Non-specific effects of rabies vaccine on the incidence of common infectious disease episodes: study protocol for a randomized controlled trial

Protocol Number: 18-04-FL

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Sponsor: Ross University School of Veterinary Medicine (RUSVM)

Sponsor's Protocol Reference Number: 18-04-FL

Co-Sponsor: Serum Institute of India Pvt. Ltd. (SIIPL)

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
Roles and responsibilities (p. 3)	Designation of Donna Barry as Manager of RUSVM Student Health Services was removed, and designation of Marshalette Smith-Antony as interim Manager of RUSVM Health Services was added.	Change in leadership of RUSVM Health Services. Dr. Donna Barry left the role when she left RUSVM, but remained on the study as study nurse practitioner
Roles and responsibilities (p. 3)	Addition of Shianne England as study nurse.	Shianne England brought in as nurse in RUSVM Health Services after Dr. Donna Barry's departure.
3.2.4	Addition of secondary objective to safety analysis (sub-group analysis for potential effect measure modification of safety endpoints by sex).	Evidence in the literature of modification of vaccine safety outcomes by sex. Alignment with secondary objective of sub-group analysis for potential effect measure modification of Primary Endpoint and Secondary Endpoints 1-7 by sex (Section 3.2.2; third bullet point).
3.2.5	Addition of secondary objective to vaccine efficacy ancillary study (sub-group analysis for potential effect measure modification of vaccine efficacy endpoint by sex). Extension of vaccine efficacy ancillary study from two to three consecutive cohorts (including Fall 2019).	Evidence in the literature of modification of vaccine efficacy outcomes by sex. Alignment with secondary objective of sub-group analysis for potential effect measure modification of Primary Endpoint and Secondary Endpoints 1-7 by sex (Section 3.2.2; third bullet point). To accommodate this secondary objective, the vaccine efficacy ancillary study was extended to a third cohort (Fall 2019) to increase the number of males in the ancillary study.

Roles and responsibilities

Darryn Knobel[1] BVSc PhD [Principal Investigator]

Anne Conan[1] DVM PhD [Study coordinator and Data analyst]

Donna Barry[1] APN DNP [Study nurse practitioner]

Marshalette Smith-Antony[1] RN [Study nurse and interim Manager of RUSVM Health Services]

Juliet Battice[1] RN [Study nurse]

Shianne England[1] RN [Study nurse]

Felix Toka[1] DVM PhD DSc [Immunology specialist]

Bradford D. Gessner[2] MD MPH [Medical advisor]

Christianah Ibironke Odita[3] DVM MVPH MSc [PhD Student]

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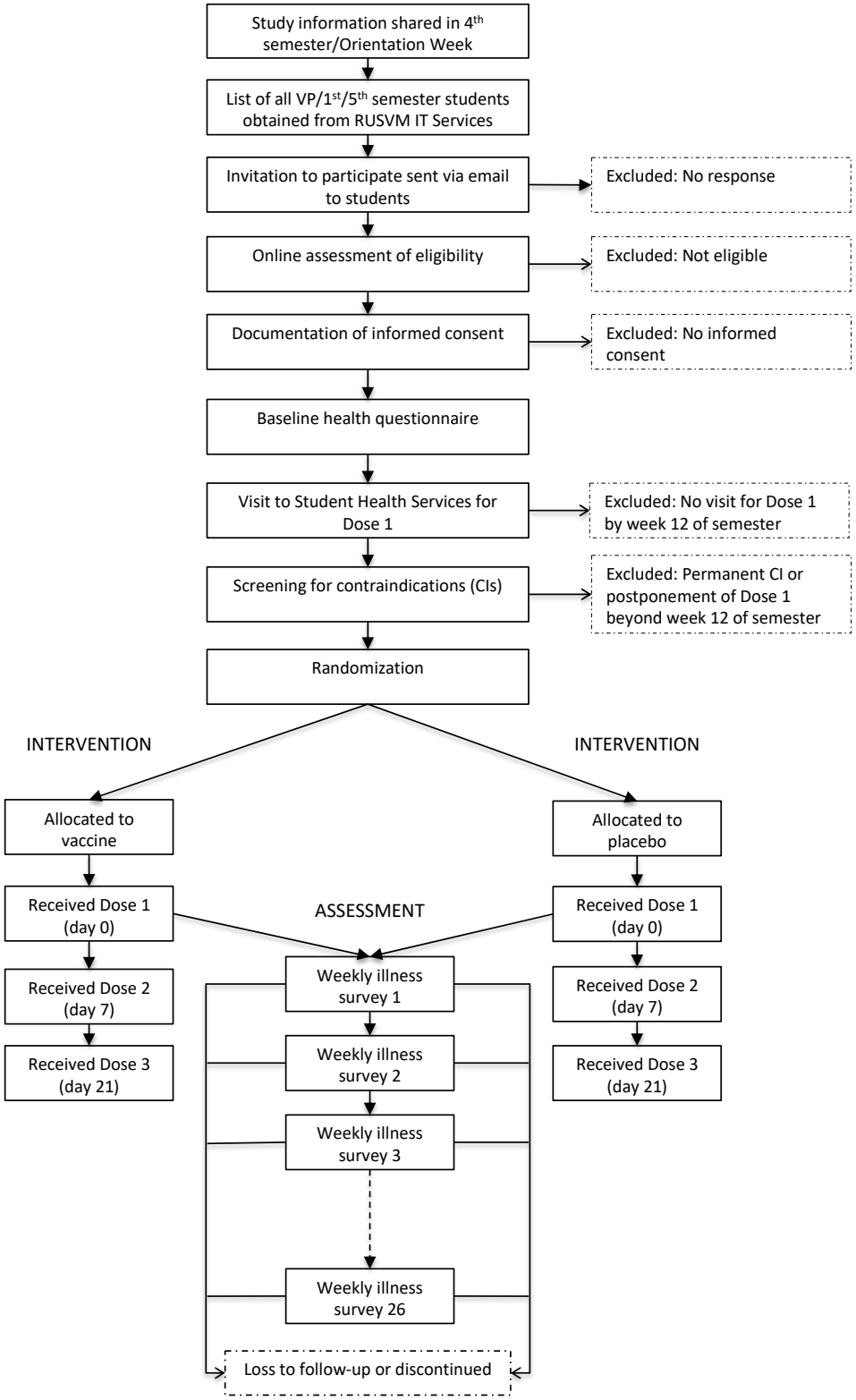
STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects

All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 STUDY SCHEME



2 INTRODUCTION

2.1 BACKGROUND AND RATIONALE

Non-specific effects (NSEs) of vaccines – also known as heterologous effects [1] – are those immune-mediated effects of vaccines on morbidity and mortality that are not explained by the prevention of the targeted disease [2]. Hypothetically, NSEs may be beneficial or detrimental. It has been proposed that this is determined by the type of vaccine [3], with NSEs of live vaccines (such as oral polio vaccine (OPV), Bacillus Calmette–Guérin (BCG) and measles-containing vaccines (MCV)) reducing all-cause mortality and NSEs of non-live vaccines (such as inactivated polio vaccine (IPV) and diphtheria tetanus and whole cell pertussis (DTP) combined vaccines) increasing all-cause mortality. It has also been reported that these NSEs on all-cause mortality are generally more pronounced in females than in males [4]. A systematic review of the evidence for NSEs of BCG, DTP and MCV on all-cause mortality in children under five years old provides support for these conclusions, although much of the evidence came from observational studies with a high risk of bias [5], and the importance and implications of these effects remain controversial [6]. There is a clear need for new data from randomised controlled trials (RCT), but there are logistic and ethical challenges to trials in children with relevant vaccines in high-mortality settings [6].

Recently, it was proposed that rabies vaccine (a non-live vaccine) has protective NSEs in people and in animals [7-9]. The higher number of cases of meningitis and of cerebral malaria among children receiving the RTS,S malaria vaccine than in controls who received rabies vaccine in a recent large RCT [10] can be explained by a protective NSE of rabies

vaccine [7, 8]. In a population-based cohort study, rabies vaccine was shown to be associated with decreased all-cause mortality in free-roaming dogs in a high-mortality setting [9]. A review of the literature revealed older studies in mice, using live attenuated rabies vaccine, that provided protection against *Klebsiella pneumonia* sepsis [11] and mortality following intracerebral injection of a neurotropic strain of herpes virus [12]. Studies have shown that part of the rabies virus (the nucleoprotein, which is present in the vaccine) acts as a non-specific immunological enhancer [13]. More recently, a controlled trial by the PI has shown that rabies vaccine reduces the risk of respiratory disease in feedlot cattle (manuscript in preparation). Further randomized controlled trials are needed to test for the effect in people.

A non-specific protective effect of rabies vaccine would have implications for prevention of rabies in endemic areas. In rabies-endemic areas, recognition of nonspecific effects of rabies vaccine in humans could affect public health decisions: Although rabies vaccine is known to be a safe and effective vaccine, its routine use as pre-exposure prophylaxis in children is not recommended as it is not cost-effective in most situations, in which the incidence of exposure to rabies is relatively low [14]. A substantial non-specific protective effect against other infections would improve the cost-comparability of routine pre-exposure prophylaxis vs. a reliance on post-exposure prophylaxis alone in areas where dog rabies is endemic. Post-exposure prophylaxis is not always affordable or readily available in many developing countries; hence incorporating rabies vaccine into routine childhood vaccination schedules could prevent rabies cases.

The aim of the proposed study is to determine if rabies vaccine reduces the incidence rate of episodes of common infectious disease syndromes in a population of veterinary students. The primary study endpoint will be a composite outcome of common infectious diseases (CID) including upper respiratory, diarrheal, and febrile illnesses. CIDs was chosen as the endpoints because of their common occurrence (and thus ability to have adequate study power), because of their public health and economic importance, and because no studies have yet documented rabies immunization effects on milder disease outcomes.

3 HYPOTHESIS, OBJECTIVES AND ENDPOINTS

3.1 PRIMARY HYPOTHESIS

Compared to an unvaccinated control group, administration of at least one dose of a three-dose course of rabies vaccine to previously-unvaccinated subjects leads to at least a 25% relative reduction in the rate of self-reported new episodes of common infectious disease syndromes (respiratory, diarrheal and febrile illness) over a 26-week period.

3.2 OBJECTIVES AND ENDPOINTS

3.2.1 PRIMARY

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine if the incidence rate of self-reported episodes of common infectious disease (CID) syndromes (respiratory, diarrheal and febrile illness) over a 26-week period is significantly different between previously-unvaccinated	Number of self-reported new episodes of acute common infectious disease (CID), defined as any of the following: upper respiratory illness (URI) *or* influenza-like illness (ILI) *or* diarrhea (DIA)	The assumption underlying the choice of a composite primary endpoint is that the biological mechanism that protects against infections that cause URI or ILI will also protect against infections that cause diarrhea or acute

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
subjects that receive at least one dose of a three-dose course of rabies vaccine and those subjects that receive a placebo injection. *Primary analysis will be based on an intention-to-treat analysis.*	*or* undifferentiated febrile illness (UFI). URI is defined as (two or more of the following: runny or blocked nose/sneezing/sore throat/cough) *and* (absence of itchy or watery eyes). ILI is defined as [fever (feeling feverish, or an axillary, oral or otic temperature of 100°F or higher)] *and* (cough or sore throat). DIA is defined as three or more loose stools within a 24-hour period. UFI is defined as [fever (feeling feverish, or an axillary, oral or otic temperature of 100°F or higher)] *and* (not meeting the case definition of URI, ILI or DIA). To be defined as a new episode, illness must be preceded by at least one week without any CID. Time Frame: Weekly self-reporting of occurrence or non-occurrence of episodes of CID for a maximum of 26 weeks, starting 10-14 days after allocation.	undifferentiated febrile illness, and therefore there is no need to separate those outcomes, and no biological basis currently for doing so. If rabies vaccine acts non-specifically to prevent some infectious diseases, its biological basis is unknown. However, the only existing data suggest it may work as a T-cell enhancer, which in theory could prevent any infectious disease. Assessment of occurrence of study syndromes will be done on a weekly basis, through completion of a weekly survey of self-reported symptoms of illness by participants. For the composite outcome of CID, a week without any illness must occur before an illness is considered a new episode; a similar requirement will exist within individual disease syndromes.

3.2.2 SECONDARY

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Secondary		

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
• To compare, between the same two groups over the same time period, 1) The rate of self-reported new episodes of respiratory illness (URI *or* ILI), DIA and UFI. 2) The rate of self-reported new episodes of a) ILI b) URI c) DIA d) UFI 3) The rate of clinically-confirmed episodes of the study syndromes 4) The rate of laboratory-confirmed episodes of the study syndromes • First event, per protocol analysis (receipt of all 3 doses) and extended per protocol analysis (receipt of at least one dose) of primary endpoint and secondary endpoints 1-7 (*primary analyses will focus on all events and intention-to-treat analyses*). • Sub-group analyses for potential effect measure modification of Primary Endpoint and Secondary Endpoints 1-7 by 1) Sex (males or females) 2) Season (spring = January cohorts; summer = May cohorts; winter = September cohorts)	1. Number of self-reported new episodes of respiratory illness (URI *or* ILI), DIA and UFI. 2. Number of self-reported new episodes of URI 3. Number of self-reported new episodes of ILI 4. Number of self-reported new episode of DIA 5. Number of self-reported new episodes of UFI 6. Number of clinically-confirmed episodes of the study syndromes, defined as an episode resulting in a visit to the RUSVM Student Health Services with a recorded ICD10 of J00 (acute nasopharyngitis); J11 (influenza due to unidentified influenza virus); R19.7 (diarrhea) or R50.9 (fever, unspecified). 7. Number of laboratory-confirmed episodes of the study syndromes, defined as clinically-confirmed episodes with laboratory diagnosis of influenza virus, respiratory syncytial virus or metapneumovirus (URI/ILI episodes) or rotavirus or norovirus (DIA episodes) .	For Secondary Endpoint 1, in any week a single participant could report one of the following: • No CID episode • One CID episode (URI *or* ILI *or* DIA *or* UFI) • Two CID episodes (either URI *or* ILI, *and* DIA) By this definition, a participant cannot experience more than two CID episodes within a week, as occurrence of URI together with ILI is considered an episode of ILI only, and occurrence of URI, ILI and/or DIA precludes the occurrence of UFI. We also will attempt to determine impact on etiologically confirmed outcomes but we expect few episodes to meet this criteria and thus study power will be low. Etiological confirmation will be done for a limited range of pathogens only (influenza virus, respiratory syncytial virus, metapneumovirus, rotavirus and norovirus) through an independent research protocol (RUSVM IRB protocol #17-11-FL) and the test results sent back to the RUSVM Student Health Services.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
3) Class (new arrivals [VP and 1st semester] vs. 5th semester) • Subset analyses of Primary Endpoint and Secondary Endpoints 1-7 in the subset of participants excluding those participants categorized as immunosuppressed (reporting use of immunosuppressive medications or treatments or occurrence of immunosuppressive medical conditions in baseline questionnaire)		

3.2.3 TERTIARY

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Tertiary/Exploratory		
• Among the vaccinated group only, to compare Primary Endpoint and Secondary Endpoints 1-7 between high responders and low responders, defined by RVNA titers measured by rapid fluorescent foci inhibition test (RFFIT) at day 180 after first vaccination.	• Primary Endpoint and Secondary Endpoints 1-7	See above
• Explore latency period and duration of effect on Primary Endpoint and Secondary Endpoints 1-5.	• Primary Endpoint and Secondary Endpoints 1-5	
• Explore the effect of dose (1 vs. 2 vs. 3) on Primary	• Primary Endpoint and Secondary Endpoints 1-5	

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Endpoint and Secondary Endpoints 1-5.		

3.2.4 SAFETY

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Safety		
• To compare, between the same two groups, 1) The rate of solicited adverse events (AEs) through 3 days after each injection (dose 1, 2 and 3) 2) The rate of unsolicited adverse events and serious adverse events (SAEs) through four weeks after first injection • As a secondary objective, sub-group analysis for potential effect measure modification of safety endpoints by sex (males or females)	1. Number of solicited self-reported events over three days following each injection of the following adverse events: a. Local reactions (limited to the site of the injection): pain, erythema, oedema, pruritus and induration. b. Systemic reactions: fever, shivering, malaise, asthenia, faintness, dizziness, headache, myalgia, arthralgia, nausea and abdominal pain. c. Hypersensitivity or allergic reactions: anaphylaxis, urticaria, rash and erythema multiforme. 2. Number of unsolicited adverse events and SAEs reported to RUSVM Student Health Services through four weeks after first injection	Solicited adverse events are those adverse events listed in the package insert of the study intervention (Rabivax-S rabies vaccine)

3.2.5 VACCINE EFFICACY ANCILLARY STUDY

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Vaccine efficacy ancillary study		
• To compare, between participants in each group over three consecutive cohorts (Spring 2019, Summer 2019 and Fall 2019)(n = ~350), concentrations of rabies virus neutralizing antibody (RVNA) before first injection (day -7) and six months after first injection (day 180). • As a secondary objective, sub-group analysis for potential effect measure modification of vaccine efficacy endpoint by sex (males or females)	• Geometric mean concentration (GMC) of RVNA titers measured by rapid fluorescent foci inhibition test (RFFIT) on sera samples collected 7 days before injection (day -7) and 6 months after first injection (day 180).	

3.2.6 IMMUNOLOGY ANCILLARY STUDY

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Immunology ancillary study		
• To compare, between 20 consecutively enrolled participants in each group (n = 40), 1) cytokine response of isolated peripheral blood mononuclear cells (PBMCs) stimulated with antigens unrelated to rabies vaccine antigens	1. Concentration of TNFα, IL-1β, IL-6, IFNγ and IL-17 by ELISA assays or Luminex system on day 0, 21 and 90 after first injection 2. Expression of 84 genes involved in innate and adaptive immune responses, using custom RT2 Profiler PCR arrays on day 0, 21 and 90 after first injection	

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
2) innate and adaptive immune gene expression profile of isolated PBMCs stimulated with antigens unrelated to rabies vaccine antigens 3) changes in the proportion of monocyte and lymphocyte subpopulations upon stimulation with antigens unrelated to rabies vaccine antigens	3. Proportion of monocyte and lymphocyte subpopulations of peripheral blood mononuclear cells analyzed in a flow cytometer on day 0, 21 and 90 after first injection	

4 STUDY DESIGN

The trial design is a single-site, two-arm, parallel-group, participant-blinded, randomized, placebo-controlled, two-sided comparative study, with an internal pilot study for blinded sample size re-estimation. Allocation to study arm will be by block randomization stratified by sex within cohort (semester) with a 1:1 allocation ratio.

5 STUDY POPULATION

5.1 STUDY SETTING

The study will take place at Ross University School of Veterinary Medicine (RUSVM) on the island of St Kitts in the Caribbean. The Doctor of Veterinary Medicine (DVM) program at RUSVM comprises a preclinical curriculum of seven semesters in St Kitts, and three semesters of clinical training at an affiliated school of veterinary medicine in the United

States, Canada, or other international location. There are three semesters per year (starting in January, May and September), and one intake (cohort) per semester. Each semester is 15 weeks, with a break of two or three weeks between semesters. RUSVM also offers a one-semester Veterinary Preparatory (VP) program. Students who successfully complete the VP program are placed into first semester classes. The study will enroll participants from the VP program and the 1st and 5th semester classes of the DVM program each semester.

Because St Kitts is free from rabies (https://www.cdc.gov/importation/rabies-free-countries.html), students are not required to receive pre-exposure rabies prophylaxis during their preclinical training (semesters 1-7). All students at RUSVM must have received a primary course of rabies vaccine before the end of their preclinical program to provide cover during their clinical training (semesters 8-10). This is based on the recommendations of the Advisory Committee on Immunization Practices for human rabies prevention [15], that veterinarians in the United States are considered a population in the "frequent" risk category for rabies exposure, for whom the pre-exposure recommendations are (i) primary course of rabies vaccine, followed by (ii) serological testing every two years, and (iii) booster vaccination if antibody titer is below acceptable level. Students who have not received a primary course of rabies vaccine by their 7th semester are able to do so through RUSVM Student Health Services, at a current cost of US$ 200 for the 3-dose course. Vaccinations are done by the RUSVM Student Health Services using Rabivax-S, produced by the Serum Institute of India Pvt. Ltd (SIIPL).

5.2 ELIGIBILITY CRITERIA

5.2.1 INCLUSION CRITERIA

A student registered at RUSVM will be eligible for inclusion in the study if s/he is in the VP program or the 1st or 5th semester of the DVM program.

5.2.2 EXCLUSION CRITERIA

A student registered at RUSVM and in the VP program or the 1st or 5th semester of the DVM program will be excluded from the study if s/he:

1) has previously received a dose of rabies vaccine, or
2) is intending to undertake activities during the course of participation in the study that would increase their risk category of rabies exposure above that of the U.S. population at large, as defined by the Advisory Committee on Immunization Practices (ACIP) for human rabies prevention [15], or
3) does not provide informed consent for participation, or
4) enrolls in the study but does not present for the first injection within the first 12 weeks of the semester (up to and including Week 12), or
5) has a contraindication to rabies vaccine as described in the Rabivax-S package insert (see **Section 5.2.3, Screen Failures** for details).

5.2.3 SCREEN FAILURES

Eligible students who consent to participate in the study and who present for the first injection within the first 12 weeks of the semester will be screened by study personnel at the RUSVM Student Health Services for contraindications to the vaccine, prior to

assignment to study intervention. Contraindications are described in the vaccine package insert and are defined as follows:

1. Contraindications requiring postponement of vaccination:
    a. Fever or an acute illness
    b. Antimalarial medication or other short-term immunosuppressive treatments
    c. Pregnancy
    d. Breastfeeding
2. Absolute contraindications:
    a. Known systemic hypersensitivity to any of the vaccine components (including neomycin)
    b. History of life-threatening reaction to a vaccine containing any of the same substances as Rabivax-S

Individuals who fail screening because of a contraindication requiring postponement of vaccination may be rescreened once the condition is resolved, so long as they still meet the inclusion criteria for the study (a student in the VP program or the 1st or 5th semester of the DVM program) and that the intervention regimen (3-dose course on Days 0, 7 and 21) could still be completed before the semester break (to comply with this requirement, the latest time at which an individual could be rescreened is in Week 12 of the semester).

Individuals who fail screening because of an absolute contraindication will be excluded from the study.

6 STUDY INTERVENTIONS

6.1 VACCINE GROUP

The study intervention is at least one dose (1 mL by intramuscular injection) of a three dose primary course of Rabivax-S. Rabivax-S is a lyophilized vaccine manufactured by Serum Institute of India Pvt. Ltd. containing inactivated purified rabies antigen (Pitman Moore, PM3218 as virus strain) produced using Vero ATCC CCL 81 cells. The diluent (sterile water for injection) is provided in a separate 1 mL ampoule. After reconstitution, a single dose of 1 mL contains an inactivated, purified rabies antigen (not less than 2.5 IU), glycine (40 mg), sucrose (40 mg) and human serum albumin (25% 10 mg).

6.2 CONTROL GROUP

The intervention (placebo) in the control group is at least one dose (1 mL by intramuscular injection) of a three dose primary course (on days 0, 7 and 21) of vaccine diluent (sterile water for injection).

6.3 DOSING AND ADMINISTRATION

Dosing and administration of the vaccine (Rabivax-S) will be according to the package insert, following the schedule for pre-exposure prophylaxis via the intramuscular route; that is, 1 mL by intramuscular injection in the deltoid area of the arm on Day 0, Day 7 and Day 21. Dosage and administration of the placebo (sterile water for injection) will follow the same schedule and route of administration; that is, 1 mL by intramuscular injection in the deltoid area of the arm on Day 0, Day 7 and Day 21. In the event of interruption of the schedule of administration (of either the vaccine or the placebo), the schedule will be

resumed without repeating a previous dose (as per Table 3 Recommendations for Interrupted or Delayed Routine Immunization – Summary of WHO Position Papers, updated April 2018; http://www.who.int/immunization/policy/immunization_tables/en/).

Vaccine and placebo will be administered to participants at the RUSVM Student Health Services by study nurses or nurse practitioner, using a 25G (0.50 x 25mm) needle and 2 mL syringe.

6.4 STUDY INTERVENTION COMPLIANCE

The number of doses received, and the date received, will be recorded by study personnel at RUSVM Student Health Services in Case Report Forms (CRF) for Visits 1-3. An entry will also be made in students' health records at RUSVM Student Health Services.

6.5 DISCONTINUATION OF STUDY INTERVENTION

Prior to administration of each dose of either the vaccine or placebo, participants will be assessed by personnel at the RUSVM Student Health Services for contraindications to the vaccine (see **Section 5.2.3, Screen Failures** for listed contraindications). Individuals with a contraindication requiring postponement of vaccination may return once the condition is resolved to resume the intervention for their allocated group (vaccine or placebo), so long as this occurs before study completion (see **Section 7, Participant Timeline** for definition of study completion). In the event of interruption of the schedule of administration (of

either the intervention or the control), the schedule will be resumed without repeating a previous dose (see **Section 6.3, Dosing and Administration** for details).

Participants for whom the study intervention was halted, due to absolute contraindications or other clinically-relevant event, or for whom intervention was postponed beyond the time of study completion, will remain in the study and complete the weekly illness surveys.

7 PARTICIPANT TIMELINE

Students will be informed about the trial prior to the start of the semester for which they are eligible (orientation week for VP and 1st semester cohorts, and at the end of 4th semester for 5th semester cohorts). Students will be recruited into the study during orientation week and/or the first week of the semester, and will receive the first injection (day 0) within the first two weeks of the semester. Participants will receive the second injection on day 7 and the third injection on day 21. If necessary, participants can begin the intervention later, so long as day 0 falls within the first 12 weeks of the semester (see **Section 5.2.2, Exclusion criteria** for details).

Weekly illness surveys will begin in study week 3 (Monday of study week 3, day 10-14 after first injection) for all participants, and will continue for 26 weeks (through week 11 of the following semester in the case of a two-week semester break, or week 10 of the following semester in the case of a three-week semester break). One week after receipt of the final (26th) weekly survey, participants will be unmasked and will exit the study. Following exit, participants who were allocated to the control group will begin a primary course of rabies

vaccine in weeks 11 or 12 (depending on 3- or 2-week semester break), to be completed by the end of the semester (weeks 14 or 15).

A participant is considered to have completed the study one week after receipt of the final (26th) weekly survey.

7.1 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request. Participants who fail to complete surveys for three consecutive weeks will be followed up and contacted to determine if they have withdrawn or wish to withdraw from the study. If so, they will be informed of their rabies vaccination status and withdrawn from the study. Participants will also be withdrawn from the study if for any reason they become aware of their study arm allocation (unblinding). The reason for participant withdrawal will be captured in the End of Study CRF.

Schedule of activities for participants in a single cohort (semester)

		1st semester						Semester break*		2nd semester					
Weeks since allocation (Study week)		Wk-1	Wk1	Wk2	Wk3	Wk4	Etc.	Wk15	Wk16	Wk17	Etc.	Wk25	Wk26	Wk27	Wk28
Weeks of semester/break		*Wk1*	*Wk2*	*Wk3*	*Wk4*	*Wk5*	*Etc.*	*Wk1*	*Wk2*	*Wk1*	*Etc.*	*Wk8*	*Wk9*	*Wk110*	*Wk11*
ENROLLMENT															
Information	X	X													
Eligibility screen	X	X													
Informed consent	X	X													
Contraindication screening			X												
Allocation			X												
INTERVENTION**															
Dose 1			X												
Dose 2				X											
Dose 3						X									
ASSESSMENTS															
Baseline		X													
Weekly illness survey (1-26)					X (S1)	X (S2)	X (Etc.)	X (S13)	X (S14)	X (S15)	X (Etc.)	X (S23)	X (S24)	X (S25)	X (S26)
Solicited adverse events			X	X		X									
Unsolicited adverse events			X	X	X	X									
Blood collection (efficacy ancillary study)		X (D-7)										X (D180)			
Blood collection (immunology ancillary study)			X (D0)			X (D21)		X (D90)							
Study exit															X

*Some semester breaks are three weeks long, in which case Weeks 27 and 28 since allocation will fall in Weeks 9 and 10 of 6th semester

**Schedule shown here assumes administration of the first dose in week 2 and administration of subsequent doses per schedule (day 7 and day 21). If necessary, participants can begin the intervention at any time in the first 12 weeks of the semester.

7.2 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if s/he fails to complete a weekly survey for more than three consecutive weeks and is unable to be contacted by the study personnel. Contact will be by email to the participant's registered RUSVM email address. The participant will be considered unreachable if s/he does not respond after 3 emails. At this point, s/he will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 SAMPLE SIZE

The estimated number of participants needed to achieve the study's primary objective is 430 (215 in each group). The sample size was calculated using the approach for comparing two negative binomial rates based on true rates (Approach 2 in [17]), implemented using the function *power.nb.test* in package 'MKmisc' in R, and assuming the following parameter values:

- Alpha level = 0.05
- Targeted power = 0.8
- Event rate for control group = 2 (expected mean number of new CID episodes [composite primary endpoint] over 26 weeks)
- Rate ratio under alternative hypothesis = 0.75
- Average length of participation (accounting for drop-out and non-response) = 21 weeks (0.8 of 26-week observation period)
- Negative binomial dispersion parameter (k in [17]) = 0.4

The three nuisance parameters (average length of participation, event rate for control group and negative binomial dispersion parameter) were estimated from data collected over 7 weeks of a pilot study of rates of CID episodes in 90 RUSVM students (40 in 1st semester and 50 in 5th semester) from 21 May to 8 July, 2018 (weeks 3 through 9 of summer semester).

An internal pilot study will be done using data from the first 300 participants to complete the study. As the expected enrollment rate is <100 participants per semester, this will allow capture of seasonal variation in event rates (≥3 semesters in internal pilot study). The three nuisance parameters will be re-estimated from the internal pilot study data and the sample size recalculated, using a blinded sample size re-estimation method for count data [18, 19], which maintains required power without an increase in the type I error. The sample size will only be increased and not decreased on the basis of the internal pilot study (that is, if the recalculated sample size is smaller than the original sample size, the original sample size will still be used).

9 RECRUITMENT

The PI or Study Coordinator will present information on the study to VP and 1st semester students during their orientation week prior to the start of their program, and to 5th semester students during their 4th semester. They will be provided with a link to a website that contains more information about the project, including the informed consent documents and contact details of the study personnel. A question-and-answer session with

the PI will be scheduled. Prospective participants will have the opportunity to ask questions via email or in person with the study investigators, either during scheduled information sessions or by appointment.

To compensate study participants for their time and inconvenience, the study will provide the primary course of vaccine at no cost to all participants (participants allocated to the control group [including participants who withdraw or are withdrawn] will receive the vaccine after exit from the study). Current cost of rabies vaccination for RUSVM students is US$ 200 for a 3-dose primary course.

10 CONSENT

A list of all VP/1st/5th semester student names and email addresses will be obtained from the RUSVM IT Department, and an invitation to join the study will be sent by email to these students. The email will contain a unique link to a survey (Appendix A), which will be used to establish eligibility and seek agreement to participate from eligible students. Initial agreement to participate in the study will be documented electronically as selection of the option, "I agree to participate in the study" for the unique survey linked to the participants identifiers (name, surname and email address). Participants who agree in the online survey will still need to sign a paper copy of the informed consent document (Appendix B) in person with a designated member of the study team (Principal Investigator, Study Coordinator or Nurse Practitioner). Only participants who sign a paper copy of the informed consent document with a member of the study team will be considered enrolled and will be allocated to a study group. A signed and dated copy of the consent form will be

given to the participant. Paper copies of signed consent forms will be collected and stored by the Principal Investigator in a locked file cabinet in an area with limited access (PI's office).

Following enrollment, participants will be given a baseline health questionnaire to complete (Baseline CRF; Appendix C) and will be given instructions to visit the RUSVM Student Health Services Office within the first two weeks of the semester for screening and allocation into one of the two study arms. The completed baseline health questionnaire will be checked by study personnel at RUSVM Student Health Services at Visit 1, collected and stored by the Principal Investigator in a locked file cabinet in an area with limited access (PI's office).

11 ALLOCATION

11.1 SEQUENCE GENERATION

Allocation of participants to study arms will be done by restricted randomization (permuted block design with stratification). Stratification will be by cohort (three cohorts per year: January, May and September) and sex (within cohort). Within strata, randomization will be done by computer-generated randomly permuted blocks of varying size (using the function *blockrand* in the package 'blockrand' in R). Enrolled students will be allocated in a 1:1 ratio to one of the two study arms.

11.2 ALLOCATION CONCEALMENT

Randomization cards will be created by the Principal Investigator or Study Coordinator (using the function *plotblockrand* in the package 'blockrand' in R), placed in opaque envelopes with a window, and sealed, with the sequential subject number (starting from 1 for each sex in each cohort) visible in the envelope window. On the first day of the semester, envelopes will be placed in the RUSVM Student Health Services Office. Envelopes will be drawn sequentially by study personnel at Student Health Services as participants come in for the first injection (day 0), and opened to determine allocation. After the injection is administered, the RUSVM Student Health Services member will complete the Case Report Form for this visit (Visit 1 CRF; Appendix D), including student name, surname, email address, allocation group, intervention actually received, date received, and the batch number and expiry date of the vaccine or diluent. An entry will be also be made in students' health records. Data from the Visit 1 CRF will be entered into a password-protected file ('Participant allocation file') in a restricted-access folder on the RUSVM network. Access to the folder will be restricted to study personnel only. When participants return for the second (day 7) and third (day 21) injections in the course, study staff at RUSVM Student Health Services will refer to the file to determine the participant's allocation. After injection, study personnel will complete CRF Visit 2 (Appendix E) or Visit 3 (Appendix F).

11.3 BLINDING (MASKING)

Participants will be blinded to their study arm allocation. The intervention procedure will be identical for both arms (intramuscular injections at Student Health Services on days 0, 7 and 21). Participants allocated to the control group will receive an intramuscular injection

of sterile water for injection using identical syringes and needles as for the vaccine group. The injection will be prepared in a separate room to maintain participant blinding.

Unblinding of participants will occur in circumstances in which there is a need to determine their rabies pre-exposure vaccination status; for example, in the event of a possible rabies exposure or if participants wish to undertake activities during the course of their participation in the study that would increase their risk category of rabies exposure. Rabies post-exposure management differs between patients who have not been previously vaccinated and those who have been previously vaccinated. The ACIP-recommended treatments for patients who have not been previously vaccinated [16] are rabies immune globulin (RIG) infiltrated around the wound (if present), and four injections of rabies vaccine (one each on days 0, 3, 7 and 14). For previously-vaccinated patients, ACIP recommends that no RIG should be administered, and only two injections of rabies vaccine (one each of days 0 and 3). Thus, in the event of a suspected rabies exposure that requires post-exposure prophylaxis, participants should establish their vaccination status as soon as possible.

Participants can determine their vaccination status by contacting the Student Health Services to request access to their health records, or by contacting study personnel with access to the 'Participant allocation file' and/or Visit 1 CRF (Principal Investigator, Study Coordinator, study personnel at Student Health Services). Steps for determining vaccination status and emergency contact details of all relevant personnel will be provided

in the informed consent document and in the weekly survey sent to participants. Participants who are unblinded will exit the study at that point.

12 DATA COLLECTION METHODS

After enrollment, participants will complete a questionnaire that will capture data on relevant baseline characteristics including sex, age, health and vaccination status. (Baseline CRF; Appendix C). Participants will complete a short survey each week, starting in study week 3, to capture self-reported episodes of illness (respiratory, diarrheal and febrile illness; Primary Endpoint and Secondary Endpoints 1-5) in the preceding week (Appendix G). Surveys will be sent by email to all participants each Monday. Participants who have not completed the survey by Wednesday afternoon will be sent a request by email to complete the survey for the preceding week. Surveys will be sent weekly, for 26 weeks. A participant is considered to have completed the study one week after receipt of the final (26th) weekly survey for that participant's cohort.

As an incentive to enhance participant retention and response rates in weekly illness surveys, all participants who complete a weekly survey will be entered into a draw each week for a chance to win a EC$25 gift voucher to a food/beverage vendor on the RUSVM campus. Selection of a winner each week will be done by computer-generated random sampling. The weekly winner will be notified by email in the week following the draw.

Clinically- and laboratory-confirmed episodes of study syndromes (Secondary Endpoints 6 & 7) will be collected by study personnel at RUSVM Student Health Services, as part of the

routine clinic visits and diagnostics. At the end of each 26-week observation period for each study cohort, data from that cohort will compiled by the director of the RUSVM Student Health Services, anonymized to protect privacy and aggregated by study arm, before being sent to the Data Analyst who will conduct the analysis.

Occurrence of expected Adverse Events (Safety Endpoint 1) will be solicited in an online survey (Appendix H) sent to participants 3 days after receiving an injection (for dose 1, 2 and 3). Occurrence of unsolicited adverse events and serious adverse events (Safety Endpoint 2) will be captured by study personnel at RUSVM Student Health Services through completion of an Adverse Event form (Appendix I; See **Section 15.3, Identification of Adverse Events** for details).

Participants in the vaccine efficacy ancillary study will have a maximum volume of 8 mL of blood collected by venipuncture prior to injection on day -7 (acceptable range -3 through -11), and again on day 180 (acceptable range 173 through 187). Serum will be separated and stored at -20 °C at RUSVM until transport to the laboratory for measurement of RVNA by RFFIT. Serum specimens will be labelled with participant study identification numbers only. No other participant information will be sent to the laboratory.

Participants in the immunology ancillary study will have a maximum volume of 10 mL of blood collected by venipuncture prior to injection on day 0, and again on days 21 and 90. Blood specimens will be immediately taken to the RUSVM Research Laboratory for

isolation of PBMCs. Blood specimens will be labelled with participant study identification numbers only. No other participant information will be sent to the laboratory.

13 DATA MANAGEMENT

Weekly survey data on illness episodes, will be collected through the Qualtrics® platform. Qualtrics safeguards all customer data, and uses secure data centers to ensure the highest protection as per the requirements of the Health Information Technology for Economic and Clinical Health Act (HITECH) (www.qualtrics.com/security-statement/). Survey data will be accessible only by study investigators. Weekly survey data will be downloaded by the data analyst from Qualtrics servers each week. Downloaded data will be stored on a password-protected computer and backed up to an encrypted external hard drive. Study participant research data for purposes of statistical analysis and scientific reporting, will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The code for re-identification of study data will be held by the data analyst in a separate password-protected database.

For the vaccine efficacy ancillary study, RFFIT RVNA titers will be sent by the laboratory to the PI or Study Coordinator, who will enter the data in the CRF.

For the immunology ancillary study, laboratory results will be sent by the Immunology Specialist to the PI or Study Coordinator, who will enter the data in the CRF. The Data

Analyst will return a deidentified dataset containing the immunology laboratory results to the Immunology Specialist for further analysis.

14 STATISTICAL METHODS

The analysis of the primary endpoint and secondary endpoints 1-7 will be conducted after all randomized participants have completed the study (one week after receipt of the final weekly survey) or have withdrawn from the study, whichever occurs first. Primary analysis will be based on intention-to-treat analysis of all events (that is, not just occurrence of first events). The comparison of incidence rates of CID episodes (primary endpoint and secondary endpoints 1-7) between the treatment arms will be performed using a negative binomial regression model, which accounts for different lengths of observation period. Participants' number of CID episodes will be modelled as a function of treatment group, with the number of weeks of observation included as an offset in the model. This analytic model estimates the rate ratio, λ_t / λ_c , which quantifies the risk of CID episodes associated with rabies vaccination (λ_t) in comparison to placebo injection (λ_c). Statistical significance will be controlled at the 2-sided, 0.05 alpha level, and the estimated rate ratio compared with 1, assuming the following statistical hypothesis:

H_0 (null hypothesis): Rate ratio = 1

H_1 (alternative hypothesis) Rate ratio ≠ 1

Treatment effect will be based on the coefficient in the model. Statistical significance at the pre-specified alpha level will be based on a Wald testing procedure. CID rates (primary

endpoint and secondary endpoints 1-7) for rabies vaccine and placebo, and the rate ratio, will be presented and will include 95% confidence intervals.

A detailed description of the statistical methods that will be used for the primary and secondary endpoints will be provided in a Statistical Analysis Plan (SAP).

15 HARMS

15.1 DEFINITIONS OF ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

Definitions of adverse events (AEs) and serious adverse events (SAEs) are taken from the Office of Human Research Protections Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events.

Adverse event means any untoward or unfavorable medical occurrence in a participant, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

A serious adverse event is any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:

1. results in death;
2. is life-threatening (places the subject at immediate risk of death from the event as it occurred);
3. requires inpatient hospitalization or prolongation of existing hospitalization;

4. results in a persistent or significant disability/incapacity;
5. results in a congenital anomaly/birth defect; or
6. any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

15.2 CLASSIFICATION OF ADVERSE EVENTS

All adverse events (AEs) will have their relationship to study intervention assessed by the Manager of the RUSVM Student Health Services (study nurse practitioner), in consultation with the study Medical Advisor, based on temporal relationship and clinical judgment.

The following guidelines will be used to describe severity:

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-

threatening or incapacitating. Of note, the term “severe” does not necessarily equate to “serious”.

The degree of certainty about causality will be graded using the categories below.

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

Adverse events will also be classified as **expected** or **unexpected**. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention. Based on the information contained in the package insert for Rabivax-S, the following adverse events are considered expected:

- Local reactions (limited to the site of the injection): pain, erythema, oedema, pruritus and induration.
- Systemic reactions: fever, shivering, malaise, asthenia, faintness, dizziness, headache, myalgia, arthralgia, nausea and abdominal pain.

- Hypersensitivity or allergic reactions: anaphylaxis, urticaria, rash and erythema multiforme.

15.3 IDENTIFICATION OF ADVERSE EVENTS

The Study Coordinator will review the Visit 1-3 CRFs daily for participants receiving injections (dose 1, 2 or 3). The study coordinator will send a link to an online Qualtrics survey (Appendix H) by email on day 3 after the date of the injection (dose 1, 2 or 3) to all participants. Information on occurrence of expected AEs will be specifically solicited in the survey, by asking the question, "Since receiving the study injection 3 days ago, did you experience any of the following…?" and providing participants with a list of expected AEs (see **Section 15.2, Classification of adverse events** for details) from which to select. Participants will also be asked to grade the severity of any AEs (mild, moderate or severe) and the outcome (recovered/resolved without sequelae, recovered/resolved with sequelae or ongoing). Information on occurrence of other AEs (unsolicited) will be sought by asking the question, "Besides the signs listed above, have you noticed anything different since starting the study?", and providing participants the opportunity to describe what they noticed or experienced, and grade the severity and outcome as above.

Participants will also be encouraged to report any adverse events to RUSVM Student Health Services (unsolicited adverse events). An adverse event form (Appendix I) will be completed for all adverse events reported to RUSVM Student Health Services, using the Vaccine Adverse Event Reporting System (VAERS) writable PDF form (https://vaers.hhs.gov/pdf/vaers_form.pdf). The Principal Investigator, together with the

Director of the RUSVM Student Health Services, will review and compare the reports of unsolicited adverse events (adverse event forms) against reports of solicited adverse events in the AE surveys, to avoid double capture.

15.4 REPORTING OF ADVERSE EVENTS

The Principal Investigator will report all serious adverse events whether or not considered study intervention related, and all nonserious unexpected adverse events considered related to the study intervention (See **Section 15.2, Classification of adverse events** for details), to the RUSVM IRB within 48 hours of his becoming aware of the event (through weekly review, or through notification from study personnel at the RUSVM Student Health Services).

The Principal Investigator will report safety data (adverse events and serious adverse events) to the vaccine manufacturer for the first two cohorts within 30 days of 80% of the cohort completing four weeks follow-up after first dose. For remaining cohorts, RUSVM will report safety data to the vaccine manufacturer for each cohort within 30 days of exit of participants from that cohort.

16 RESEARCH ETHICS APPROVAL

Approval for the study will be sought from the RUSVM Institutional Review Board (IRB). The protocol, informed consent form, recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the

protocol will require review and approval by the IRB before the changes are implemented to the study. Subsequent to initial review and approval, the study will be subject to annual continuing review by the RUSVM IRB.

17 TRIAL MONITORING

Trial monitoring will be conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s). Monitors will be appointed by the RUSVM Institutional Officer. Details of trial monitoring are documented in a Trial Monitoring Plan (TMP). The TMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

18 PROTOCOL AMENDMENTS

Any modifications to the protocol that may impact on the conduct of the study, potential benefit of the patient or may affect patient safety, including changes of study objectives, study design, patient population, sample sizes, study procedures, or significant administrative aspects will require a formal amendment to the protocol. Such amendments will be submitted to the RUSVM IRB for review and approval prior to implementation.

Administrative changes of the protocol are minor corrections and/or clarifications that have no effect on the way the study is to be conducted. These administrative changes will be documented in a memorandum. During annual continuing review of the study, the RUSVM IRB will be notified of administrative changes made to the protocol since the previous review.

Protocol version will be in the form *x*.x. Formal amendments to the protocol will change the first number (e.g. version 1.1 to 2.1). Minor administrative changes will change the second number (e.g. version 1.1 to 1.2). All minor changes made after a formal amendment will be subsumed in any subsequent formal amendment, so that the second number reverts to 1 for each formal amendment (e.g. version 1.2 to 2.1).

19 CONFIDENTIALITY

All study-related information will be stored electronically in password-protected databases in file folders with restricted access on the RUSVM network or on password-protected laptops with back-up to encrypted external hard drives. Any paper copies with participant information will be stored in locked file cabinets in areas with limited access. Biological specimens will be identified by a coded identification number. All records that contain names or other personal identifiers will be stored separately from the code list.

20 DECLARATION OF INTERESTS

The protocol was shared with Serum Institute of India Pvt Ltd (SIIPL) as co-sponsor but input was limited to the vaccine safety and efficacy components of the protocol.

Further, role of SIIPL in this study is restricted to supply the products (Rabivax-S and Sterile Water for Injections) for this study and to be used as per study protocol. The cost for immunogenicity testing of sera samples by RFFIT assay will be borne by SIIPL.

Dr. Gessner worked for AMP through 2017 and currently advises the company; AMP receives grant support from Sanofi Pasteur, a manufacturer of rabies vaccine. Dr. Gessner currently serves as the Global Medical Lead for Pneumococcal Vaccines at Pfizer.

21 ACCESS TO DATA

The Principal Investigator and Study Coordinator will have access to the full dataset. Deidentified data may be shared with the vaccine manufacturers (SIIPL) and other institutions upon request to the PI.

22 DISSEMINATION POLICY

Results of the internal pilot study (sample size recalculation), vaccine efficacy ancillary study and related safety data for participants up to this point, and immunology ancillary study may be released prior to release of the main study results (Primary Endpoint and Secondary Endpoints 1-7).

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