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Elucidating the difference in the kinetics of antibody titres of infants in Belgium and Vietnam

Thao Mai Phuong Tran [a,*], Kirsten Maertens [b], Ha Thi Thu Hoang [c], Pierre Van Damme [d], Elke Leuridan [b], Niel Hens [a,b]

[a] I-Biostat, Data Science Institute, Hasselt University, Martelarenlaan 42, 3500 Hasselt, Belgium
[b] Center for the Evaluation of Vaccination, Vaccine & Infectious Diseases Institute, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[c] National Institute of Hygiene and Epidemiology, Yersin Street 1, Hanoi 10000, Viet Nam
[d] Centre for Health Economics Research & Modelling Infectious Diseases, Vaccine and Infectious Disease Institute, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

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ABSTRACT

Serological results obtained in a single laboratory from twin-studies on maternal immunisation, in Vietnam and Belgium offer the opportunity to compare antibody kinetics in infants before and after infant vaccination in the presence of vaccine-induced maternal antibodies. Nonlinear mixed-effects models (NLMMs) making use of a hypothesised dynamic evolution that captures the change in antibody titres over time, were employed to model anti-PT and anti-Prn antibody dynamics. Our proposed modelling approach provided useful insight into understanding the differences in the infants' antibody kinetics in both countries since NLMMs offer the possibility of pooling all data in one analysis and incorporate relevant covariates of interest.

In both controlled cohort studies, pregnant women were vaccinated with a tetanus, diphtheria, acellular pertussis (Tdap) vaccine (Boostrix®, Belgium; Adacel®, Vietnam), and children were followed before and after primary vaccination, and before and after booster vaccination (Infanrix hexa®). From our models, both anti-PRN and anti-PT antibody titres at birth of Vietnamese infants were significantly lower than those of Belgian infants born to vaccinated women groups. Even though the antibody titres in the cord at birth of Belgian infants were also higher than those of Vietnamese infants born to the control women groups, the difference was not significant. The significant difference between infants born to vaccinated women in the two countries was likely due to the use of different vaccine brands in pregnant women and the different vaccination histories of women in these two countries.

Our analyses also suggested that the blunting effect was present during the primary immunisation but went away afterward for anti-PT data. In contrast, for anti-PRN antibodies, the blunting effect persisted after the primary vaccination and possibly went away after the booster dose. Countries should be aware of the regional situation in view of recommending maternal immunization.

1. Introduction

Recently, much attention has been paid to the evaluation of (pertussis) vaccination during pregnancy, to protect pregnant women and their infants from infectious diseases. Pertussis (whooping cough) is an acute respiratory disease caused by the bacterium *Bordetella pertussis*. People of all ages can be infected by the disease, and mainly in infants, the disease can be severe and even life-threatening [1]. Since pertussis is a vaccine-preventable disease, the most effective way to control the disease is through vaccination. Despite the availability of longstanding global vaccination programmes against *Bordetella pertussis*, there is an increase in the number of reported cases, especially in high-income countries [2,3]. Most of these cases are seen in infants, who are too young to be protected by the available vaccines and vaccination schedules. While there have been a lot of surveillance data available for high-income countries, surveillance data in low- and middle-income countries are mainly missing, leading to a lack of data on pertussis epidemiology in these countries [4].

Currently, pertussis vaccination in pregnancy is recommended to protect infants from pertussis in an increasing number of countries. This protection is achieved through the transfer of maternal antibodies from the mother to the child via the placenta during

* Corresponding author.
E-mail address: maiphuongthao.tran@uhasselt.be (T.M.P. Tran).

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pregnancy and via lactation afterwards. This strategy has been implemented, since 2012, in many countries, including the UK, the US, Belgium and New Zealand. Consequently, many studies have been conducted to investigate the immunogenicity, safety, and effectiveness of the maternal vaccination strategy with regard to the protection of infants from the disease. In general, basic statistics have usually been used to interpret the results of these (randomised) controlled trials, using geometric mean concentrations (GMCs) and their 95% confidence intervals (CIs), simple (paired) t-tests and (multiple) regression analyses.

Although these analyses are useful, their drawback is that they do not take the longitudinal nature of the data into account. In many vaccine trials measuring antibody titres to evaluate the efficacy of a vaccination programme, the repeated nature of the data has not been considered. However, several authors have shown that considering both inter- and intra-subject variability offers more insights into antibody dynamics. For example, Auranen et al. [5] employed hierarchical Bayesian modelling to study the decline in hepatitis B immunity, Teunis et al. [6] made use of a hyperbolic response model to describe the kinetics of the IgG antibody response to the pertussis toxin, and Goeyvaerts et al. [7] studied the maternal antibody decay of several diseases using nonlinear growth mixed models accounting for censoring. Recently, Maertens et al. [8] used a nonlinear mixed-effects model to describe the kinetics of antibody titres before and after vaccination in Belgian infants. Among the approaches mentioned above, only the work of Teunis et al. [6] and Maertens et al. [8] made use of a dynamic model to capture the evolution of antibody titres over time.

In this article, nonlinear mixed-effects models were employed to model longitudinal data of antibody titres before and after infant vaccinations in two countries: Belgium and Vietnam. A hypothesised dynamic model that captures the change in antibody titres after birth, before and after the primary vaccination series, and before and after the booster dose was used. Our goal is to analyse data on infant antibody titres, which are useful for understanding the kinetics of antibodies in infants before and after infant primary and booster vaccination against pertussis in the presence of vaccine-induced maternal antibodies.

2. Materials and methods

Data on antibody concentrations collected from two vaccine trials in Belgium (2014) and Vietnam (2013) form the basis for the analysis. The first trial in Belgium was a prospective controlled cohort study, while the second study in Vietnam was a randomised controlled study. The local ethics committees approved both studies. They were conducted in accordance with the Helsinki Declaration, Good Clinical Practice (GCP), and the Belgian and Vietnamese laws, respectively. Informed consent forms were obtained from all participants and both parents of participating infants. Readers are referred to Maertens et al. [9,10] for more details regarding the study in Belgium, Hoang et al. [11] and Maertens et al. [12] for the one in Vietnam.

2.1. Study design

In total, 57 healthy pregnant women were in the vaccine group (vaccinated with Boostrix®, GSK Biologicals, Rixensart, Belgium), and 42 women were in the control group (no pertussis-containing vaccine for at least ten years) in the study in Belgium. In the Vietnamese study, 52 women were vaccinated with Adacel® (Sanofi Pasteur, Canada) (Tdap group), and 51 women were inoculated with a tetanus-only vaccine (TT group). According to the protocol, all infants should be vaccinated with hexavalent pertussis-containing vaccines (Infanrix® Hexa, GSK Biologicals, Rixensart, Belgium) at two, three, and four months of age (see Fig. S1). A booster dose was planned at 15 months of age. However, in reality, vaccinations in infants were administered at different moments in Vietnam due to a delay in ethical committee approval [11,12].

2.2. Sample collection

In all infants, cord blood was collected at delivery. In Belgium, blood samples were collected from the infants before starting the primary vaccination schedule (week eight with a variation of ±4 days), at month five (28–35 days after the third vaccine dose), at month 15 (right before the booster dose) and month 16 (28–35 days after the booster dose). In Vietnam, blood samples were collected from the infants at two months of age, before the start of the primary pertussis vaccination schedule. The first dose of priming, however, was administered one month later than planned in the protocol. The blood sample post-priming was taken at a mean interval of 26–29 days after the third vaccine dose [11]. The booster vaccine dose was administered at a mean age of 22.18 months, four months later than foreseen, and blood samples were obtained on average 30.2 days after the fourth vaccine dose [12]. There was no blood sample drawn before the booster dose in Vietnam. Consequently, a maximum of five blood samples were obtained from Belgian infants, and only a maximum of four blood samples were available from Vietnamese infants.

2.3. Statistical analysis

There is no good correlate of protection for pertussis [13]. However, low antibody levels are associated with susceptibility to pertussis infection [14,15]. Moreover, high anti-PRN and anti-PT antibody levels correlate with protection [16]; hence, we modelled only anti-PT and anti-PRN data. Before fitting nonlinear mixed-effects models to our data, we first performed a simulation study to investigate the performance of the proposed method with respect to the number of repeated measures for each infant. This simulation study was motivated by the limited number of observations collected per subject and per time point in our vaccine trials.

2.3.1. Modelling antibody titres using nonlinear mixed-effects models

The modelling approach was based on the hypothesised dynamics of antibody concentrations in infants (see Fig. 1). The cord antibody titre is denoted as A_0. We assume that antibodies in infants first decrease with the decay rate β right after birth, until the first vaccine dose. Then antibodies increase with the rate γ reflecting the overall rising-rate after three vaccination doses. After the third dose of priming, antibodies continue to go up, until the time point h. Then, they decrease again with the decay rate α. At month 15 (in Belgium) or month 22 (in Vietnam), infants received a booster dose. Immediately after the administration of the booster vaccine, antibodies increase at the rate ω. Presumably, antibodies are still increasing one month after the booster dose.

To account for within-subject heterogeneity, we allowed random effects for some parameters. We assumed either a log-normal or a gamma distribution for these random effects since all parameters take only positive values. Consider a subject-specific parameter $\theta_i = f(\theta, \eta_i)$, where θ represents the population parameter, η_i denotes the subject random effect, and f is the functional form describing the relationship between θ and η_i, which reflects some underlying distributions for the subject-specific parameter θ_i. For a log-normal distributed random effect, one assumes that $\theta_i = \theta \exp(\eta_i)$, where $\eta_i \sim N(0, \sigma^2)$ which leads to $\theta_i \sim LN(\log(\theta), \sigma^2)$ (LNdenotes the log-normal distribution). This parameterisation implies that

$$t \le t_{vacc_1} : \frac{dA}{dt} = -\beta A$$
$$t_{vacc_1} < t \le h : \frac{dA}{dt} = \gamma A$$
$$h < t \le t_{vacc_{boost}} : \frac{dA}{dt} = -\alpha A$$
$$t_{vacc_{boost}} < t : \frac{dA}{dt} = \omega A$$

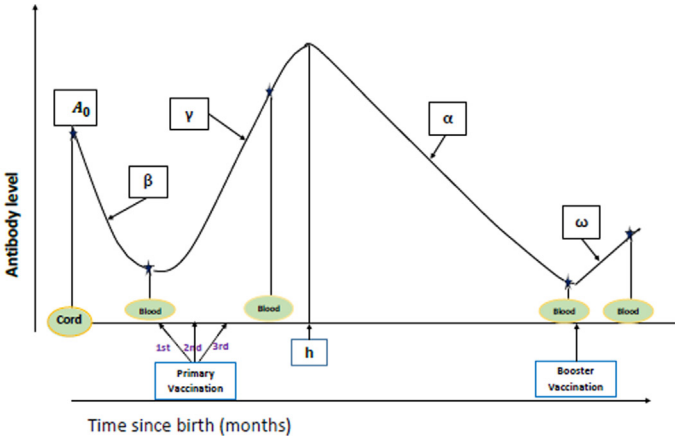

Fig. 1. Dynamics of antibody titres: $t_{vacc,1}$ represents the moment of the first vaccine dose, $t_{vacc,boost}$ denotes the moment of the booster dose (left-panel). The hypothesised dynamics of antibody titres in infants is expressed in different phases due to vaccination. Green circles indicate blood sample moments (right-panel). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$E(\theta_i) = \exp[\log(\theta) + \sigma^2/2] = \theta\exp(\sigma^2/2)$. For a gamma-distributed random effect, $\theta_i = \theta\eta_i$, where $\eta_i \sim Gamma(1/\sigma_\eta, 1/\sigma_\eta)$ with shape and inverse scale parameterisation. This construction implies that $\theta_i \sim Gamma(1/\sigma_\eta, 1/(\theta\sigma_\eta))$. Consequently, one has $E(\theta_i) = \theta$.

2.3.2. Univariate modelling

First, the kinetics of anti-PT and anti-PRN IgG data were modelled separately. We considered two important factors. The first one is the group of infants, denoted as *group*. This covariate is a binary variable indicating whether infants were born to vaccinated pregnant women (*group = 0*) or to unvaccinated pregnant women (*group = 1*). The second factor is country, denoted as *country*, a binary variable taking the value of 0 for Belgian infants, and 1 for Vietnamese infants. The interaction between *country* and *group* was also considered. Since there was a maximum of five samples for each Belgian infant and four samples for each Vietnamese infant, the richest model that we considered was the model with subject-random effects for parameters A_0, β and γ. Antibody titre data on a log-scale were fitted using an additive residual error model with a constant variance, that is: $\log(A_{obs,ij}) = \log(A_{pred,ij}) + \epsilon_{ij}$, where $\epsilon_{ij} \sim N(0, \sigma^2)$. Here, $A_{obs,ij}$ and $A_{pred,ij}$ denote the observed and predicted antibody titres for an individual i ($i = 1,\ldots, n$ with n the number of infants in the sample) at time point j ($j = 1, 2, 3, 4, 5$). The predicted antibody titre at time t_{ij} can be defined as:

$$t_{ij} \le t_{vacc1,i} : \quad A_{pred,ij} = A_{0,i}\exp\left[-\beta_i t_{ij}\right],$$

$$t_{vacc1,i} \le t_{ij} \le h_i : \quad A_{pred,ij} = A_{0,i}\exp\left[-\beta_i t_{vacc1,i} + \gamma_i(t_{ij} - t_{vacc1,i})\right],$$

$$h_i \le t_{ij} \le t_{vacc_{boost},i} : \quad A_{pred,ij} = A_{0,i}\exp\left[-\beta_i t_{vacc1,i} + \gamma_i(h_i - t_{vacc1,i}) - \alpha_i(t_{ij} - h_i)\right],$$

$$t_{ij} \ge t_{vacc_{boost},i} : \quad A_{pred,ij} = A_{0,i}\exp\left[-\beta_i t_{vacc1,i} + \gamma_i(h_i - t_{vacc1,i}) - \alpha_i(t_{vacc_{boost},i} - h_i) + \omega_i(t_{ij} - t_{vacc_{boost},i})\right].$$

All model parameters that capture the antibody kinetics $(A_0, \beta, \gamma, \alpha, \omega)$ were estimated under the constraint of non-negativity. The time point h was estimated under the constraint that $h \ge 4$ (months).

2.3.3. Joint modelling

We assume the same dynamics for anti-PT and anti-PRN IgG antibodies in infants as specified before. The time point h is assumed to be the same for the two antigens. The antibody levels at birth and the increase and decay rates are assumed to be antigen-specific. For anti-PT data, we denote: AT_0 (anti-PT IgG antibodies at birth), β_1, γ_1, α_1 and ω_1. For anti-PRN data, we denote: AR_0 (anti-PRN IgG antibody levels at birth), β_2, γ_2, α_2, and ω_2. Two covariates *group* and *country* were also taken into account. Antibody concentrations on the log-scale were fitted. The statistical model is defined as follows:

$$\begin{bmatrix}\log(AT_{obs,ij})\\ \log(AR_{obs,ij})\end{bmatrix} \sim \mathbb{N}\left\{\begin{bmatrix}\log(AT_{pred,ij})\\ \log(AR_{pred,ij})\end{bmatrix}, \sum\right\},$$

where $AT_{obs,ij}, AT_{pred,ij}, AR_{obs,ij}, AR_{pred,ij}$ are the observed and predicted antibody titres for anti-PT, and anti-PRN for infant i ($i = 1, 2,\ldots, n$) at time point j ($j = 1, 2, 3, 4, 5$), respectively. Here, $\sum$ is the 2×2 variance–covariance matrix having the form $\begin{bmatrix}\sigma_1^2 & \rho\sigma_1\sigma_2\\ \rho\sigma_1\sigma_2 & \sigma_2^2\end{bmatrix}$. We used the Cholesky decomposition for the variance–covariance matrix to stabilize the estimate of its elements.

All models were fitted in **R** using the *rstan* package [17]. Model comparison was performed using leave-one-out predictive performance based on elpd_loo [18]. The model with a higher value of elpd_loo is to be preferred.

2.3.4. Investigation of the performance of NLMMs concerning the number of repeated measurements

Here, we investigated the performance of nonlinear mixed-effects models when fitting longitudinal data involving the dynamics of antibody titre data in infants concerning the number of repeated measurements per subject. Data were simulated using a dynamic model similar to our data application model, where infants received a booster dose at month 18. The country effect was assumed to significantly affect the antibody levels in the cord between the two countries. Subject-specific parameters were assumed to follow a log-normal distribution. A denotes the antibody titres in infants, A_0 denotes the antibody titres in the cord at birth, and β, γ, α, ω denote the decay and rates of increase at different stages. The model base for the simulation study is specified as follows:

$$\begin{aligned} t \le 2 &: dA/dt = -\beta t,\\ 2 < t \le h &: dA/dt = \gamma A,\\ h < t \le 18 &: dA/dt = -\alpha A,\\ t > 18 &: dA/dt = \omega A. \end{aligned}$$

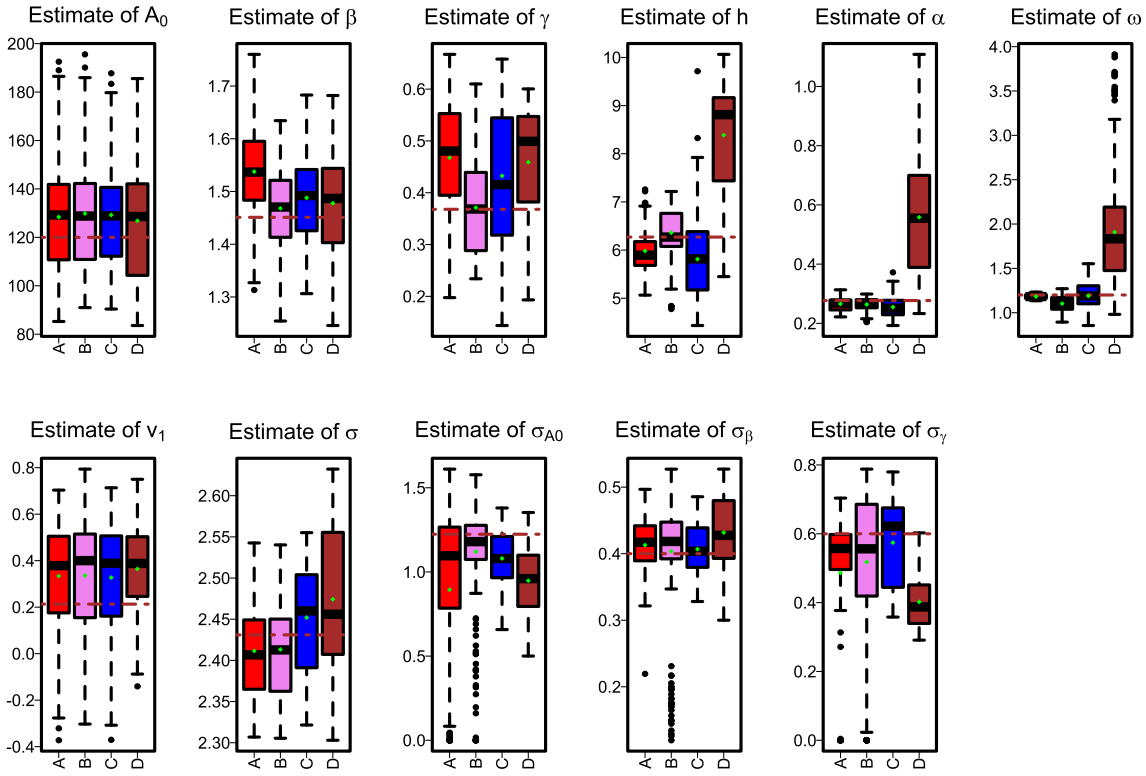

Fig. 2. Box plots of the distributions of parameter estimates across 300 simulated data sets. The horizontal brown dashed lines indicate the true values. The green dots show the mean estimates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

We assume that :

$$A_{0,i} = A_0 exp\left(v_1 country_i + \eta_{A_0,i}\right), \text{ where, } \eta_{A_0,i} \sim N\left(0, \sigma^2{}_{A_0}\right).$$

$$\beta_i = \beta \exp\left(\eta_{\beta,i}\right), \text{ where } \eta_{\beta,i} \sim N\left(0, \sigma^2{}_{\beta}\right).$$

$$\gamma_i = \gamma exp\left(\eta_{\gamma,i}\right), \text{ where } \eta_{\gamma,i} \sim N\left(0, \sigma^2{}_{\gamma}\right).$$

$$y_{obs.ij} \sim N\left(y_{pred.ij}, \sigma^2\right),$$

where $y_{pred.ij} = \log(A_{pred.ij})$ and$y_{obs.ij} = \log\left(A_{obs.ij}\right)$.

Four scenarios were considered (see Table S1). The first scenario (scenario A) simulated 11 observations: at birth ($t = 0$), and subsequently at ten equidistant time points per profile. The second scenario (B) contained 11 observations per profile and closely captured the kinetics of the antibody titres. In the last two scenarios, fewer observations for each infant were considered, but these observations firmly followed the timing of vaccination in infants. More specifically, eight observations per subject were generated for the third scenario (C), and only five observations were generated in the last scenario (D). The last scenario is the most similar to the available data under our analysis. The set of true values was $A_0 = 120, \beta = 1.451, \gamma = 0.368, \alpha = 0.277, h = 6.270, \omega = 1.200, \sigma = 2.431, \sigma_{A_0} = 1.224, \sigma_\beta = 0.400, \sigma_\gamma = 0.600,$ and $v_1 = 0.213$. For each scenario, 300 data sets were generated with a sample size of 115 infants for each data set. This sample size was inspired by the sample size that we observed in our case study. The proportion of Belgian infants and Vietnamese infants is 45:55. The simulation was done using the *mlx* package in R [19].

3. Results

3.1. Simulation outputs

In this section, we report simulation results regarding the analysis of 300 simulated data sets for each scenario. For each run, the posterior median was used as an estimate for the parameter of interest (see Fig. 2). While the second and third scenarios performed quite well in estimating the different parameters capturing the antibody titre dynamics ($A_0, \beta, \gamma, h, \alpha, \omega$), this was not the case for the first and fourth simulated schemes. More specifically, the first scenario performed reasonably well in estimating A_0, α, and ω but did not perform well when estimating β and γ. These results were expected since, based on the sampling time, there was no measurement between time point 0 and at two months, which might capture the decay of antibody titres after birth. Surprisingly, although there were two measurements between month two and *h*, the estimate of γ (in scenario A) was not well-captured since its 95% credible interval (CI) did not contain the true value. Generally, the model seemed to overestimate the overall rate of increase γ. The last scenario included only five samples per profile and gave a reasonable estimate for A_0 and β only, while 95% CIs of γ, h, α, and ω did not cover the true values. Overall, the model overestimated all parameters describing the dynamics of antibodies.

For the estimation of the variability, i.e., the parameters $\sigma, \sigma_{A_0}, \sigma_\beta$, and $\sigma_\gamma$and the covariate effect v_1, the first three scenarios had acceptable performance. In contrast, the last scenario performed poorly, especially for the estimation of $\sigma_{A_0}$and σ_γ,in which these two dispersion parameters were underestimated.

Moreover, we show the coverage probabilities (CPs) of the different parameters in Table S2. Generally, the second and third sce-

Table 1
Parameter estimates and 95% CIs for models of anti-PT and anti-PRN (Univariate modelling).

Anti-PT									
Parms	A_0	β	γ	α	ω	h	v_{1,A_0}	v_{2,A_0}	v_{3A_0}
Median	96.38	1.01	0.30	0.22	1.76	6.34	−2.12	−1.70	1.30
95% CI	[73.48; 126.41]	[0.88; 1.13]	[0.23; 0.40]	[0.16; 0.31]	[1.53; 1.99]	[5.16; 7.69]	[−2.62; -1.63]	[−2.22; −1.19]	[0.56; 2.08]
Parms	$v_{1,\beta}$	$v_{2,\beta}$	$v_{1,h}$	$v_{2,h}$	$v_{1,\gamma}$	$v_{2,\gamma}$	$v_{3,\gamma}$	$v_{1,\alpha}$	$v_{1,\omega}$
Median	0.37	−0.31	−0.59	1.52	1.59	0.79	−1.09	0.03	0.23
95% CI	[0.20; 0.54]	[−0.51; −0.12]	[−1.91; 0.61]	[0.98; 1.91]	[1.17; 2.20]	[0.53; 1.17]	[−1.82; −0.44]	[−0.07; 0.13]	[−0.18; 0.63]
Anti-PRN									
Parms	A_0	β	γ	α	ω	h	v_{1,A_0}	v_{2,A_0}	v_{3A_0}
Median	644.13	0.62	0.01	0.32	2.81	4.10	−619.96	−472.51	462.84
95% CI	[494.92; 835.65]	[0.47; 0.78]	[0.00; 0.05]	[0.29; 0.35]	[2.48; 3.15]	[4.00; 4.46]	[−811.33; −469.40]	[−658.72; −300.67]	[290.72; 649.34]
Parms	$v_{1,\beta}$	$v_{2,\beta}$	$v_{1,h}$	$v_{2,h}$	$v_{1,\gamma}$	$v_{2,\gamma}$	$v_{3,\gamma}$	$v_{1,\alpha}$	$v_{1,\omega}$
Median	−0.53	−0.04	1.09	4.05	0.63	0.49	−0.56	−0.07	−0.62
95% CI	[−0.71; −0.30]	[−0.25; 0.10]	[0.06; 2.74]	[3.04; 5.53]	[0.41; 0.95]	[0.28; 0.76]	[−0.88; −0.29]	[−0.14; 0.06]	[−1.26; −0.03]

narios did perform well with large coverage probabilities (close to 100%) for almost all parameters. The exception was the estimate of the peak h by the third scenario. The first and last scenarios performed less well compared with the other two scenarios but still did a reasonable job since the CP values were reasonably high for almost all parameters (except for γ in the first scenario and σ_{A_0} for the first and the last scenarios).

3.2. Case study

3.2.1. Univariate modelling

For anti-PT antibody titre data, we ran the predefined model with four chains of 4000 iterations (2000 burn-in iterations). We first ran the model without any covariates to perform model selection. Different models with gamma or lognormal random effects were considered. The starting model was the one with random effects for A_0, β, and γ. In the end, the model with gamma random effects of γ and lognormal random effects of $A_0$and β performed best in terms of the predictive performance. Hence, the best model was specified as:

- $A_{0,i} = A_0 \exp\left(v_{1,A_0} group_i + v_{2,A_0} country_i + v_{3,A_0} group_i country_i + \eta_{i,A_0}\right)$,
- $\beta_i = \beta \exp\left(v_{1,\beta} group_i + v_{2,\beta} country_i + v_{3,\beta} group_i country_i + \eta_{i,\beta}\right)$,
- $\gamma_i = \left(\gamma + v_{1,\gamma} group_i + v_{2,\gamma} country_i + v_{3,\gamma} group_i country_i\right)\eta_{i,\gamma}$,
- $h_i = h + v_{1,h} group_i + v_{2,h} country_i$,
- $\alpha_i = \alpha + v_{\alpha} group_i$, and $\omega_i = \omega + v_{\omega} group_i$,

where $\eta_{i,A_0} = \sigma_{A_0}\epsilon_{i,A_0}$ and $\epsilon_{i,A_0} \sim N(0,1)$; $\eta_{i,\beta} = \sigma_\beta \epsilon_{i,\beta}$ and $\epsilon_{i,\beta} \sim N(0,1)$; $\eta_{i,\gamma} \sim Gamma(1/\sigma_\gamma, 1/\sigma_\gamma,)$. Because no blood sample was taken from Vietnamese infants before the booster dose, we did not consider the country effect for the α and ω parameters.

The same model-building procedure was performed for anti-PRN antibody concentration data. The model with gamma random effects for A_0, β and γ produced the best predictive performance. Hence, the best model for anti-PRN can be specified as follows:

- $A_{0,i} = \left(A_0 + v_{1,A_0} group_i + v_{2,A_0} country_i + v_{3,A_0} group_i country_i\right)\eta_{i,A_0}$,
- $\beta_i = \left(\beta + v_{1,\beta} group_i + v_{2,\beta} country_i + v_{3,\beta} group_i country_i\right)\eta_{i,\beta}$,
- $\gamma_i = \left(\gamma + v_{1,\gamma} group_i + v_{2,\gamma} country_i + v_{3,\gamma} group_i country_i\right)\eta_{i,\gamma}$,
- $h_i = h + v_{1,h} group_i + v_{2,h} country_i$,
- $\alpha_i = \alpha + v_{\alpha} group_i$, and $\omega_i = \omega + v_{\omega} group_i$,

where $\eta_{i,A_0} \sim Gamma(1/\sigma_{A_0}, 1/\sigma_{A_0},)$, $\eta_{i,\beta} \sim Gamma(1/\sigma_\beta, 1/\sigma_\beta,)$, and $\eta_{i,\gamma} \sim Gamma(1/\sigma_\gamma, 1/\sigma_\gamma,)$.

In both data sets, the interaction terms related to parameter β were not statistically significant; hence, we removed them from the final model for making the inference. Fig. S2 shows the plot between observed and predicted values indicating a good fit. The Pearson correlation coefficients between the observed and predicted values were 0.97 (for anti-PT data) and 0.90 (for anti-PRN data). The model fit for anti-PT data seems to show more agreement between observed and predicted values of individual antibody titres at different time points.

The parameter estimates for the two models are shown in Table 1. For anti-PT, the groups of infants and countries did not play a significant role in estimating the peak h. Nevertheless, these two factors were statistically significant in the case of anti-PRN data, although the group effect seems to be trivial. According to the protocol, the first vaccination dose should be performed at two months of age in both countries. However, in reality, this timeline was true only in Belgium. In Vietnam, due to some logistical difficulties, the first vaccine dose was performed one month later than planned in the protocol, at a mean age of 3 months [11]. Hence, the time point h in Vietnamese infants should theoretically be one month later than in Belgian infants. The estimate of the country effect on h for anti-PT is 1.52 (months) with 95% CI containing 1, indicating a non-significant difference between the two countries. In the meanwhile, the estimate for anti-PRN was 4.05 (months) with 95% CI of [3.04; 5.53] (months) that did not contain 1. This estimate indicates that the peak h was comparable for anti-PT but roughly three months later for anti-PRN in Vietnam compared to Belgium.

The effects of *country* and *group*, as well as their interaction, were statistically significant for the estimation of γ. These effects were in the same direction for both anti-PT and anti-PRN. More specifically, the overall rates of increase were significantly higher in Vietnamese infants than in Belgian infants and higher in infants born to women in the control group in comparison to infants born to vaccinated women. There was no significant difference between two infant groups in the decay rate after primary vaccination α for both antibody types and in the rate of increase after the booster vaccine dose ω for anti-PT. For anti-PRN, the rate of increase after the booster dose was significantly lower in Vietnamese infants than in Belgian infants.

Similarly, both *group* and *country* effects and their interaction, played a significant role in the estimates of cord anti-PT and anti-PRN antibodies. In Belgium, the mean antibody titre at birth in infants born to vaccinated women was significantly higher than

Table 2
Estimates of antibody titres in the cord in infants and their 95% CIs for anti-PT and anti-PRN: $A_{0_{00}}, A_{0_{01}}, A_{0_{10}}, A_{0_{11}}$ represent the antibody titres in cord in Belgian infants of vaccinated women, Vietnamese infants of vaccinated women, Belgian infants of women in the control group, and Vietnamese infants of women in the control group, respectively.

Parms	Anti-PT	Anti-PRN
$A_{0_{00}}$	132.69 [90.32; 202.23]	644.13 [494.92; 835.56]
$A_{0_{01}}$	24.31 [15.55; 38.71]	169.67 [112.02; 264.08]
$A_{0_{10}}$	15.84 [10.32; 24.90]	23.88 [16.74; 34.69]
$A_{0_{11}}$	10.64 [6.86; 17.19]	14.00 [9.78; 20.30]

that in infants born to control women (132.69 [90.32; 202.23] vs. 15.84 [10.32; 24.90] for anti-PT, and 644.13 [494.92; 835.56] vs. 23.88 [16.74; 34.69] for anti-PRN) (see Table 2). In Vietnam, antibody titres in the cord in infants born to vaccinated women were also higher than those in infants born to women in the control group. While this difference is significant for anti-PRN (169.67 [112.02, 264.08] vs. 14.00 [9.78, 20.30]), it is not the case for anti-PT since the 95% CI of anti-PT antibody titres in these two groups of infants showed some overlap (24.31 [15.55, 38.71] vs. 10.64 [6.86, 17.19]). In terms of country difference, the mean antibody titres in the cord of Belgian infants at birth were significantly higher than those of Vietnamese infants for both antigens in infants born to vaccinated pregnant women. In infants born to control women groups, although the cord antibody titres were higher in Belgian infants, the 95% CI of antibody titres in the cord of Belgian and Vietnamese infants overlapped. Both factors statistically significantly affected the estimate of the decay rate β right after birth for anti-PT, but only *group* effect was significant for β in anti-PRN data.

A correlate of protection for pertussis is not defined; however, higher antibody concentrations correlate with better protection against the disease. Fig. 3 shows the decrease in maternal antibody concentrations during the first months of life in infants when no infant vaccination was assumed. In Belgian infants, approximately 3.3 (vaccinated group) and 2.1 months (control group) were required so that their anti-PT concentrations fell below 5 (IU/ml). However, less than one month in both groups of Vietnamese infants was required for the same event to happen. Anti-PRN antibody concentrations had much longer half-lives; hence, more than six months in two groups of Belgian infants was demanded for a similar outcome to be observed. In Vietnamese infants born to vaccinated women, after 18 months, the anti-PRN started to fall below the same threshold. This duration in infants born to women in the control group was approximately 29 months. This observation can be explained by the fact that while the cord antibody concentrations in infants born to vaccinated women were higher than the cord antibody concentrations of infants in the control group, their decay rates were also higher.

Finally, we show in Table S3 the half-lives (time for antibody titres to decline 50%) of antibody titres in infants after being born and before the start of the vaccination programme. Generally, the half-lives in infants born to vaccinated women were statistically longer than those in infants born to women in the control group (for anti-PT), but the reverse held for anti-PRN. When compared between countries, the half-lives in Vietnamese infants were longer than those in Belgian infants, but this difference was minimal. While the half-lives for anti-PT were short for all groups of infants, the numbers for anti-PRN were quite high for infants born to women in the control group.

3.2.2. Joint modelling of anti-PT and anti-PRN antibody titres

In this part, we first fitted the model with lognormal distributions for random effects of AT_0, β_1 and gamma distributions for random effects of $\gamma_1, AR_0, \beta_2, \gamma_2$. This model, however, did not show

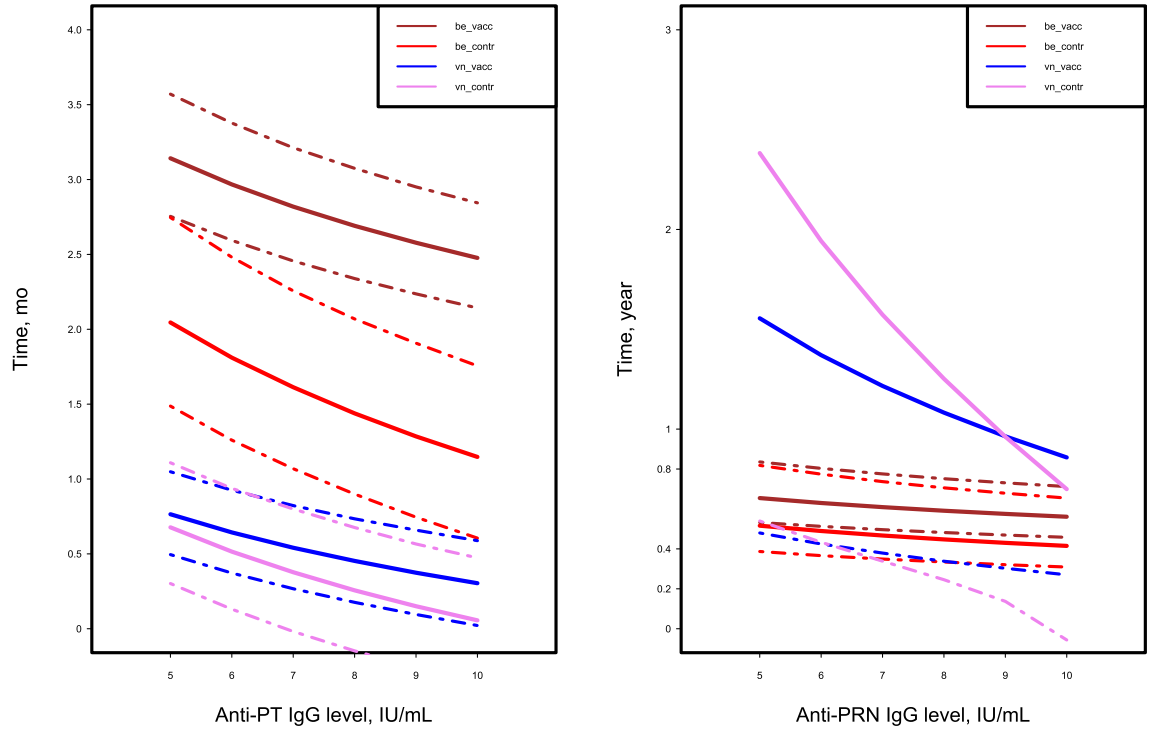

Fig. 3. The decline in maternal antibody levels of antibody titres during first months of life in infants: Belgian infants born to vaccinated women (brown lines), Belgian infants born to women in the control group (red lines), Vietnamese infants born to vaccinated women (blue lines), and Vietnamese infants born to women in the control group (violet lines). We reported median time points (interquartile ranges) at which maternal IgG antibodies in infants started to decline below a prespecified threshold of 5–10 enzyme-linked immunosorbent assay units (IU/ml). Data for anti-PT (left panel) were expressed in **months**, and data for anti-PRN (right-panel) were plotted in **years**. The plots were based on the assumption of no infant vaccination. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

a well-behaved mixing for some parameters. Hence, we proceeded further with the bivariate model, where we assumed all random effects followed a gamma distribution.

Generally, the joint model gave similar results compared to the univariate models for both anti-PT and anti-PRN. The time point h is still significantly higher in Vietnamese infants than in Belgian infants (with a median of 0.88 months). The effect of *group* became significant to the decay rate α_2 after the time point h but not significant to the increasing rate ω_2 after the booster dose anymore (for anti-PRN). This output suggests that the blunting effect faded away after the booster dose. Finally, a positive correlation between two types of measurements indicated that anti-PT and anti-PRN changed in the same direction over time in infants.

4. Discussion

We present a modelling framework to analyse longitudinal data collected from vaccine trials with the focus on antibody titre data using nonlinear mixed-effects models. A hypothesised dynamic model for antibody titres in infants capturing their evolution from birth, before and after primary vaccination, and before and after the booster dose was the base for our modelling approach. We analysed data from two countries: Belgium and Vietnam. With the proposed method, the methodological discrepancies between both studies were successfully incorporated into the models. For anti-PRN, antibody titres at birth in control infants were significantly lower compared to infants born to vaccinated women in both Vietnam and Belgium. These results were in agreement with the results obtained using the conventional t-test to test for the difference in Geometric Mean Concentrations (GMCs), as shown in Maertens et al. [9] and Hoang et al. [11]. For anti-PT data, this conclusion only held in Belgium, as also shown by Maertens et al. [9] In Vietnam, although the cord anti-PT antibody concentrations were higher in infants born to vaccinated women than in the control group, the difference was not considered to be significant. This observation contradicted the conclusion of Hoang et al. [11]. A possible explanation is that our modelling approach considered all data together and hence produced more variability than the method of Hoang et al. [11].

Additionally, we observed that in infants born to vaccinated women, both anti-PT and anti-PRN antibody titres in the cord were significantly higher in Belgium than in Vietnam. However, this result did not hold for infants born to the control group for both types of antigens. Given that cord antibody titres in infants born to control women were comparable in the two countries, the difference we observed in those infants born to vaccinated women was likely due to the use of different vaccine brands given in pregnancy and to the difference in vaccination history between the women in Belgium and Vietnam. This conclusion, however, does not exclude possible effects of other important factors such as gestational age at the time of immunisation and the interval between vaccination and giving birth. Recently, Wanlapakorn et al. [20] showed that the interval between Tdap administration and delivery affected the cord titres significantly. The current analysis did not consider this factor since these models did not achieve convergence. Future studies need to be conducted to understand more about the differences between countries and regions. The peak h was estimated higher in Vietnamese infants compared to Belgian newborns. The difference in maternal vaccines (different manufacturers in two countries), which might explain the difference in the overall increasing rate γ, can partially explain the later peak in Vietnamese infants. Antibodies in this population rose faster, which resulted in a later peak [21].

Another essential feature of the maternal vaccination strategy seen in many vaccine trials is the interference or the blunting effect. This blunting effect refers to the fact that maternal antibodies may inhibit antibody generation in the infant after primary vaccination and, to a lesser extent, after booster vaccination. The inhibition results in lower pertussis-specific antibody titres in infants from vaccinated women compared to infants from women in the control group after the immunization in infants. This blunting effect has been observed before [22,9,11,23,24]. The univariate modelling approach showed that the *group* effect was statistically significant for estimating β, γ in anti-PT data and for estimating h, β, γ and ω rates in anti-PRN data. The joint analysis gave the same results for anti-PRN except that the *group* effect was no longer significant for the rate of increase after the booster dose (ω_2) but turned out to affect significantly the decay rate α_2. These results suggested that the blunting effect was present during primary vaccination for anti-PT but went away afterward. For anti-PRN, on the other hand, the blunting effect remained after the primary immunisation but no longer persisted after the booster dose.

One of the main limitations of our analysis is that the number of observations per infant was somewhat limited. More specifically, there were maximally five time-points for each Belgian infant and four blood samples for each Vietnamese infant. Our simulation study showed that due to little information received from these longitudinal profiles, the estimates of some parameters (especially the parameters expressing the within- and between-subject variability, and parameters of the dynamics of antibody titres after the primary vaccination) were considered biased. Hence, the interpretation regarding the output of nonlinear mixed-effects models needs to be done with care. This simulation study also suggests that in future research, more blood samples need to be collected, if possible, to enhance the performance and reliability of the nonlinear mixed-effects model approach. Currently, it is advised to have minimum eight observations per individual. The timing of blood samples also needs to carefully follow the moment of infant vaccination to achieve reasonable estimates for all model parameters.

5. Conclusions

The lower antibody concentration at birth in Vietnam is possibly related to the use of different Tdap vaccines or different maternal vaccination history. The difference between both countries persisted after primary vaccination. The difference in the overall increase of antibodies after primary immunisation in Vietnamese infants was possibly due to the use of maternal and infant vaccines of different brands or the lower pre-vaccination maternal antibody titre in Vietnamese infants. The nonlinear mixed-effects modelling framework offers the possibility of pooling all data together and employs a hypothesised kinetics model for antibodies. Hence, this approach enables researchers to investigate the evolution of antibodies over time and incorporate many covariates of interest. As a result, the proposed method relies on sufficient data being available. Future study design needs to pay more attention to the timing of the blood samples as well as the number of blood samples so that conclusions drawn from a comprehensive modelling approach are guaranteed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of Competing Interest statement

The authors do not have commercial or other association that might pose a conflict of interest (e.g., pharmaceutical stock ownership, consultancy, pharmaceutical board membership, relevant patents, or research funding).

Contribution to authorship

All contributed to the writing of the manuscript. EL and KM were responsible for the data collection for the study in Belgium. EL and TTHH were responsible for the data collection for the study in Vietnam. TMPT and NH performed the statistics.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.09.003.

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