Antibody in breastmilk following pertussis vaccination in three-time windows in pregnancy

Olwenn Daniel[1]*, Myles Loughnan[1,2,8]*, Miranda Quenby[1,3]*, Krina Chawla[1], Vanessa Greening[1,3], Paul Heath[1,3], Christine E. Jones[4,5], Asma Khalil[1,3], Laxmee Ramkhelawon[1], Anna Calvert[1,3]^, Kirsty Le Doare[1,3,6,7]^

On behalf of the MAMA/OpTIMUM breastmilk study group: Agnieszka Burtt, Emily Cornish, Danielle Hake, Tom Hall, Uzma Khan, Nicki Martin, Robin Parsons, Laura Sparks, Fiona Walbridge, Susan J. Wellstead

*Joint first author

^Joint senior author

[1]Centre for Neonatal and Paediatric Infection and Vaccine Institute St George's, University of London, London UK

[2]Department of General Medicine, The Royal Children's Hospital, Melbourne 3052, Australia

[3]St George's University Hospitals NHS Foundation Trust, London UK

[4]NIHR Southampton Clinical Research Facility and Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust, Southampton UK

[5]Faculty of Medicine and Institute for Life Sciences, University of Southampton, Southampton, UK

[6]Makerere University-Johns Hopkins University Research Collaboration, Kampala P.O. Box 23491, Uganda.

[7]Pathogen Immunology Group, UK Health Security Agency, Porton Down, Salisbury SP4 0JG, UK.

Corresponding author

Olwenn Daniel

Institute for Infection and Immunity

St George's University of London

Cranmer Terrace, SW170RE, London

UNITED KINGDOM

odaniel@sgul.ac.uk

Running head

Breastmilk antibodies and pertussis vaccine

Key words

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Conflict of interest and financial disclosure

The authors have declared no conflict of interest or financial disclosure.

Abstract

Background

Pertussis-containing vaccines are routinely offered in the UK at 16-32 weeks of gestation and have been shown to be safe and effective, but there remains debate about the best timing for vaccination. Most research into this has focussed on serological immunity, but breastmilk is also important in infant immunity and the amount of IgA in breastmilk may impact on mucosal immunity. It is important to understand if the timing of vaccination in pregnancy affects the concentration of IgA in breastmilk.

Methods

Participants recruited as part of the MAMA and OpTIMUM trials received a pertussis-containing vaccine during pregnancy, either before 24 weeks, between 24-27+6 weeks or between 28-31+6 weeks. Samples of colostrum within 24 hours of delivery, and breastmilk at 14 days were collected. Pertussis toxin (PT), Pertactin (PRN), Tetanus toxoid (TT) and Diphtheria toxoid (DT) specific IgA levels were measured using a multiplex immunoassay.

Results

There was no difference in specific IgA levels against PT, PRN, TT and DT between the groups vaccinated within different time periods. For all antigens there was decay in antigen-specific IgA levels between colostrum and breastmilk at 14 days.

Conclusion

Our results suggest that the timing of administration of a pertussis-containing vaccine in pregnancy does not impact on antigen-specific IgA concentration in colostrum or breastmilk at 14 days.

Background

Pertussis is a vaccine-preventable respiratory infection caused by the bacterium *Bordetella pertussis*. Infection can result in significant morbidity and mortality, primarily in infants less than three months of age born to mothers unvaccinated with a pertussis containing vaccine in pregnancy and who have not yet received the benefits of their own vaccinations. (1, 2) Despite well-established vaccination programmes against pertussis, there was an increase in the number of cases seen in many countries from 2005, associated with increased hospitalisations and deaths in young infants. (3) In response to this, many high-income countries, including the United-Kingdom (4-6), introduced antenatal pertussis vaccine programmes which have been shown to be safe and effective (7).

Recommendations about the timing of antenatal pertussis vaccination vary between countries, and there has been debate about the optimal timing of vaccination for the best serological protection of the infant. Two vaccine effectiveness studies have reported better protection after vaccination in the third rather than second trimester (8, 9) and a third has shown no impact of timing on effectiveness (10), whereas several immunogenicity studies have suggested that vaccination earlier in the third trimester results in higher antibody concentrations than vaccination later in the third trimester (11-13). Vaccination was initially recommended between 28-32 weeks gestation in the UK, but this advice was updated in 2016 to 16-32 weeks and extending this time window has been shown to have advantages for increasing opportunities to vaccinate, and for preterm infants. (14)

Although most research has focussed on serological immunity, antenatal vaccination has been shown to increase antibody titres in breastmilk against pertussis. One study found an approximately threefold increase in the geometric mean concentration (GMC) of IgA to pertussis toxin (PT) in the breastmilk at 8–9 weeks postpartum of antenatally vaccinated

women compared to women not vaccinated in the preceding five years. (15) Another study found greater filamentous haemagglutinin-specific IgA in colostrum and breastmilk at two weeks, in Tdap-vaccinated women after 20 weeks of gestation than in unvaccinated women. (16) A retrospective Belgian study of 234 participants found significantly higher PT-specific IgG GMC in the colostrum of vaccinated women delivering at term compared with unvaccinated controls. (17)

Whilst the exact mechanism of protection from antibody in breastmilk is uncertain, increasing antibody titres in breastmilk plausibly increases protection against pertussis in the neonate through enhancement of mucosal immunity. Using mice intracerebrally challenged with *B.pertussis*, a study has shown the effect of IgA contained in human colostrum on bacteria neutralisation. (18) PT-specific IgA and IgG from maternal breastmilk has also been shown to remain stable in the infant gastrointestinal tract during digestion. (19) Increasing pertussis-specific IgA in breastmilk through maternal vaccination could plausibly increase protection against pertussis in the neonate, but the impact of timing of vaccination on pertussis antibody concentration has not previously been explored. In this analysis we studied IgA concentrations in colostrum and breastmilk at 14 days, after vaccination either before 24 gestational weeks, between 24-27^{+6} gestational weeks, or between 28-31^{+6} gestational weeks in pregnancy.

Methods

The participants included in this analysis were recruited from two studies in the United-Kingdom: Maternal Antibody in Milk After vaccination (MAMA, NCT03982732) and Optimising the Timing of whooping cough Immunisations in mums (OpTIMUM, NCT03908164).

The MAMA study was a single-centre observational study in which women were recruited postnatally. Participants were eligible if they had a singleton pregnancy, had received pertussis vaccination between 16 and 32 gestational weeks and were planning to breastfeed. Women were excluded if they had a known immunodeficiency. All participants had received a pertussis containing vaccine as part of routine antenatal care and the timing of vaccination was self-reported by participants, being checked with medical records if participants were uncertain. Participants were assigned to three groups according to the time at which they had received vaccination (<24 weeks, 24-27^{+6} weeks and 28-31^{+6} weeks). Participants provided a sample of colostrum within 48 hours of delivery, and a breastmilk sample at 14 and 42 days.

The OpTIMUM study was an equivalence study in which participants were randomised into three gestational age groups (<24 weeks, 24-27^{+6} weeks and 28-32 weeks). Randomisation was on a 1:1:1 ratio using a computerised block randomisation list. Participants were eligible if they were pregnant and had not yet received pertussis vaccination, willing and able to comply with study procedures and provide informed consent, and had received a 20-week anomaly scan with no life limiting congenital anomalies identified. Exclusion criteria were maternal age of less than 16, confirmed or suspected pertussis in previous five years, known diagnosis of immune deficiency, receiving immunosuppressive medication within six months of enrolment, or in the opinion of the investigator was unlikely to complete follow up. This was a multicentre study run in six sites. Participants in two sites (St. George's, University of London and University Hospitals Southampton NHS Foundation Trust) were asked if they wished to take part in the breastmilk sub-study. All participants in the OpTIMUM study had a cord blood sample taken at delivery and participants in the breastmilk sub-study gave a sample of colostrum within 48 hours of delivery. A further breastmilk sample was collected at 14 and 42 days.

Due to the COVID lockdowns the number of participants that attended the 42 day visit in the OpTIMUM study were very low (Figure 1) and this time point was therefore excluded from further analysis.

Participants in both studies received Boostrix-inactivated poliovirus vaccine (GlaxoSmithKline; London, UK). Boostrix-IPV contains pertussis toxin (8μg), filamentous haemagglutinin (8μg), pertactin (2.5μg), diphtheria toxoid (not less than two international unit), tetanus toxoid (not less than 20 international units), and inactivated polio virus types 1-3 (type-1 40 D-antigen unit, type-2 8 D-antigen unit, and type 3 32 D-antigen unit).

Ethical approval for the MAMA study was given by West of Scotland Research Ethics Committee (18/WA/0171) and for the OpTIMUM study was given by York and Humber Research Ethics Committee (19/YH/0050).

Sample processing

Colostrum and breastmilk samples were centrifuged first at 1000g at 4°C for 10 minutes and then at 10000g at 4°C for 30 minutes to separate the lipid fraction from whey which was then stored at -80°C.

IgA measurement

An in-house Multiplex assay was used to measure antigen-specific colostrum and breastmilk IgA. MagPlex microspheres (Luminex DiaSorin, Italy) were conjugated to four antigens: pertussis toxin, pertactin, diphtheria toxoid, and tetanus toxoid (181, 187, 151 and 191B respectively, List Biological Laboratories, Campbell, United-States).

Colostrum and breastmilk samples were diluted to 1:100, 1:500 and 1:2.500, next to a curve prepared from the Pertussis Antiserum WHO International standard (1:60 dilution and 2-fold

serial dilution, NIBSC 06/140, UK). Samples and microspheres were incubated overnight at 300rpm and 2-8°C, and R-Phycoerythrin-conjugated goat anti-human IgA secondary antibody (1:200, 50ul/well, 109-115-011, Jackson ImmunoResearch, Ely, UK) was added for 1 hour 30 minutes at 300 rpm at room temperature. Plates were read with Bio-Plex 200 (Bio-Rad, Hercules, United-States).

Statistical analysis

Mean fluorescence intensity (MFI) were interpolated into concentrations (Arbitrary units) with Bio-Plex Manager Software 6.2. IgA results under the assay limits of quantification were attributed 0.001 AU. On Graph Pad Prism 10.2.2, the GMC levels of IgA were plotted. Because of the impact of the COVID-19 pandemic on collection of the final breastmilk samples we included only the timepoints of colostrum at delivery and breastmilk at 14 days.

Results

This analysis was performed on samples collected from two studies: MAMA and OPTIMUM (Figure 1). There were 104 participants with either a colostrum sample, or a breastmilk sample at 14 days, or a sample at both time points (43 MAMA, 61 OpTIMUM). These are included in the analysis.

Overall, there were more participants vaccinated at <24 gestational weeks (GW) (n=41) and 24-27^{+6} GW (n=40) than at 28-31^{+6} GW (n=23). Demographic details are described in Table 1.

IgA in colostrum and breastmilk

IgA concentrations against any of the tested antigens between women vaccinated in either of the three gestational age groups were similar for colostrum (Figure 2) and breastmilk at 14 days (Figure 3).

Colostrum PT and PRN specific-IgA is compared to cord serum PT and PRN specific-IgG collected in the OpTIMUM study in supplementary figure 1 and no correlation was found.

Antibody decay

Antibody concentrations were greater in colostrum than in breastmilk collected at 14 days, with IgA decay between these two time points. The decay percentages were similar between study groups (**Pertussis toxin:** Percent decay <24GW= 99.89%, 24-27^{+6} GW= 99.98%, 28-31^{+6} GW= 99.67%; **Pertactin:** Percent decay <24GW= 96.37%, 24-27^{+6} GW= 95.97%, 28-31^{+6} GW= 96.95%; **Tetanus toxoid:** <24GW=99.56%, 24-27^{+6} GW= 99.70%, 28-31^{+6} GW= 99.75%; **Diphtheria toxoid:** <24GW= 97.25%, 24-27^{+6} GW= 98.45%, 28-31^{+6} GW= 98.70%).

A high number of breastmilk samples at 14 days had no quantifiable IgA: 69.1% (67/97) for Pertussis toxin, 48.5% (47/97) for Tetanus toxoid and 16.5% (16/97) for Diphtheria toxoid. All breastmilk samples had quantifiable Pertactin-specific IgA.

Discussion

To our knowledge, this is the first time that the impact of timing of pertussis vaccination in pregnancy has been explored in relation to antibody in breastmilk. We found similar concentrations of pertussis toxin, pertactin, tetanus toxoid, or diphtheria toxoid specific-IgA in colostrum or breastmilk at 14 days in women who had received vaccination in different time windows, all within the period recommended for vaccination in the UK.

Human breastmilk is a complex and dynamic source of nutrition which plays an important immunological role, particularly in the first few months of life as infants are developing their adaptive immunity (20). Breastfeeding has been established as protective for infants in preventing various respiratory infections (21), however there is disagreement in the literature looking at breast feeding and clinical pertussis cases. Exclusive breastfeeding was not a protective factor for pertussis in hospitalised infants under 6 months (22), and the incidence and prevalence of breastfeeding at the time of hospital admission were not different between pertussis-like cases and controls (23). In contrast a study shows that breastfed infants had decreased pertussis odds compared to infants receiving more formula (24), and another study states that breastfeeding had a protective effect against pertussis in infants from both unvaccinated and vaccinated mothers (25).

IgA is the dominant antibody found in breastmilk and supports the developing mucosal immunity by lining mucosal surfaces, including the respiratory tract (26-28). The significance of this in mediating protection against disease is uncertain, but it can be hypothesised that an increase in antibody concentration could result in increased protection for infants. Antenatal vaccinations have been shown to help increasing breastmilk IgA concentration for pertussis (15,16), pneumococcus (29-31), meningococcus (32, 33), influenza A (34) and SARS-CoV-2 (35).

Our finding that the timing of administration of a pertussis containing vaccine in pregnancy did not result in any difference in PT and PRN specific-IgA concentrations in breastmilk is interesting. Similarly in the primary analysis of the OpTIMUM trial (36) we have shown equivalence of PT and PRN-specific IgG concentration in the cord blood of term infant at birth, regardless of the timing of antenatal vaccination. Equally, although there is no serocorrelate of protection for pertussis, recent effectiveness data from the UK show that

there is similar effectiveness against pertussis in infants born to mothers vaccinated in both the second and third trimesters (37).

Our study showed that specific-IgA concentrations were higher in colostrum than in 14 days breastmilk for all antigens. Similarly, multiple studies report that vaccine-specific IgA were higher in colostrum and lower in breastmilk at subsequent time points (15, 16, 17, 38). Notably a study comparing the colostrum and breastmilk at 2, 4 and 8 weeks postpartum, of unvaccinated and Tdap-vaccinated women after 20 weeks of gestation, found a significant decline in PT-specific IgA after two weeks in both groups (16). This is also consistent with the fact that total IgA levels are known to be higher in colostrum than in breastmilk (39).

It is interesting that a high number of participants had IgA concentrations are below the lower limit of detection for pertussis toxin (69.1%) in transitional breastmilk at 14 days. A similar finding was made in a study using an ELISA assay at 1:101 milk dilution as per manufacturer instructions (16). Our multiplex bead assay requires a similar coefficient of dilution at 1:100. Although there are lower rates of samples with concentrations below the lower limit of quantification in two other studies using ELISA assays (15, 17), breastmilk was only diluted at 1:5 or 1:10. The highest coefficient of dilution in our assay and in study (16) can explain the higher rate of samples below the limit of detection. We would recommend exploring a lower coefficient of dilution for transitional and mature breastmilk in contrast to colostrum, while being mindful of potential matrix effects.

Limitations

This analysis includes participants from two separate studies with different methodologies for the recruitment and vaccination of participants. In the MAMA study, participants were recruited postnatally, and their vaccinations had been given as part of routine care. This may

have led to inaccuracy in information about timing of vaccination, although if there was any uncertainty from participants, the details were confirmed from medical records. The laboratory analysis for samples from both trials was conducted in the same laboratory using the same assays. Because we combined the data from two trials, we are only able to include results which were collected in both trials, which limited our ability to report on all the planned objectives. We also did not have an unvaccinated control group with which to compare our results.

Although we had originally planned to collect samples from participants 6 weeks after delivery, there was significant disruption in collecting these samples because of restrictions which took place because of the COVID-19 pandemic and there were limited samples available at this time point which made meaningful analysis impossible. This has limited our period of longitudinal assessment which is unfortunate as this is a weakness in previous studies (17, 40).

Finally, this was a small study which was not powered to show a difference between the study groups, and the conclusions we are able to draw are therefore limited and require further investigation in a larger study.

Conclusion

In this study looking at different timing of administration of a pertussis-containing vaccine in pregnancy, no differences were identified on the specific-IgA antibody concentration in colostrum or breastmilk at 14 days for PT, PRN, DT or TT. Further work is required to investigate the relationship between timing of vaccination and breastmilk protection. We suggest a study of larger scale with an unvaccinated control group, looking at breast feeding,

vaccination and clinical outcomes, different immune components present in milk including antibodies and their functionality such as bactericidal activity and opsonophagocytosis.

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Ethics approval and patient consent

Ethical approval for the MAMA study was given by West of Scotland Research Ethics Committee (18/WA/0171) and for the OpTIMUM study was given by York and Humber Research Ethics Committee (19/YH/0050). All participants gave informed consent for their participation in the trials.

Data availability statement

Data can be made available for suitable applications submitted to the corresponding author.

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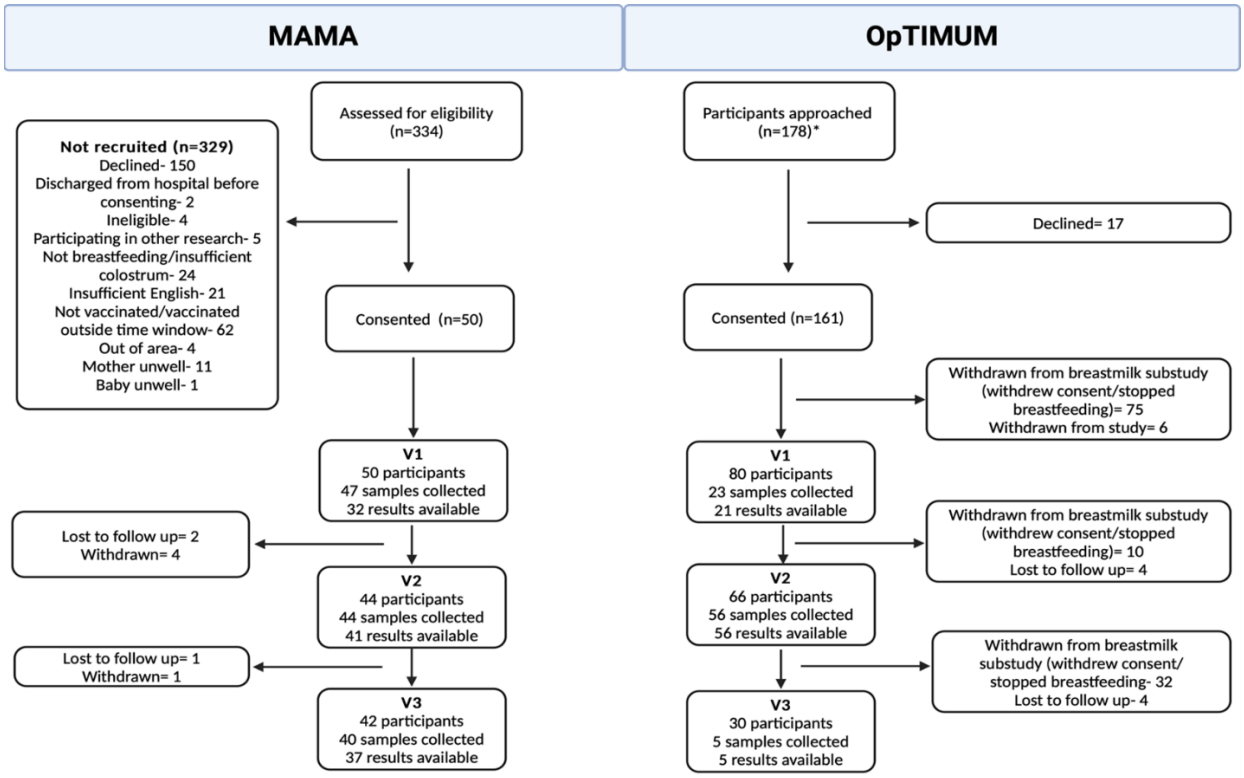
406 **Figures**

407 **Table 1: Demographic details about participants who received vaccination in the three**

408 **gestational age windows.**

	Group 1 (<24 GW) (n= 41)	**Group 2 (24-27^{+6} GW) (n=40)**	**Group 3 (28-31^{+6} GW) (n=23)**
Median gestational age at vaccination (IQR)	21^{+5} (17^{+0}-23^{+6})	$24^{+6.5}$ (24^{+0}-27^{+6})	28^{+4} (28^{+0}-31^{+6})
Pertussis vaccination in a previous pregnancy (%)	19/41 (46.3)	23/40 (57.5)	12/23 (52.2)
White ethnicity (%)	33/41 (80.5)	35/40 (87.5)	21/23 (91.3)
Median gestational age at delivery (IQR)	40 (37^{+6}-41^{+6})	40^{+2} (37^{+2}-42^{+1})	40^{+1} (38^{+4}-41^{+6})

409 IQR= interquartile range; GW= gestational weeks.



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411 **Figure 1: Consort flow diagram.** Participant recruitment, visits and sample collection for

412 the MAMA study and the OpTIMUM study.

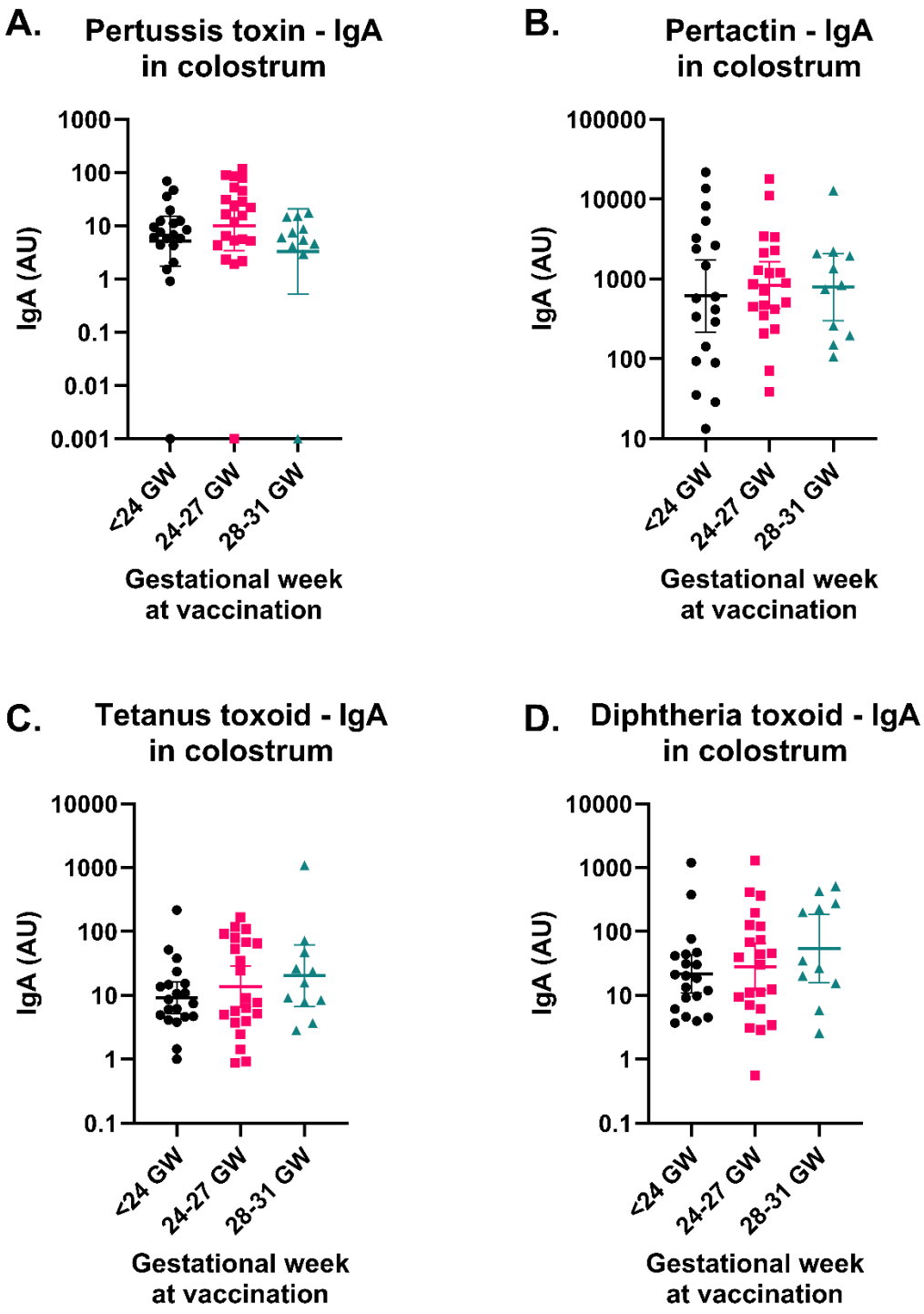
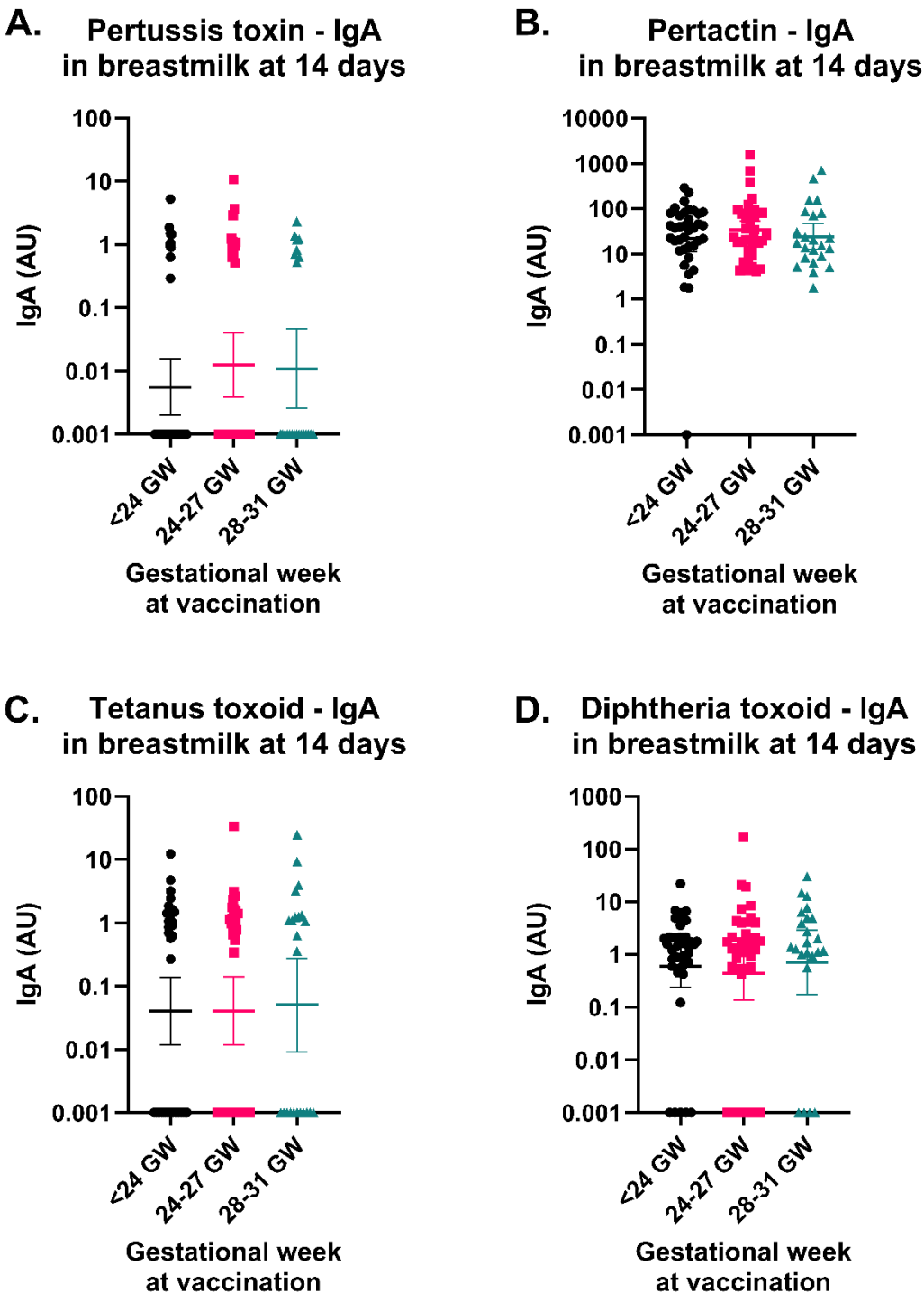


Figure 2: GMC of IgA against Pertussis toxin (A), Pertactin (B), Tetanus toxoid (C) and Diphtheria toxoid (D) in colostrum according to gestational window at vaccination

Geometric mean concentration and 95% confidence interval of antigen-specific IgA (AU). <24 GW (n=20), 24-27 GW (n=22), 28-31 GW (n=11). GW= gestational weeks.



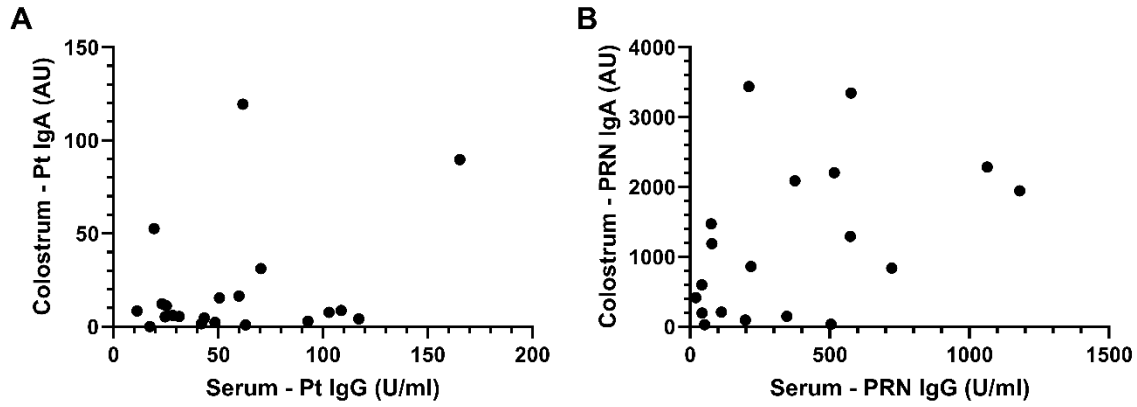
418

419 **Figure 3: GMC of IgA against Pertussis toxin (A), Pertactin (B), Tetanus toxoid (C) and**

420 **Diphtheria toxoid (D) in breastmilk at 14 days according to gestational window at**

421 **vaccination**. Geometric mean concentration and 95% confidence interval of antigen-specific

422 IgA (AU). <24 GW (n=37), 24-27 GW (n=37), 28-31 GW (n=23). GW= gestational weeks.



423

424 **Supplementary figure 1: Colostrum IgA and cord serum IgG, specific to Pertussis toxin**

425 **(PT, A) and to Pertactin (PRN, B).** Cord serum was collected at delivery and colostrum

426 within 48 hours after delivery. For PT n=21 participants, for PRN n=19 participants.