was associated with absence of seroconversion after the 4-dose Essen (scenario I); −0.007 (95% CI -0.011; −0.003). Age groups were categorized in 15-years intervals and inputted into the model as categorical data. For



	4-dose cohort		
	Sample size (n)	RVNA GMT (IU/ml)	RVNA Range (IU/ml)
HRIG administered	456	1.857	0.1 – 71.0
HRIG not administered	72	5.119	0.14 – 72.20

	4-dose individuals qualified for 5th dose administration		
	Sample size (n)	RVNA GMT (IU/ml)	RVNA Range (IU/ml)
HRIG administered	72	0.267	0.1 – 0.49
HRIG not administered	7	0.247	0.11 – 0.48
	5-dose cohort		
	Sample size (n)	RVNA GMT (IU/ml)	RVNA Range (IU/ml)
HRIG administered	72	2.176	0.29 – 31.0
HRIG not administered	7	2.797	0.3 – 15.4

Fig. 2. Association of RVNA titer with age and administration of HRIG. RVNA titer values used for Spearman correlation analysis were log transformed in order to achieve normal distribution. (A) RVNA titer is significantly associated with age of patients immunized via reduced (4-dose) Essen PEP; (B) RVNA titer is not associated with age of patients immunized via 5-dose Essen PEP; (C) Seroconversion rate is significantly associated with HRIG administration during reduced Essen PEP. Patients who received HRIG with 1st vaccine dose more frequently failed to reach seroconversion (RVNA titer ≥0.5 IU/ml) compared to patients where HRIG was not administered; bars are representing range intervals; Table is presenting GMT and range of RVNA titers for cohorts with and without HRIG administration and corresponds to chart above(D) Seroconversion rate is not associated with HRIG administration during 5-dose Essen PEP; bars are representing range intervals; Table is presenting GMT and range of RVNA titers for cohorts with and without HRIG administration before and after 5th dose administration and corresponds to chart above. RVNA – rabies virus neutralizing antibodies, *$p < 0.05$, *ns* – not significant.

the 4-dose regimen, all age categories were associated with absence of seroconversion, with two age groups (45–60 and 75–92) having the highest risk (Fig. 3A).

The mean age of patients who did not achieve seroconversion after 4-dose Essen PEP was 55.3 years (95% CI: 51.8–58.8). To predict whether a patient will require 5th vaccine dose to achieve RVNA titer ≥0.5 IU/ml, we used the lower boundary of the 95% CI of age from the group of unsuccessfully immunized patients after 4-dose Essen PEP (i.e., 51 years of age). After implementing this threshold and stratifying the patients into <51 and ≥51 years of age groups, we found statistically significant

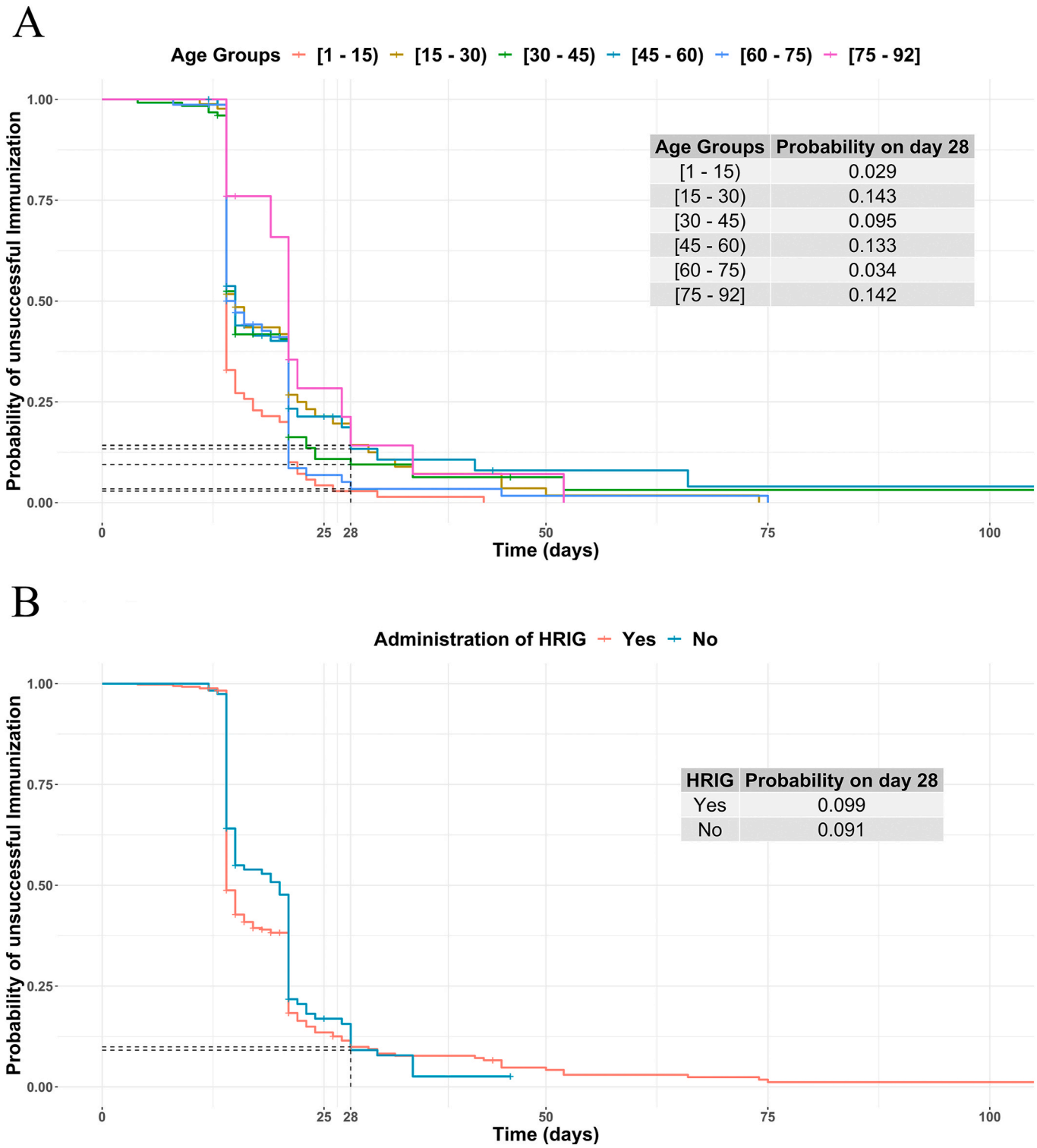


Fig. 3. A) Kaplan–Meier curve of probability for unsuccessful immunization after reduced Essen PEP for specific patient age groups in the context of time. Cox proportional hazards mixed model identified age groups 45–60 and 75–92 to have the highest risk of unsuccessful immunization after 4-dose Essen PEP B) Kaplan–Meier curve of probability for unsuccessful immunization after reduced Essen PEP in the context of time according if HRIG was administrated. Cox proportional hazards mixed model identified significant difference in probability of patient achieving seroconversion (i.e. immunization being successful) depending if patient received HRIG as part of 4-dose Essen PEP.

difference ($\chi2(1) = 16.341, p < 0.0001$) in the frequency of patients who achieved seroconversion. In addition, there was significance in the residuals of the patients above and below 51 years who failed to achieve seroconversion (Bonferroni adjustment, $p < 0.05$), indicating that more people above 51 years failed to reach seroconversion after 4-dose Essen PEP.

3.3. Effect of HRIG and vaccine potency on 4-dose and 5-dose essen PEP outcome

HRIG was administered to 456 cases (75.87%) as part of the 4-dose Essen protocol and 72 cases (72/79; 91.13%) as part of the 5-dose Essen protocol. Seroconversion was achieved in 83.55% (381/456) and 95.83% (69/72) of patients, respectively. Patients who achieved seroconversion (i.e., had RVNA titer ≥0.5 IU/ml) after the 4-dose Essen protocol received an average of 1345.62 IU (95% CI: 1275.6–1415.5) of HRIG, while those who achieved seroconversion after the 5-dose Essen protocol received an average of 1438 IU (95% CI: 1350.8–1535.5). Patients who failed to reach seroconversion after 4- and 5-dose Essen protocols (i.e., had RVNA titer <0.5 IU/ml) received a higher amount of HRIG: 1456 IU (95% CI: 1382.8–1529.9) and 1480 IU (95% CI: 153.5–2806.4), respectively. Patients who failed to reach seroconversion after the 4-dose Essen regimen received a significantly higher amount of HRIG compared to subjects who achieved seroconversion (Mann-Whitney; $U = 9812.5, p < 0.05$). In addition, patients who hadn't received HRIG in the 4-dose Essen cohort developed significantly higher RVNA titer values compared to those where HRIG was included (Mann-Whitney; $U = 74.5$, $p < 0.05$) (Fig. 2C). These differences were not observed in the 5-dose Essen cohort (Mann-Whitney; $U = 154.5$, $p > 0.05$ and $U = 142.5$, $p > 0.05$, respectively). Despite the observed differences in the 4-dose Essen cohort, no correlation emerged between specific RVNA titer values and the administered amount of HRIG in both the 4-dose and 5-dose Essen protocols ($r = -0.07052$, $p > 0.05$, and $r = -0.1461$, $p > 0.05$).

The Cox proportional hazards mixed model also revealed that the administration of HRIG was associated with unsuccessful immunization for the 4-dose Essen regimen (−0.27 (−0.515; −0.024)) (Fig. 3B).

Over a 3-year period, seven PVRV vaccine lots were used for PEP in Republic of Serbia (i.e., K1391-2: 3.6 IU/dose; L1262-2:10.5 IU/dose; M16661V: 6.5 IU/dose, N1G972V: 9.7 IU/dose, N1J313V: 9.2 IU/dose, P1C531V: 10.8 IU/dose, and P1D271V: 6.2 IU/dose). However, we found no relationship between vaccine lot and seroconversion rate in either the 4-dose ($\chi2(5) = 10.02, p > 0.05$) or the 5-dose Essen protocol ($\chi2(5) = 3.96$, $p > 0.05$). Additionally, there was no significant difference in vaccine potency between patients who achieved seroconversion and those who did not for both the 4-dose (Mann-Whitney; $U = 19{,}039$, $p > 0.05$) and 5-dose Essen protocols (Mann-Whitney; $U = 179$, $p > 0.05$). Furthermore, no correlation was found between vaccine potency and RVNA titer for both the 4-dose and 5-dose Essen protocols ($r = -0.007$, $p > 0.05$ and $r = 0.046$, $p > 0.05$, respectively).

4. Discussion

This study provides valuable insights into the seroconversion rate after PEP against rabies and examines factors that may influence the effectiveness of immunization in real-world setting within one European country. It is important to know that the data here come from non-homogenous cohort where various common comorbidities are expected (e.g., hypertension, diabetes mellitus, various hormonal dysbalances, etc.) making them different from majority of studies that focus on healthy individuals in controlled settings. Nevertheless, we believe that data from real-world settings are more representative and can be highly valuable for decision-making regarding PEP against rabies.

In our research on the Serbian population immunized against rabies, the seroconversion rates were found to be 84.5% and 97.46% after 4-dose and 5-dose Essen PEP, respectively. Patient gender and PVRV vaccine potency were not found to be significant factors affecting seroconversion rate in 4- or 5-dose Essen PEP. Accordingly, current WHO recommendations of 4-dose intramuscular PEP [49] could be considered as inadequate for specific individuals within this representative cohort.

The patient's age was identified as one on the few factors that is related with humoral response during 4-dose Essen PEP. Older patients showed lower RVNA production, consistent with previous studies [26,50–52]. Different age-specific conditions may be the reason for this phenomena, such as development of auto-antibodies to type I interferons, that may modulate the immune response to vaccines in older individuals [53–55].

Furthermore, our seroconversion rates differed from those reported in Indian patients [23], possible due to differences in sample size and age distribution between the two studies. To compare the two studies, let's first look at the participant demographics. The Indian study enrolled 70 subjects, divided into reduced and complete Essen PEP groups, with an average age of 37.86 years for the 4-dose Essen group and 34.91 years for the 5-dose Essen group [23]. In contrast, our study included 601 patients who received the 4-dose Essen PEP and 79 patients who received the 5th vaccine dose, with average ages of 48.88 and 54.75 years, respectively.

A recent publication acknowledged that older adults tend to have lower levels of antibodies compared to younger adults [56]. However, it's important to note that the antibody levels observed in the study were significantly higher than the 0.5 IU/mL threshold, and as a result, these findings were not considered clinical significant. In addition, there's a long-standing assumption that individuals who have received modern cell culture vaccines for rabies are not experiencing breakthrough infections. It's worth noting that the threshold of 0.5 IU/mL for RVNA titer is somewhat arbitrary, and it's possible that even lower levels of antibodies may still offer protection against rabies.

On the other hand, it's important to recognize that for cases classified as WHO Category III exposure to rabies, there isn't a requirement to confirm rabies exposure before initiating PEP. This makes it challenging to accurately determine the real-world incidence of breakthrough infections. Although the "Law on measures for early detection, diagnosis and prevention of Rabies in Serbia"(Official Gazette of Republic of Serbia No. 78/2009) provides detailed instructions for animal surveillance and analysis on rabies after WHO category III exposure, it is extremely challenging to form a cohort of patients bitten by proven rabid animals in order to quantify incidence of breakthrough infections.

Another important factor affecting seroconversion was the administration of HRIG, which was found to be related to lower RVNA values. This interference with vaccine immunogenicity was more pronounced in the 4-dose Essen PEP group, as previously reported [57,58]. Additionally, we observed that HRIG-related interference was greater among individuals with higher body weight, who consequently received larger doses of HRIG. Although the precise mehanism leading to this phenomenon is still not completely understood, intereference has been linked with the half-life of administered anti-RABV antibodies. As it was described previously, titer of RVNA derived from passive immunization is peaking within first 4 days and declining progresively until day 21 [59,60], with intereference period possibly lasting up to day 28 [58]. Therefore, 5th vaccine in the case 4-dose Essen PEP extension is facing no intereference with antibodies administered via RIG, allowing patient to achieve expected RVNA titer values. Implementing WHO recommendations to adjust the amount of RIG administered may help mitigate this interference and reduce costs of PEP [49]. More precisely, it was recommended that amount of RIG administered should be adjusted to achieve the maximal infiltration of the wound and not to rely on a body weight as a parameter to determine the RIG volume necessary for each patient [61]. In this way, patient still receives effective passive immunization, while intramuscular administration of RIG is avoided and intereference with rabies vaccine immunogenicity is reduced, as well as the costs and the amount of RIG required for PEP. The current PEP

guideline in Serbia recommends dosing RIG according to body weight. Modifications may be made based on the WHO 2018 recommendation in cases where multiple PEPs are initiated in a single antirabies station during a shortage of RIG stock.

Our study's strengths lie in the large sample size and high degree of completeness, making the results representative of real-world data. However, some limitations should be acknowledged, such as the inability to assess the number and location of lesions requiring HRIG infiltration to identify local interference with vaccine particles, as well as the lack of evaluation of other PEP protocols. Although all patients reported no prior immunization against rabies, it is essential to note that the limitation of this observational study lies in the absence of RVNA titer verification for all subjects on day 0. This verification is crucial to substantiate that the ongoing PEP indeed serves as a primary immunization.

5. Conclusion

This study highlights age and HRIG administration as factors linked to seroconversion after 4-dose Essen PEP. We found that odds of immunization result in seroconversion is decreased by 3.18% for each additional year of patients age, as well as that age of 51 years is a threshold from which Serbian patient is expected to require 5th vaccine dose to achieve seroconversion. Understanding these factors can help optimize PEP strategies for rabies immunization in real-world settings. Future research should investigate the relationship between HRIG administration and vaccine immunogenicity in various PEP protocols (e.g, comparison of (i) 0,3,7,14; (ii) 0,3,7,28 and (iii) 0,3,7,14,28 shemes).

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CRediT authorship contribution statement

Pavle Banović: Writing – review & editing, Writing – original draft, Visualization, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dragana Mijatović:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Verica Simin:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Nenad Vranješ:** Writing – review & editing, Resources, Data curation, Conceptualization. **Eleftherios Meletis:** Writing – review & editing, Visualization, Software, Methodology, Formal analysis, Data curation. **Polychronis Kostoulas:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Dasiel Obregon:** Writing – review & editing, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Alejandro Cabezas-Cruz:** Writing – review & editing, Validation, Supervision, Software, Methodology, 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.

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[62] jgraph/drawio. Statements Ethical statement: this study was conducted in full accordance all applicable Pasteur Institute Novi Sad Research Policies and Serbian state laws and regulations, including the Patient Rights Law. 2023. The research was approved by the Ethical Committee of Medical Faculty Novi Sad (Approval number 01-39/72/1, dated 30th June 2021).