Non-specific effects of rabies vaccine on the incidence of self-reported common infectious disease episodes: A randomized controlled trial

Christianah I. Odita[a], Anne Conan[b,c], Marshalette Smith-Antony[b], Juliet Battice[b], Shianne England[b], Donna Barry[b], Bradford D. Gessner[d] , Darryn L. Knobel[a,b,*]

[a] Department of Veterinary Tropical Diseases, University of Pretoria, Pretoria, South Africa

[b] Department of Biomedical Sciences, Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis

[c] Center for One Health Research and Policy Advice, City University of Hong Kong, Kowloon, Hong Kong Special Administrative Region

[d] EpiVac Consulting Services, Anchorage, AK, United States

*Corresponding author at: Ross University, School of Veterinary Medicine, P.O. Box 334, Basseterre, Saint Kitts and Nevis. E-mail address: dknobel@rossvet.edu.kn (D.L. Knobel)

Highlights

- Phase IV, single-site, participant-blinded, randomized, placebo-controlled trial.
- No effect of rabies vaccination on the incidence of self-reported illness episodes.
- Little evidence that sex modifies the effect of vaccine on evaluated outcomes.

Abstract

Vaccines may affect recipients' immune systems in ways that change morbidity or mortality rates to unrelated infections in vaccinated populations. It has been proposed that these non-specific effects differ by type of vaccine and by sex, with non-live vaccines enhancing susceptibility of females to unrelated infections, and live vaccines enhancing resistance in both sexes. Rabies vaccine–a non-live vaccine–has been associated with protection against unrelated central nervous system infections. Data from randomized controlled trials are needed to assess this effect against other illnesses. This phase IV, single-site, participant-blinded, randomized, placebo-controlled trial in a population of veterinary students on the rabies-free island of St. Kitts assessed the effect of a primary course of rabies vaccine on the incidence rate of weekly self-reported new episodes of common infectious disease (CID) syndromes, defined as a new episode of any one of the following syndromes in a particular week: upper respiratory illness (URI), influenza-like illness (ILI), diarrheal illness (DIA) or undifferentiated febrile illness (UFI). As a secondary objective, we tested for modification of the effect of rabies vaccine on study outcomes by sex. 546 participants were randomized (274 to rabies vaccine and 272 to placebo). No statistically significant differences between groups were observed for any study outcomes: CID incidence rate ratio (IRR) 0.95 (95% CI 0.77–1.18); URI IRR 1.15 (95% CI 0.86–1.54); ILI IRR 0.83 (95% CI 0.54–1.27); DIA IRR 0.93 (95% CI 0.70–1.24) and UFI IRR 1.09 (95% CI 0.48–2.44). In a secondary analysis, there was little evidence that sex modified the effect of vaccination on any of the evaluated outcomes, although the power to

detect this was low. In conclusion, rabies vaccine did not provide protection against mild self-reported illness among a young and healthy group of adults attending veterinary school.

Clinical trial registration. ClinicalTrials.gov: NCT03656198.

Keywords: Rabies vaccine; Non-specific effects of vaccines; Sex; Influenza-like illness; Upper respiratory disease; Diarrhea; Fever

1 Introduction

The current vaccinology paradigm presumes that vaccines reduce morbidity and mortality rates in populations only by conferring specific protection to target infections through an enhanced adaptive immune response, and that any detrimental effects are due to known, expected adverse events[1]. However, there is evidence that vaccines have important effects on the health of populations that cannot be explained under this paradigm[1]. These effects have been termed "non-specific"[2]. General hypotheses of non-specific effects have been proposed from observations accumulated through epidemiological studies[1]; among these, that live vaccines enhance resistance towards unrelated infections[3-12], whereas non-live vaccines enhance susceptibility of females to unrelated infections[13-23], although many of these studies are considered at high risk of bias[24]. There is a lack of randomized trials of non-specific effects of non-live vaccines.

Rabies vaccine–a non-live vaccine–has been associated with a reduced incidence of unrelated central nervous system (CNS) infections in children. This observation was made following the RTS,S/AS01 malaria vaccine trial, in which rabies vaccine was used as a comparator vaccine in the older age group (5–17 months), while a younger age group (6–12 weeks) received serogroup C meningococcal conjugate vaccine as a comparator[25]. Among the older age group, the incidence of meningitis was reduced by 90% and of cerebral malaria by 50% in the control arm, whereas rates were similar in both arms of the younger age group and the treatment arm in the older age group[26] . Another study reported reductions in herpes simplex virus CNS infection in mice[27]. These observations raise the possibility that rabies vaccine may have beneficial non-specific effects, at least against severe CNS infections.

Routine use of rabies vaccine for pre-exposure prophylaxis is not recommended as cost-effective, even in regions where the risk of exposure to the virus is relatively high, particularly among children[28]. While replication of data on reducing non-rabies CNS infections would provide a strong incentive for rabies vaccine use, the rarity of these outcomes would require a large and expensive trial. Another option is to assess if rabies vaccine might provide protection against more common childhood diseases, such as respiratory, diarrheal or febrile illness. Demonstration of a non-specific protective effect of rabies vaccine against unrelated common infectious disease syndromes would provide supportive evidence for the design of similar studies in children in populations with a high burden of these illnesses.

We conducted a randomized controlled trial of the protective effects of rabies vaccine on the incidence of self-reported infectious disease syndromes in a population of veterinary students on the island of St. Kitts in the Caribbean. The primary objective of our study was to determine whether the incidence rate of self-reported new episodes of common infectious disease (CID) syndromes (respiratory, diarrheal and febrile illness) over a 26-week period differed between

previously unvaccinated subjects who received at least one dose of a three-dose course of rabies vaccine and those subjects who received an identical course of placebo injections. Secondary objectives were to compare the incidence rate of individual syndromes (upper respiratory illness, influenza-like illness, diarrheal illness and undifferentiated febrile illness), and to test for modification of the effect of the vaccine on study outcomes by sex.

2 Materials and methods

2.1 Trial design and participants

The trial design was 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 was by block randomization stratified by sex within cohort with a 1:1 allocation ratio. The trial (ClinicalTrials.gov: NCT03656198) was conducted between 29 August 2018 and 27 July 2020 at Ross University School of Veterinary Medicine (RUSVM) in St. Kitts. The study was reviewed and approved by the RUSVM Institutional Review Board (protocol 18–04-FL) and the Interim Ethics Review Committee of the Ministry of Health, St. Kitts and Nevis (approval code IERC-2019-11-032). The trial protocol is published

. Major changes to the published protocol were early closure of enrollment before the target sample size was met due to Covid-19 related restrictions, and a change in the placebo intervention from vaccine diluent (sterile water) to sterile saline.

We enrolled students registered at RUSVM in either the Veterinary Preparatory (VP) or the Doctor of Veterinary Medicine (DVM) programs. Original inclusion criteria for DVM students were enrollment in the first or fifth semester of the program, to keep enrollment per cohort at a manageable level and to extend enrollment over at least one year to capture seasonal variation in CID rates. In January 2020 eligibility was expanded to all students in the DVM program. Exclusion criteria were (i) previous vaccination with rabies vaccine; (ii) intent at the time of enrollment to undertake activities during the course of participation in the study that would increase the risk category of rabies exposure above that of the general population of the United States, as defined by the Advisory Committee on Immunization Practices (ACIP) for human rabies prevention[30]; or (iii) having a contraindication to rabies vaccine, as described in the vaccine package insert. Each participant provided written informed consent before enrollment.

2.2 Randomization and blinding

We randomly allocated participants to receive rabies vaccine or placebo. Allocation was done by restricted randomization (permuted block design with stratification). Stratification was by cohort (reflecting three student intakes per year, in January, May and September) and sex (within cohort). Within strata, randomization was done by computer-generated randomly permuted blocks of size 2, 4 or 6, using the function *blockrand* in the package "blockrand"[31] in R software[32]. For concealment, allocation information was placed in opaque, sequentially-numbered envelopes. Envelopes were selected sequentially within cohort and sex by study nurses after participants were screened for potential contraindications. Study nurses prepared and administered the intervention according to the allocation information. Participants remained blind to their allocation status until study exit. Study personnel responsible for data analysis had access to allocation information, for use in the event that unblinding would be

needed (such as a need to determine rabies pre-exposure vaccination status in the event of a possible rabies exposure).

2.3 Interventions

Participants in the rabies vaccine group were assigned to receive three doses (1 mL each) of rabies vaccine on days 0, 7 and 21, as per the vaccine package insert. Each rabies vaccine dose (Rabivax-S, Serum Institute of India, batch numbers 148T70080Z, 148T70110Z and 1488 T016) contained not less than 2.5 international units (IU) of inactivated, purified rabies antigen (Pitman Moore PM3218 virus strain produced using Vero ATCC CCL 81 cells), glycine (40 mg), sucrose (40 mg) and human serum albumin (25% 10 mg). Participants in the control group were assigned to receive three doses (1 mL each) of rabies vaccine diluent (sterile water, Serum Institute of India, batch numbers 0658S1004Z and 0659S4002), on days 0, 7 and 21. All doses were injected intramuscularly in the deltoid muscle. On 13 September 2019, the placebo intervention was changed to sterile saline (sodium chloride 0.9% w/v intravenous infusion, B. Braun, Melsungen, Germany, batch numbers 170578141, 640064P and 01018JT) due to occurrence of adverse events associated with use of sterile water.

Table 1
Definition of study syndromes. The primary outcome (CID) was defined as the occurrence of any one of the study syndromes within a particular week.

Syndrome	Definition
Upper respiratory illness (URI)	Two or more of the following: • runny or blocked nose • sneezing • sore throat • cough AND Absence of itchy or watery eyes
Influenza-like illness (ILI)	Fever (feeling feverish or an axillary, oral or otic temperature of 100°F/37.8 °C or higher) AND Cough or sore throat
Diarrhea (DIA)	Three or more loose stools within a 24-hour period
Undifferentiated febrile illness (UFI)	Fever (feeling feverish or an axillary, oral or otic temperature of 100°F/37.8 °C or higher) AND Not meeting case definition of any of URI, ILI or DIA

2.4 Outcomes

The primary outcome measure was self-reported new weekly episodes of acute common infectious disease (CID) syndromes, defined as any one of the following: upper respiratory illness (URI), influenza-like illness (ILI), diarrhea (DIA) or undifferentiated febrile illness (UFI)(Table 1). Participants provided self-reports of occurrence or non-occurrence of illness in response to email surveys. Surveys were sent weekly for 26 weeks, starting on the Monday of the second week after the first injection. By definition, a participant could experience a maximum of one CID episode in a week. To be defined as a new episode, illness must have been preceded by a week in which non-occurrence of illness was reported (that is, a reported

CID episode-free week); consequently, the first week of the survey for each cohort was considered a 'run-in' as no new episodes could occur by definition. Similarly, the week following a non-response to the survey was not considered a week at risk of a new episode, as no event could meet the definition of a new episode.

To allow for more than one CID episode in a week, we predefined a secondary outcome measure, CID2, as self-reported new weekly episodes of respiratory illness (URI *or* ILI), DIA and/or UFI. By this definition, in any week a participant could report one of the following: no CID2 episode, one CID2 episode (URI or ILI or DIA or UFI) or two CID2 episodes (either URI or ILI, and DIA, but not UFI, as occurrence of UFI is predicated on the absence of other study syndromes). Other secondary outcome measures were self-reported new weekly episodes of individual study syndromes (URI, ILI, DIA and UFI), and clinically confirmed episodes of study syndromes reported to RUSVM Health Services using the following International Classification of Diseases, version 10 (ICD-10) codes to define syndromes:

(a) URI: J00 (acute nasopharyngitis)

(b) ILI: J11 (influenza due to unidentified influenza virus)

(c) DIA: R19.7 (diarrhea)

(d) UFI: R50.9 (fever, unspecified)

Safety outcomes were self-reports of solicited adverse events occurring within 3 days after each injection, and unsolicited adverse events through 4 weeks after first injection. Self-reports of solicited adverse events were collected in an online survey emailed to participants 3 days after receiving an injection (for dose 1, 2 and 3). Solicited adverse events were categorized as local reactions limited to the site of the injection (pain, erythema, edema, pruritus and induration), systemic reactions (fever, shivering, malaise, asthenia, faintness, dizziness, headache, myalgia, arthralgia, nausea and abdominal pain) and hypersensitivity or allergic reactions (anaphylaxis, urticaria, rash and erythema multiforme). Modifications were made to the survey after the first cohort, to allow participants to self-report outcome (recovered/resolved without sequelae; recovered/resolved with sequelae; ongoing) and severity. Severity was classified as mild (discomfort noted, but no disruption of normal daily activities; slightly bothersome; relieved with or without symptomatic treatment), moderate (discomfort sufficient to reduce or affect normal daily activity to some degree; bothersome; interferes with activities; only partially relieved with symptomatic treatment) or severe (discomfort sufficient to reduce or affect normal daily activity considerably [prevent regular activities for at least 24 h]; not relieved with symptomatic treatment). Unsolicited adverse events were any other events reported in the survey, or any events reported by participants to Campus Health Services.

2.5 Sample size and statistical analyses

The initial target sample size estimated to achieve the study's primary objective was 430 (215 in each group), based on an estimated event rate for the control group of 2 (expected mean number of new CID episodes over 26 weeks), a rate ratio under the alternative hypothesis of 0.75, average length of participation (accounting for drop-out and non-response) of 21 weeks, negative binomial dispersion parameter of 0.4, alpha level of 0.05 and targeted power of 0.8. The effect size (rate ratio 0.75, or 25% relative reduction) is based on the range of effect sizes seen for other outcomes in people and in animal studies. 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 from 21 May to 8 July 2018. The planned blinded sample size re-estimation was conducted on 22 November 2019, based on data from the first three cohorts to complete the study (n = 351), and resulted in a recalculated target sample size of 584[29] . The increase in sample size was largely due to a lower-than-expected rate of new episodes of CID. This may have been due to the definition of a new episode, which required a preceding week in which no CID episode was reported. This definition was not applied in the pilot study.

The primary analysis was based on intention-to-treat (ITT). For the CID, CID2 and individual syndrome outcome measures, we used negative binomial regression models to estimate the incidence rate ratio (with 95% CIs) comparing the vaccine group to the placebo group. We included sex and cohort in the models as stratification factors in the randomization, and the logarithm of the number of weeks at risk as an offset. To test for effect modification by sex, we used a likelihood ratio test of the significance of an interaction term between treatment and sex in each model. For the clinically confirmed episodes outcome measure, data were only provided in aggregate for each treatment group, by cohort and sex. We used a Poisson model with treatment, cohort and sex as group-level covariates and logarithm of group size as an offset. For the comparison of safety data between groups, we used a logistic regression model to estimate the odds ratio of occurrence for each dose and event type . All analyses were performed using R statistical software[32].

3 Results

Between 29 August 2018 and 23 January 2020, we enrolled 546 participants from five cohorts (Fall 2018, Spring 2019, Summer 2019, Fall 2019 and Spring 2020). All enrolled participants were randomized (n = 274 in rabies vaccine group; n = 272 in placebo group) and received the first dose of the allocated treatment. One participant allocated to the vaccine group was inadvertently administered the placebo for their third dose; otherwise all participants received the interventions according to protocol. One participant allocated to the placebo group was found to have high pre-injection rabies virus neutralizing antibody levels in the immunogenicity ancillary study; on investigation they reported previous vaccination and thus did not meet the inclusion criteria. Exclusion of these two participants in a per-protocol analysis resulted in very minor changes in effect estimates compared to the ITT analysis; here we report the ITT results only. Fig. 1 shows the participant flow diagram. Baseline characteristics were comparable between groups (Table 2). Enrollment was halted early due to the Covid-19 pandemic, which prompted a switch to remote learning by RUSVM on 16 March 2020, meaning that no new cohorts of students could be approached for enrollment beyond this date. Because closure of the campus affected access to Health Services, the Spring 2020 cohort was excluded from the analysis of clinically confirmed episodes.

Table 2
Characteristics of participants at randomization.

	Placebo group (n = 272)	Vaccine group (n = 274)
Mean age, years (sd)	24.8 (3.3)	24.9 (3.1)
Sex, n (%)		
Male	46 (17)	44 (16)
Female	226 (83)	230 (84)
Cohort, n (%)		
Fall 2018	68 (25)	68 (25)
Spring 2019	56 (21)	56 (20)
Summer 2019	51 (19)	51(19)
Fall 2019	57 (21)	56 (20)
Spring 2020	40 (15)	43 (16)
Months since start at RUSVM, n (%)		
<1 month	155 (57)	140 (51)
1–12 months	10 (4)	11 (4)
13–24 months	102 (38)	117 (43)
>24 months	4 (1)	5 (2)
Missing/Prefer not to answer	1 (0.4)	1 (0.4)
Healthcare visits in preceding 6 months, n (%)		
0	34 (13)	39 (14)
1	78 (29)	75 (27)
2	62 (23)	77 (28)
3	44 (16)	41 (15)
4 or more	53 (19)	37 (14)
Missing/Prefer not to answer	1 (0.4)	5 (2)
Presence of a chronic medical condition, n (%)		
Any	47 (17)	55 (20)
Diabetes mellitus	1 (0.4)	1 (0.4)
Asthma	15 (6)	21 (8)
Autoimmune condition	6 (2)	5 (2)
Other	31 (11)	32 (12)
Immunosuppressed, n (%)		
No	264 (97)	268 (98)
Yes	3 (1)	2 (1)
Missing/Prefer not to answer	5 (2)	4 (1)
Self-reported health status, n (%)		
Excellent	66 (24)	60 (22)
Very good	135 (50)	149 (54)
Good	66 (24)	62 (23)
Fair	4 (1)	3 (1)
Poor	1 (0.4)	0 (0)
Tobacco smoker, n (%)		
No	267 (98)	261 (95)
Yes	4 (1)	10 (4)
Missing/Prefer not to answer	1 (0.4)	3 (1)
Vaccinations received in the preceding 6 months, n (%)		
Influenza	37 (14)	43 (16)
Tetanus	74 (27)	73 (27)
Meningococcus	13 (5)	9 (3)
Measles	19 (7)	12 (4)
Hepatitis A	30 (11)	38 (14)
Hepatitis B	32 (12)	18 (7)

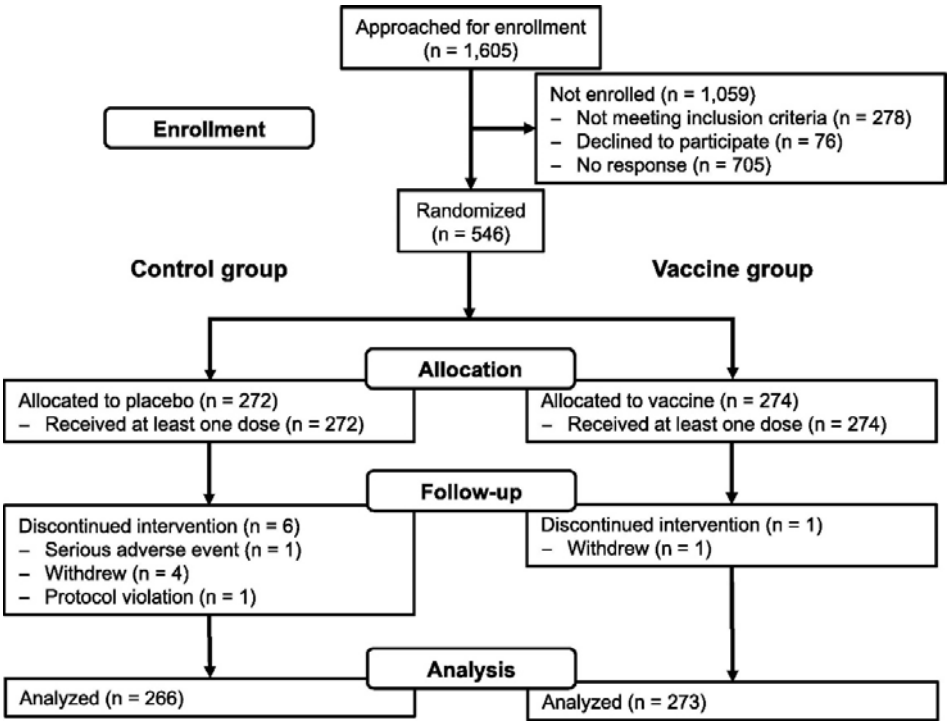

Fig. 1. Participant flow diagram.

Participants self-reported a total of 578 new weekly CID episodes (290 in the vaccine group and 288 in the placebo group) over a total of 11,363 participant-weeks at risk (5828 in the vaccine group and 5535 in the placebo group). Table 3 shows the incidence rates by group and the incidence rate ratio comparing the rabies vaccine group to the placebo group for the primary objective (self-reported CID episodes) and the secondary objectives (self-reported episodes of individual syndromes and clinically confirmed episodes). No overall directionality of results was evident and no individual associations were statistically significant at the 95% confidence level. The results of the analysis for the secondary objective of effect modification by sex are shown in Fig. 2 and Supplementary Table 1. Models were not fit for syndromes with zero counts in any category of treatment group by sex (URI for self-reported and clinically confirmed episodes, and ILI for clinically confirmed episodes). As with other outcomes, no consistent directionality was seen and no individual associations were statistically significant at the 95% confidence level. For DIA the incidence rate ratio of the effect of rabies vaccine on self-reported DIA episodes in males was 0.52 (95% CI 0.24–1.12) and in females was 1.03 (95% CI 0.76–1.39) but the likelihood ratio test p-value remained non-significant ($p = 0.11$).

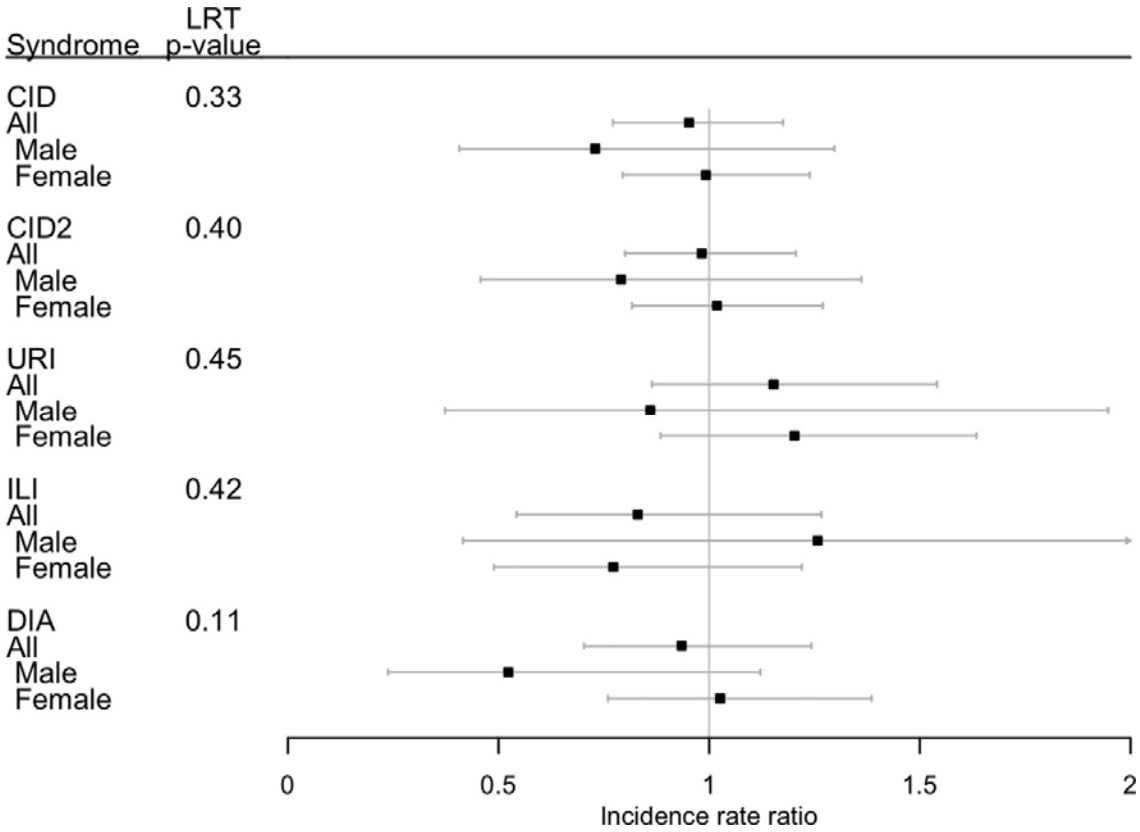

Fig. 2. Forest plot of the incidence rate ratio comparing vaccine group to placebo for self-reported episodes of study syndromes, stratified by sex. LRT, likelihood ratio test of the significance of the interaction term between treatment group and sex. CID, common infectious disease (primary outcome; URI, upper respiratory illness; ILI, influenza-like illness; DIA, diarrhea.

Independently of treatment group, females consistently reported higher incidence rates than males for all syndromes, but the difference was not statistically significant for most syndromes: CID (IRR 1.48; 95% CI 1.09–2.02), CID2 (IRR 1.33; 95% CI 0.99–1.79), URI (IRR 1.48; 95% CI 0.97–2.34), ILI (IRR 1.17; 95% CI 0.66–2.21), DIA (IRR 1.31; 95% CI 0.87–1.97) and UFI (IRR 1.60; 95% CI 0.29–3.84). Compared to the Spring 2019 cohort, the incidence rate of self-reported illness episodes was substantially lower in the Spring 2020 cohort for CID (IRR 0.56; 95% CI 0.38–0.82), CID2 (IRR 0.53; 95% CI 0.36–0.77), URI (IRR 0.47; 95% CI 0.24–0.87), ILI (IRR 0.43; 95% CI 0.16–1.01) and DIA (IRR 0.64; 95% CI 0.39–1.04), likely reflecting the movement restrictions implemented in response to the Covid-19 pandemic.

3.1 Safety results

Solicited adverse events reported in the three days following each dose are shown in Table 4. Participants in the control group were more likely to report pain (OR 2.5, $p < 0.001$), edema (OR 2.6, $p = 0.004$), pruritus (OR 2.6, $p = 0.048$), faintness (OR 3.9, $p < 0.001$), asthenia (OR 4.6, $p < 0.001$), dizziness (OR 3.2, $p = 0.004$), arthralgia (OR 2.7, $p = 0.03$), myalgia (OR 1.7, $p = 0.007$) and nausea (OR 3.6, $p < 0.001$) after the first dose, and asthenia (OR 8.4, $p = 0.046$) and arthralgia (OR 5.3, $p = 0.03$) after the third dose compared to the vaccine group, whereas participants in the vaccine group were more likely to report pruritus (OR 2.6, $p = 0.03$) and induration (OR 7.7, $p = 0.007$) after the third dose compared to the control group. Differences for other doses and other AEs were not statistically significant between groups. Unsolicited

reports of adverse events were also higher in the control group (Table 4). Five unsolicited adverse events in the control group were classified as severe, one of which was a serious adverse event, while no severe or serious unsolicited adverse events occurred in the vaccine group. All severe adverse events were episodes of syncope related to administration of the placebo, and lead to the change in placebo from sterile water to sterile saline. The main results of the RCT separated by the two periods in which different placebos were used are shown in Supplementary Table 2. The occurrences of adverse events for the cohorts and doses for which different placebos were used are shown in Supplementary Tables 3 and 4. Occurrence of adverse events among the placebo group reduced after the change was made. Compared to saline placebo, the vaccine group were more likely to report pain at the site of injection after the second (OR 3.8, $p < 0.001$) and third (OR 5.1, $p < 0.001$) doses, but not after the first dose (OR 2.2, $p = 0.13$). Occurrence of all other adverse events was not statistically significantly different between the groups, for any dose.

4 Discussion

In a group of healthy young adults attending veterinary school, we did not find evidence that rabies vaccine reduced the incidence rate of self-reported new episodes of common infectious disease syndromes over a 26-week period. Moreover, we did not find substantial evidence that sex modified the effect of rabies vaccine on the outcomes.

A previous study in children demonstrated an association between rabies vaccine and protection from all-cause meningitis and cerebral malaria[25,26]. Older studies in mice using modified live rabies virus vaccines reported protection from herpes encephalitis[27] and *Klebsiella pneumoniae* sepsis[33]. In a randomized controlled trial of an inactivated animal rabies vaccine in young dogs[34], we previously demonstrated that vaccination substantially increased all-cause mortality rates in females, while male mortality was reduced, although not statistically significantly. It may be pertinent to note that, while most animal rabies vaccines contain alum adjuvant, human rabies vaccines (including the ones used in this and a previous study[25]) are not adjuvanted, as alum adjuvant is known to suppress pro-inflammatory immune responses[35].

The move to remote learning and subsequent indefinite closure of campus as a result of the Covid-19 pandemic forced us to halt enrolment into the study early, after 546 (93%) of the target 584 participants were enrolled. Based on the parameter estimates from the blinded sample size re-estimation, we estimate that the final power of our study to detect an effect size of 0.75 (25% relative reduction) was 75%, and that, to maintain 80% power, the minimum detectable effect size was 0.74. Thus it is unlikely that the primary objective of our study was affected by early closure of enrolment. A more significant impact is likely on the secondary objectives of detection of effect modification. This effect is compounded by the significantly lower rate of self-reported episodes of illness following the onset of Covid-19 movement restrictions, as well as the low proportion of males in our study population, which mirrors the sex ratio skew in the population of students at RUSVM and among North American veterinary students generally. Our study thus likely had low power for its secondary objective, to detect a difference by sex on the non-specific effect of rabies vaccine on any study outcome, even if such an effect were large. Consequently, we cannot make definitive statements about any modification of rabies vaccine effects by sex.

Our study was limited to healthy young adults and mild illness, and thus our effect estimates may not apply to populations with a different age distribution or more severe illness. We did

not evaluate the effect of treatment on severity of illness, only incidence. The observation which prompted this study[25,26] was a possible reduction in severe central nervous system infections (meningitis and cerebral malaria) in children, and the results of our study do not preclude this possibility. A further limitation of our study was the absence of investigator blinding, but the risk of investigator bias was reduced by reliance on subject-reported (rather than investigator-determined) illness symptoms, predefined algorithms to classify these symptoms into study outcomes, and maintenance of analytical blinding until completion of data collection.

Our study does not support the ability of rabies vaccine to provide non-specific protection against mild respiratory and gastrointestinal illness among healthy young adults, although we cannot rule out small effects or the possibility that sex modifies the efficacy of rabies vaccine against these outcomes. Our study does not inform whether rabies vaccine provides protection against more severe illness, such as CNS infections. Future studies thus should focus on the possibility of rabies vaccine preventing these infections and determine if a potential biological mechanism can be identified.

CRediT authorship contribution statement

Anne Conan: Data curation, Formal analysis, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: BDG worked for Agence de Médecine Préventive (AMP) through 2017 and currently advises the company. AMP has received grant support from Sanofi Pasteur, a manufacturer of rabies vaccine. BDG currently serves as the Global Medical Lead for Pneumococcal Vaccines at Pfizer, but Pfizer has no role in the current study and BDG participated in the reported trial outside of his duties with Pfizer. The other authors declare that they have no competing interests.

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References

1. Benn CS, Fisker AB, Rieckmann A, Sørup S, Aaby P. Vaccinology: time to change the paradigm? Lancet Infectious Diseases 2020. July 6, 2020 https://doi-org.uplib.idm.oclc.org/10.1016/S1473-3099(19)30742-X.
2. Benn C.S., Netea M.G., Selin L.K., Aaby P.: A small jab – a big effect: nonspecific immunomodulation by vaccines. Trends Immunol 2013; 34: pp. 431-439.
3. Aaby P., Garly M.L., Bal C., et. al.: Survival of previously measles-vaccinated and measles-unvaccinated children in an emergency situation: an unplanned study. Pediatr Infect Dis J 2003; 22: pp. 798-805.
4. Aaby P., Martins C.L., Garly M.L., et. al.: Non-specific effects of standard measles vaccine at 4, 5 and 9 months of age on childhood mortality: randomised controlled trial. BMJ 2010; 341:

5. Biering-Sørensen S., Aaby P., Lund N., et. al.: Early BCG-Denmark and neonatal mortality among infants weighing <2500 g: a randomized controlled trial. Clin Infect Dis 2017; 65: pp. 1183-1190.
6. Jayaraman K., Adhisivam B., Nallasivan S., et. al.: Two randomized trials of the effect of the Russian strain of bacillus Calmette-Guérin alone or with oral polio vaccine on neonatal mortality in infants weighing <2000 g in India. Pediatr Infect Dis J 2019; 38: pp. 198-202.
7. Roth A.E., Benn C.S., Ravn H., et. al.: Effect of revaccination with BCG in early childhood on mortality: randomised trial in Guinea-Bissau. BMJ 2010; 340:
8. Lund N., Andersen A., Hansen A.S., et. al.: The effect of oral polio vaccine at birth on infant mortality: a randomized trial. Clin Infect Dis 2015; 61: pp. 1504-1511.
9. Aaby P., Ravn H., Benn C.S., et. al.: Randomized trials comparing inactivated vaccine after medium- or high-titer measles vaccine with standard titer measles vaccine after inactivated vaccine: a meta-analysis. Pediatr Infect Dis J 2016; 35: pp. 1232-1241.
10. Fisker A.B., Nebie E., Schoeps A., et. al.: A two-center randomized trial of an additional early dose of measles vaccine: effects on mortality and measles antibody levels. Clin Infect Dis 2018; 66: pp. 1573-1580.
11. Andersen A., Fisker A.B., Rodrigues A., et. al.: National immunization campaigns with oral polio vaccine reduce all-cause mortality: a natural experiment within seven randomized trials. Front Public Health 2018; 6: pp. 13.
12. Giamarellos-Bourboulis E.J., Tsilika M., Moorlag S., Antonakos N., Kotsaki A., Domínguez-Andrés J., et. al.: ACTIVATE: Randomized Clinical Trial of BCG Vaccination against Infection in the Elderly. Cell 2020; 183: pp. 315-323.e9.
13. Mogensen S.W., Andersen A., Rodrigues A., Benn C.S., Aaby P.: The introduction of diphtheria-tetanus-pertussis and oral polio vaccine among young infants in an urban african community: a natural experiment. EBioMedicine 2017; 17: pp. 192-198.
14. Aaby P., Mogensen S.W., Rodrigues A., Benn C.S.: Evidence of increase in mortality after the introduction of diphtheria-tetanus-pertussis vaccine to children aged 6–35 months in Guinea-Bissau: a time for reflection?. Front Public Health 2018; 6: pp. 79.
15. Aaby P., Jensen H., Gomes J., Fernandes M., Lisse I.M.: The introduction of diphtheria-tetanus-pertussis vaccine and child mortality in rural Guinea-Bissau: an observational study. Int J Epidemiol 2004; 33: pp. 374-380.
16. Aaby P., Ravn H., Benn C.S.: The WHO review of the possible nonspecific effects of diphtheria-tetanus-pertussis vaccine. Pediatr Infect Dis J 2016; 35: pp. 1247-1257.
17. Aaby P., Ravn H., Fisker A.B., Rodrigues A., Benn C.S.: Is diphtheriatetanus- pertussis (DTP) associated with increased female mortality? A meta-analysis testing the hypotheses of sex-differential non-specific effects of DTP vaccine. Trans R Soc Trop Med Hyg 2016; 110: pp. 570-581.
18. Aaby P., Rodrigues A., Kofoed P.E., Benn C.S.: RTS, S/AS01 malaria vaccine and child mortality. Lancet 2015; 386: pp. 1735-1736.
19. Klein S.L., Shann F., Moss W.J., Benn C.S., Aaby P.: RTS, S Malaria Vaccine and Increased Mortality in Girls. MBio 2016; 7: pp. e00514-e516.
20. Aaby P., Garly M.L., Nielsen J., et. al.: Increased female-male mortality ratio associated with inactivated polio and diphtheria-tetanuspertussis vaccines: observations from vaccination trials in Guinea-Bissau. Pediatr Infect Dis J 2007; 26: pp. 247-252.
21. Garly M.L., Jensen H., Martins C.L., et. al.: Hepatitis B vaccination associated with higher female than male mortality in Guinea-Bissau: an observational study. Pediatr Infect Dis J 2004; 23: pp. 1086-1092.
22. Fisker A.B., Biering-Sørensen S., Lund N., et. al.: Contrasting female-male mortality ratios after routine vaccinations with pentavalent vaccine versus measles and yellow

fever vaccine. A cohort study from urban Guinea-Bissau. Vaccine 2016; 34: pp. 4551-4557.
23. Hanifi S.M.A., Biering-Sørensen S., Jensen A.K.G.J., Aaby P., Bhuiya A.: Penta is associated with an increased female-male mortality ratio: cohort study from Bangladesh. Human Vaccines Immunotherap 2020;
24. Higgins J.P.T., Soares-Weiser K., López-López J.A., Kakourou A., Chaplin K., Christensen H., et. al.: Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. BMJ 2016; 355:
25. RTS,S Clinical Trials Partnership. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. Lancet 2015; 386: 31–45.
26. Gessner B.D., Knobel D.L., Conan A., Finn A.: Could the RTS, S/AS01 meningitis safety signal really be a protective effect of rabies vaccine?. Vaccine 2017; 35: pp. 716-721.
27. Gorshunova L.P., Bektemirov T.A., Gumennik A.E., Gogbaidze G.A.: Non-specific defense reactions in the central nervous system following antirabies vaccination. Vopr Virusol 1970; 15: pp. 610-612.
28. Kessels J.A., Recuenco S., Navarro-Vela A.M., Deray R., Vigilato M., Ertl H., et. al.: Pre-exposure rabies prophylaxis: a systematic review. Bull World Health Organ 2017; 95: pp. 210-219C.
29. Knobel D, Odita CI, Conan A, Barry D, Smith-Antony M, Battice J, et al. Non-specific effects of rabies vaccine on the incidence of common infectious disease episodes: study protocol for a randomized controlled trial. Trials 2020;21:534. https://doi-org.uplib.idm.oclc.org/10.1186/s13063-020-04467-z.
30. Centers for Disease Control and Prevention. Human rabies prevention - United States, 2008: Recommendations of the Advisory Committee on Immunization Practices. MMWR Morbidity and Mortality Weekly Report. 2008;57(RR-3):1-28.
31. Snow G. blockrand: Randomization for block random clinical trials. R package version 1.3. 2013. https://CRAN.R-project.org/package=blockrand.
32. R Core Team : R: A language and environment for statistical computing.2020.R Foundation for Statistical ComputingVienna, Austria https://www.R-project.org/
33. Brossat J.Y., Cerf P., Zeller H., Coulanges P.: Evidence for a non-specific immunostimulating effect on anti-rabies vaccine of the Fermi type on the *Klebsiella pneumoniae* system - mice. Arch Inst Pasteur Madagascar 1981; 48: pp. 269-278.
34. Arega S., Conan A., Sabeta C.T., Crafford J.E., Wentzel J., Reininghaus B., et. al.: Rabies vaccination of 6-week-old puppies born to immunized mothers: a randomized controlled trial in a high-mortality population of owned, free-roaming dogs. Trop Med Infect Dis 2020; 5: pp. 45.
35. Oleszycka E., McCluskey S., Sharp F.A., Muñoz-Wolf N., Hams E., Gorman A.L., et. al.: The vaccine adjuvant alum promotes IL-10 production that suppresses Th1 responses. Eur J Immunol 2018; 48: pp. 705-715.