
FOURTEENTH INTERNATIONAL
ROTAVIRUS SYMPOSIUM
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Maternal breast milk secretor phenotype does not affect infant susceptibility to rotavirus diarrhea

International Rotavirus Symposium
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Acknowledgements

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Objectives

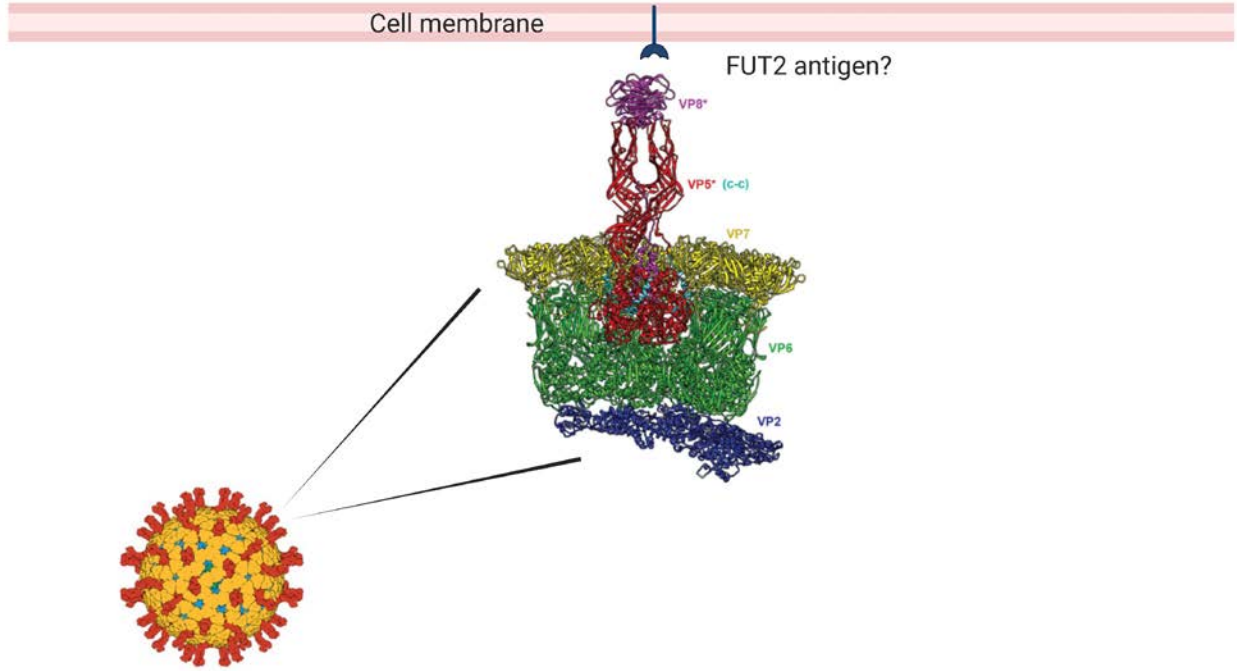
- Basic introduction to secretor status
  - Infant secretor status in rotavirus diarrhea
  - Infant and maternal secretor status in rotavirus vaccination
- Maternal secretor status in rotavirus diarrhea
- Conclusions

Overview of secretor status

- Individuals are either secretors or non-secretors, based on ability to express specific oligosaccharides
  - Exocrine secretions (e.g. saliva, breast milk)
  - Mucosal surfaces (e.g. gut)
- Secretor status is determined by *FUT2* α(1,2)-fucosyltransferase expression

Overview of secretor status

- *FUT2*-dependent antigens may act as cellular receptors, facilitating infection in a genotype-specific manner
  - Non-secretors less susceptible to P[8], P[4] infection



Adapted from Settembre et al 2011. PMID 21157433.
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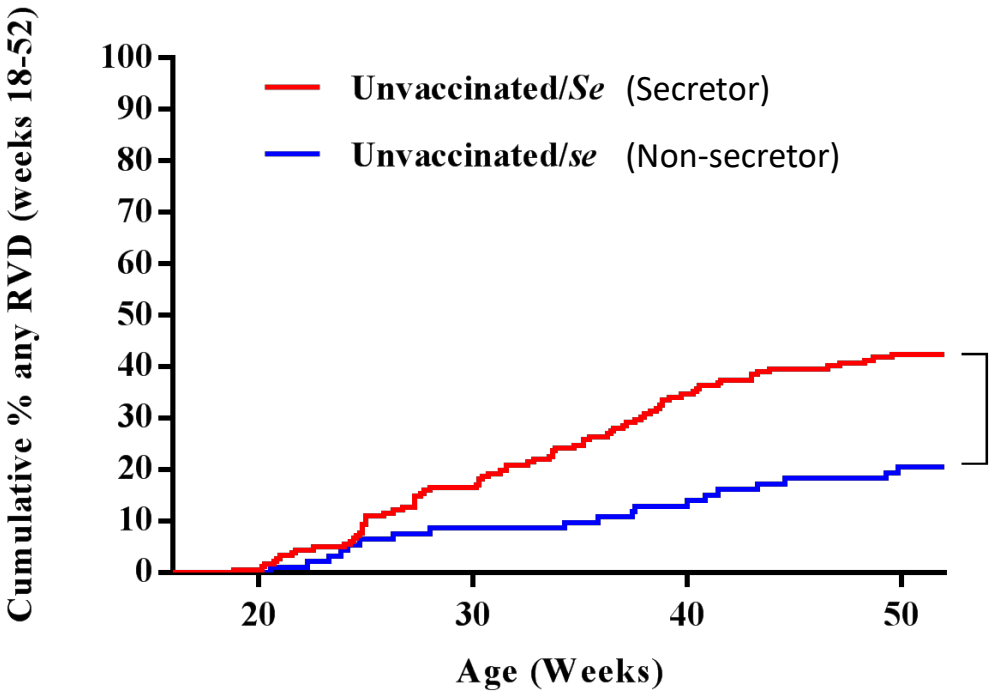
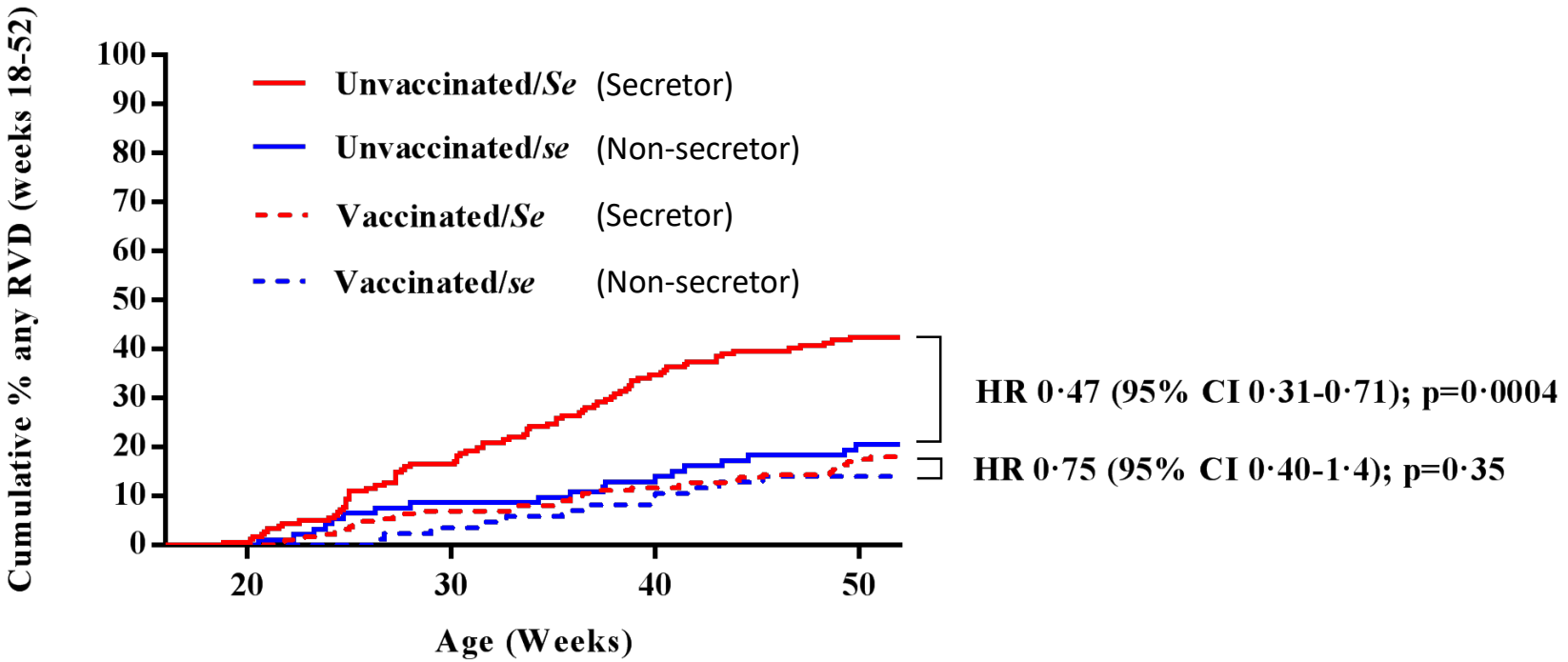
Infant secretor status affects susceptibility to rotavirus PROVIDE study (Dhaka, Bangladesh)



HR 0·47 (95% CI 0·31-0·71); p=0·0004

Lee et al 2018. PMID 29390150

Infant secretor status affects susceptibility to rotavirus PROVIDE study (Dhaka, Bangladesh)



Lee et al 2018. PMID 29390150

Infant secretors have improved responses to Rotarix

Table 1. Summary of different studies investigating the association between rotavirus vaccine take and secretor and Lewis status. Updated and modified from Reference [2].

Vaccine	Country	Secretor Status	Lewis Phenotype	Measurement	Reference
				Seroconversion	
Rotarix	Nicaragua	Non-secretors less seroconversion	Lewis A no seroconversion	After 1 dose	[27]
	Pakistan	Non-secretors less seroconversion	No association	After 3 doses	[28]
	Ghana	Non-secretors less seroconversion	No association	After 2–3 doses	[29]
	Malawi	Non-secretors less seroconversion	No association	After 2 doses	[30]
RotaTeq	Nicaragua	No association	Lewis A no seroconversion	After 1 dose	[27]
RV3-BB	New Zealand	No association	No association	Cumulative	[24]
				Vaccine shedding	
Rotarix	Nicaragua	Non-secretors no shedding	No shedding in Lewis A	After 1 dose	[31]
	Malawi	Non-secretors less shedding	No association	After 1 dose	[30]
	Malawi	No association	No association	After 2 doses	[30]
	South Africa	Non-secretors less shedding	Lower shedding in Lewis A	After 1 dose	[32]
	Brazil	Non-secretors less shedding	Lower shedding in Lewis A	After 1 dose	[7]
RotaTeq	Nicaragua	No association	No shedding in Lewis A	After 1 dose	[31]
RV3-BB	New Zealand	No association	No association	Cumulative	[24]

Sharma and Nordgren 2021. PMID 34198720.

Infant secretors have improved responses to Rotarix

	Rotavirus IgA seroconversion at age 14 weeks			
	n/N (%)	Univariable p value	Multivariable RR (95% CI)	Multivariable p value
Rotavirus IgG titres at age 6 weeks (tertiles)				
80–1280 (ref)	129/173 (75%)	..	..	..
2560	89/132 (67%)	0·17	0·6 (0·4–1·1)	0·09
5120–10 240	99/169 (59%)	0·01	0·4 (0·2–0·6)	0·01
Secretor phenotype				
Non-secretor (ref)	80/135 (59%)	..	..	..
Secretor	230/329 (70%)	0·03	1·8 (1·2–2·8)	0·01

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Velaszquez et al 2021. PMID 35961362

What about maternal status?

- Secretor-dependent antigens are present in breast milk in the form of fucosylated human milk oligosaccharides (HMO)
- Fucosylated HMOs inhibit *in vitro* infectivity of P[4] and P[8] rotaviruses

Laucirica et al 2017. PMID 28637685

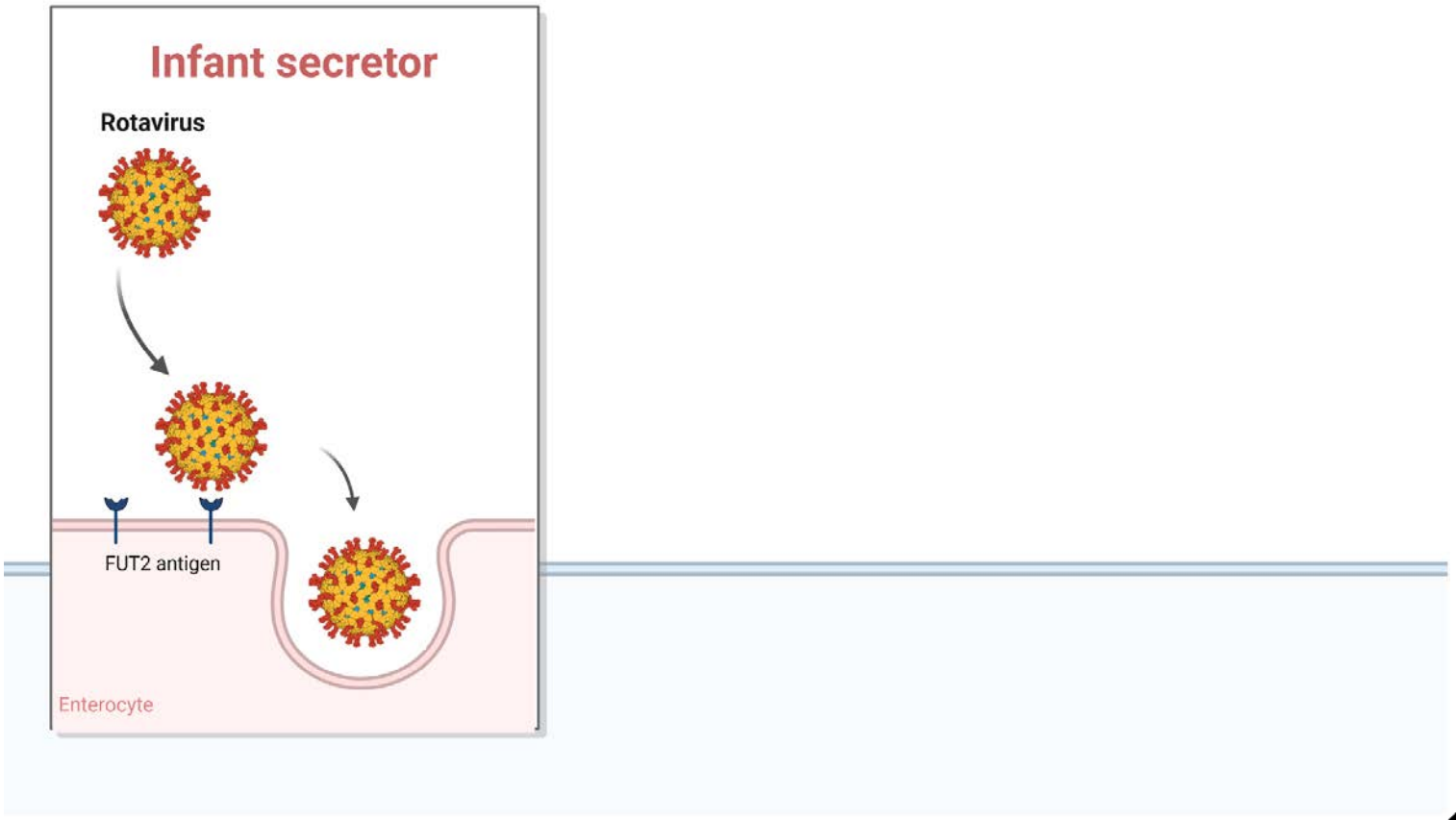
Maternal non-secretor status was associated with improved Rotarix seroconversion (PROVIDE study, Bangladesh)

Table 2. Seroconversion by Secretor Phenotype

Secretor Phenotype	Total No.	Seroconversion, No. (%)	RR (95% CI)
All infants	246	72 (27)	
Secretor mother	172	40 (23)	
Nonsecretor mother	74	29 (39)	1.69 (1.14–2.50)
Secretor infant	175	51 (29)	
Nonsecretor infant	71	18 (25)	0.82 (.53–1.27)
Secretor infants			
Secretor mother	144	34 (24)	
Nonsecretor mother	31	17 (55)	2.32 (1.50–3.59)
Nonsecretor infants			
Secretor mother	28	6 (21)	
Nonsecretor mother	43	12 (28)	1.30 (.55–3.07)

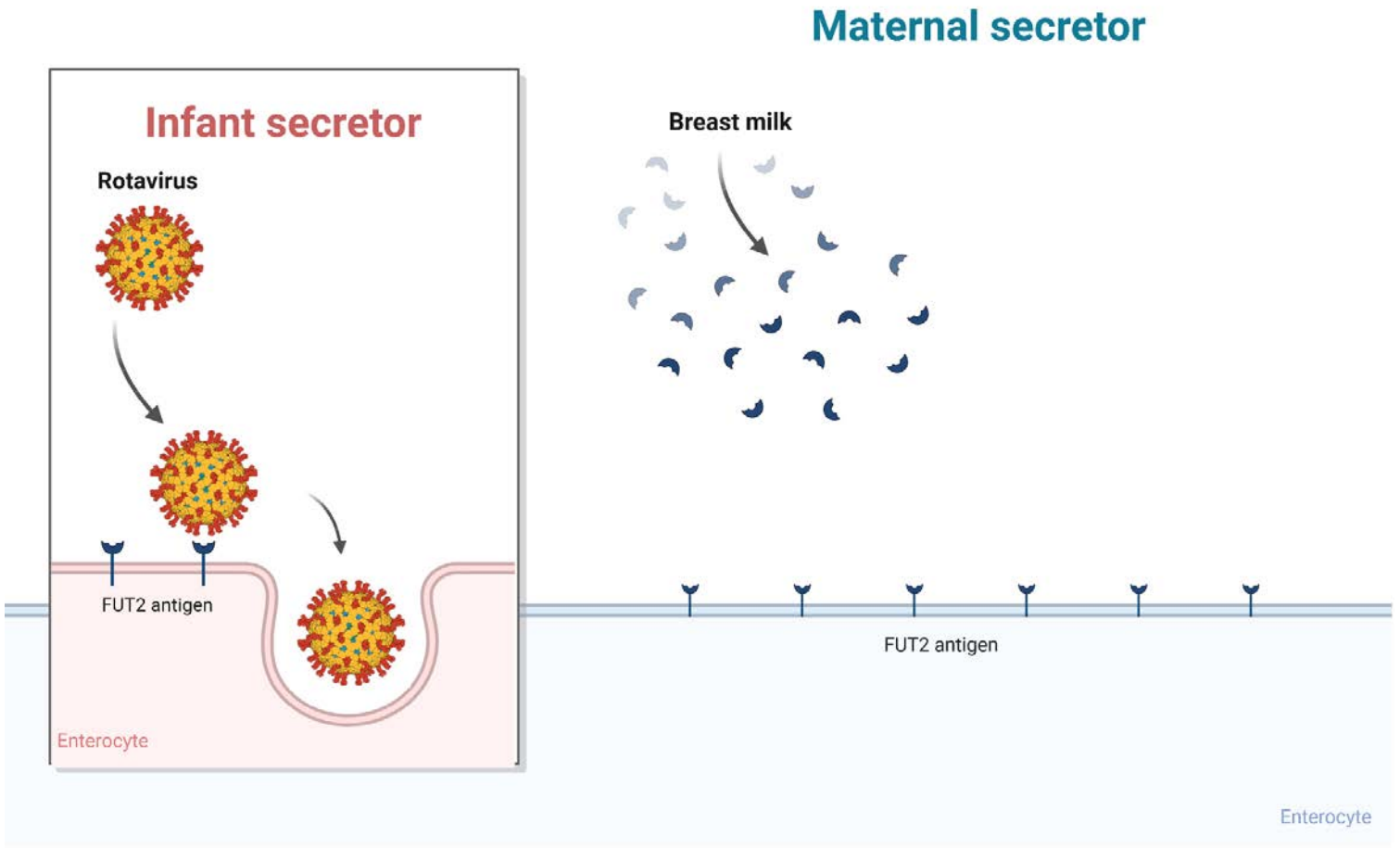
Williams et al 2021. PMID 32157282

Secretor status and vaccine response



Adapted from "Therapeutic Treatments for COVID-19 Targeting Viral Entry Mechanism (Layout)", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates

Secretor status and vaccine response



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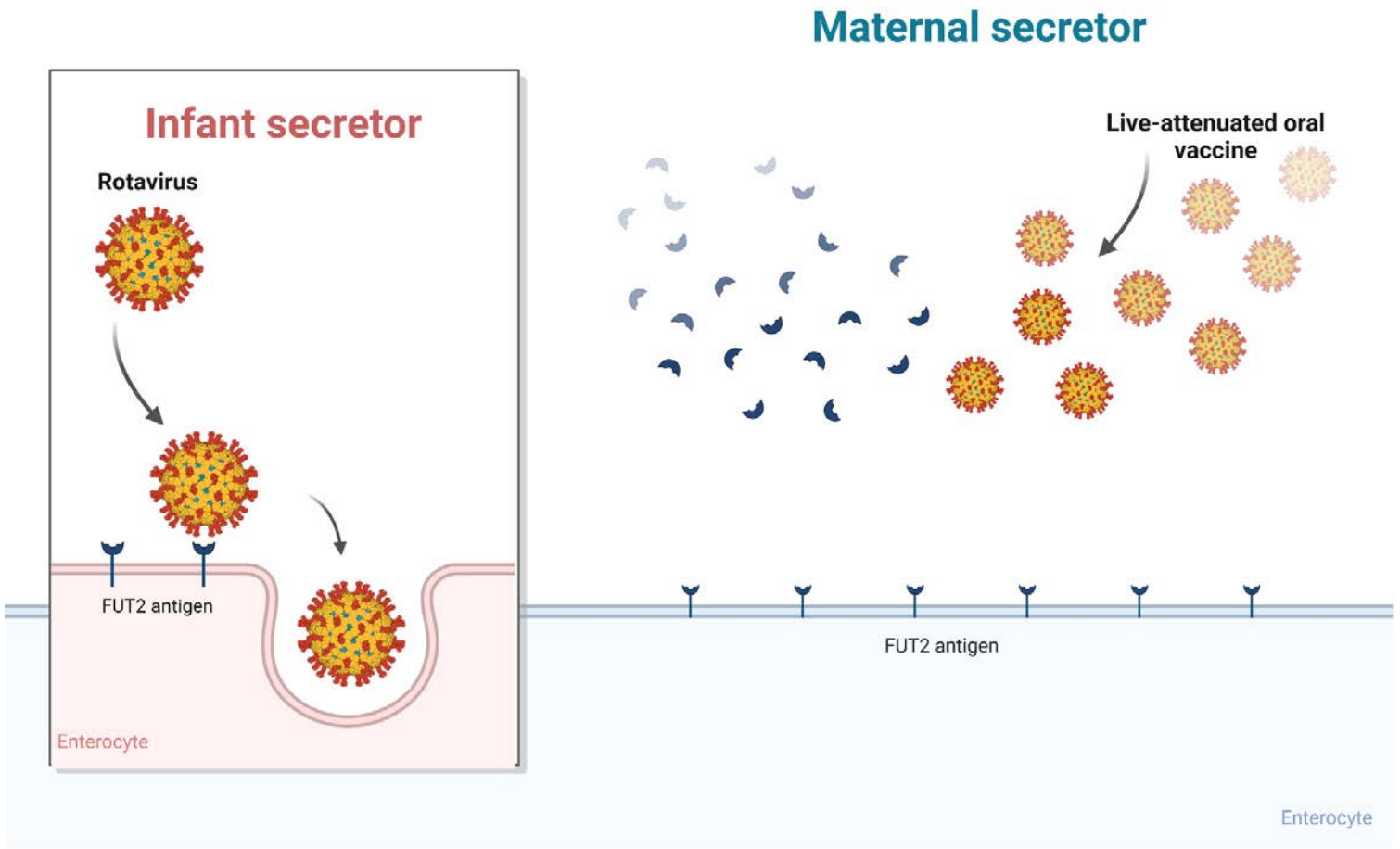

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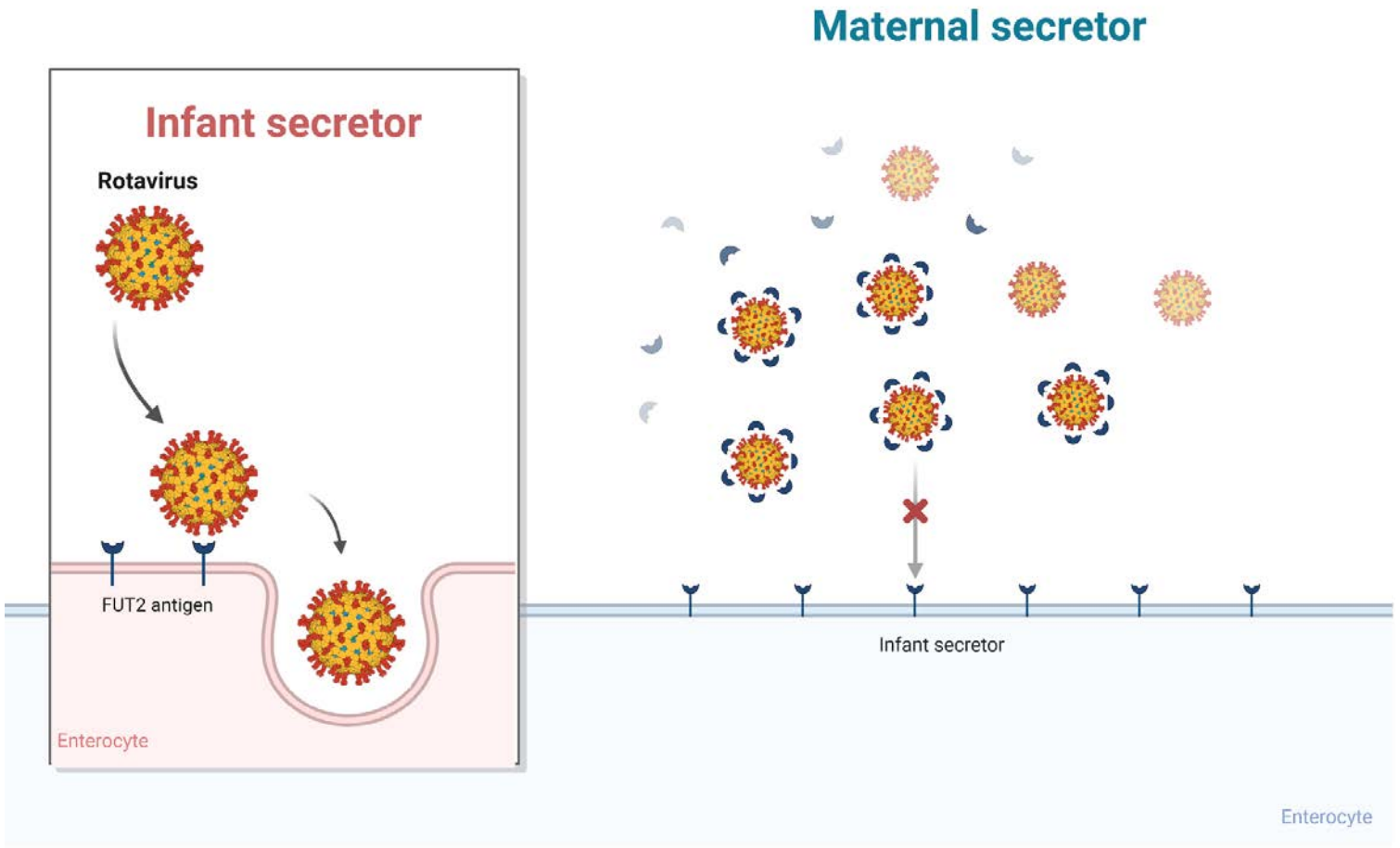
Secretor status and vaccine response



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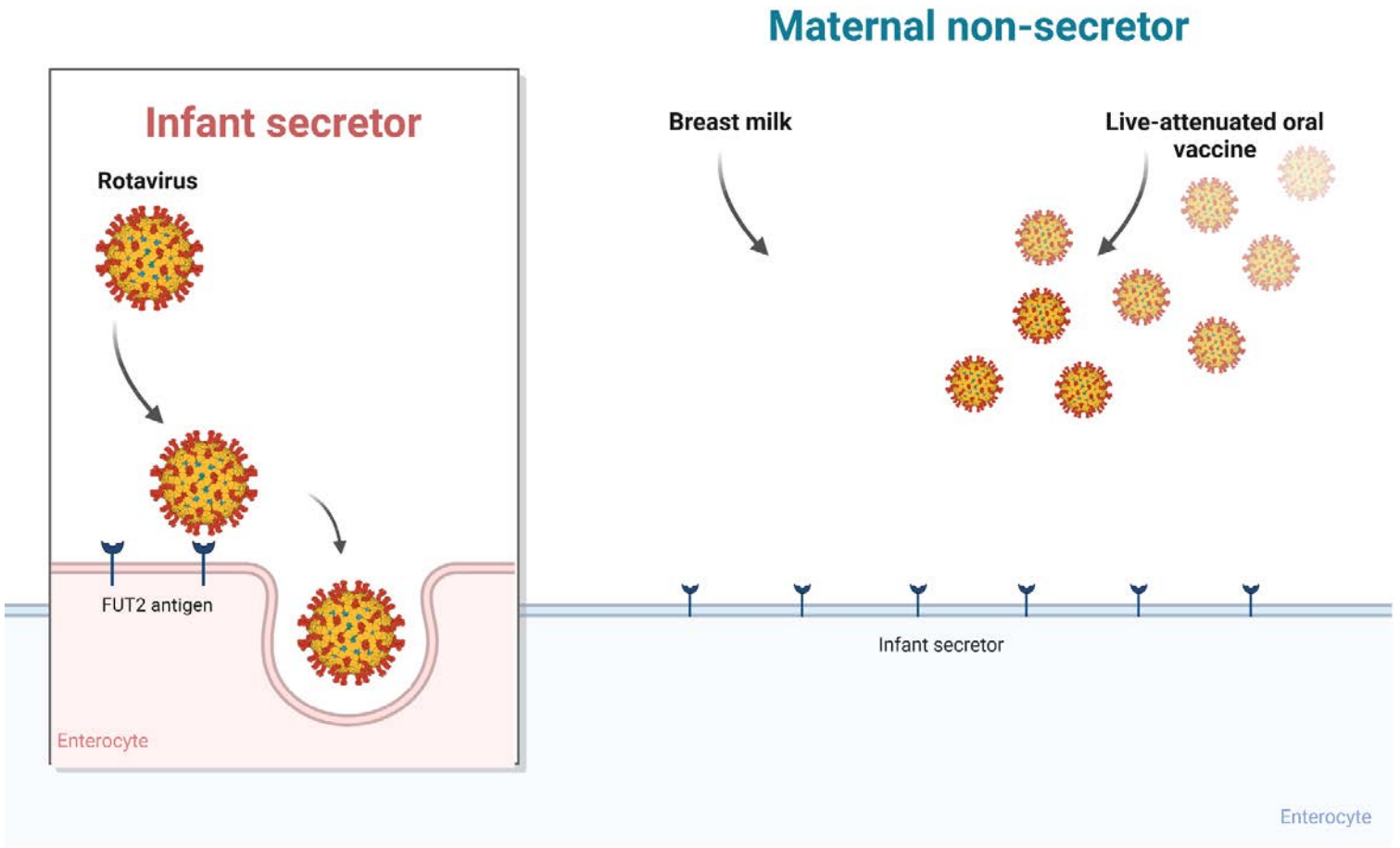
Secretor status and vaccine response



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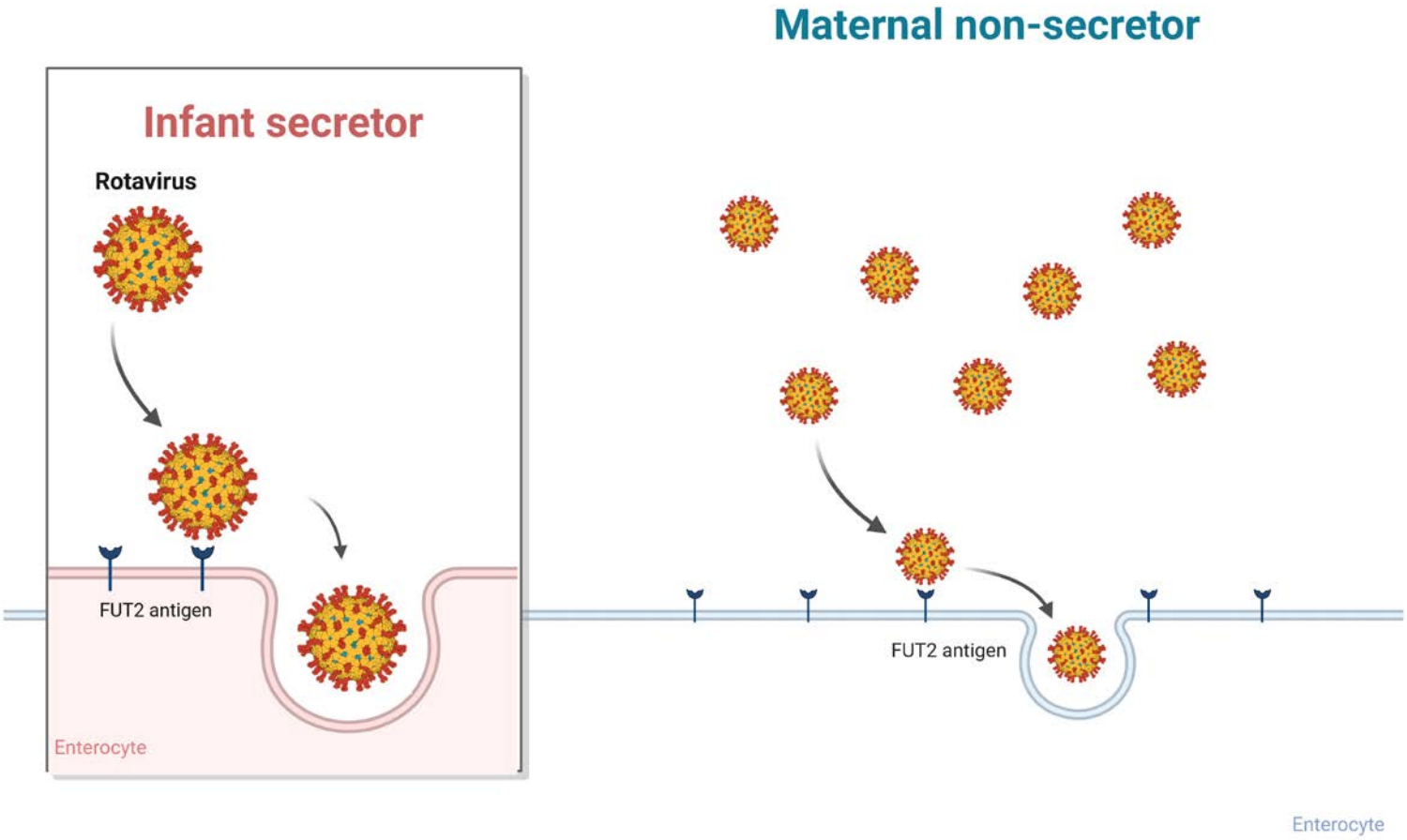
Secretor status and vaccine response



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What is the effect of maternal non-secretor status on rotavirus diarrhea risk?

- Hypothesis: Maternal non-secretor status is associated with decreased risk for rotavirus diarrhea in infants compared to infants of maternal secretors, due to increased RV-IgA seroconversion following oral vaccination

Study design: PROVIDE

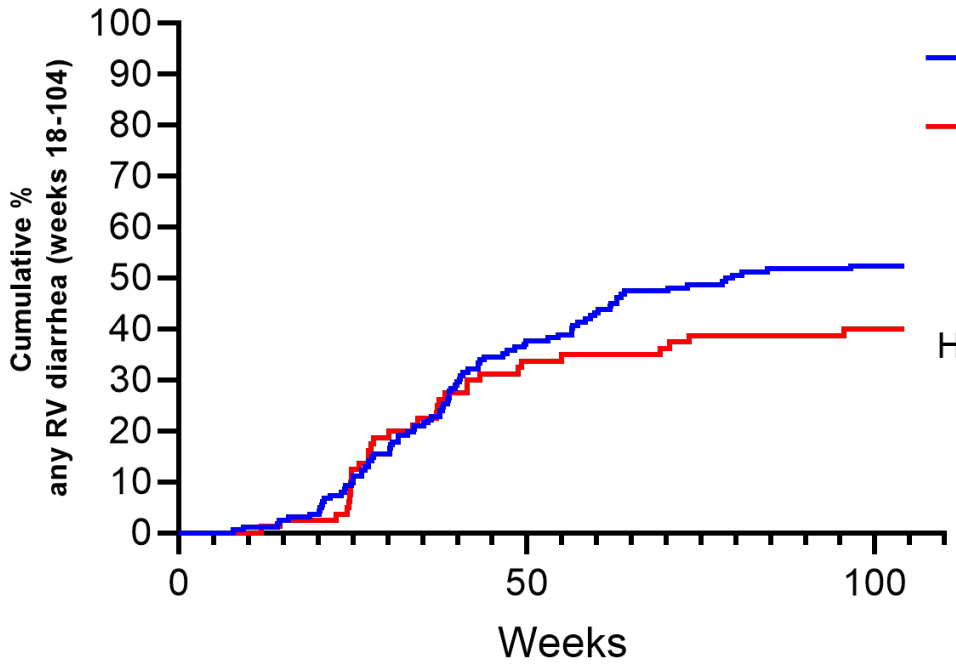
- Rotarix efficacy trial in Dhaka, Bangladesh 2011-14[1]
  - Vaccine vs no vaccine (1:1 randomization) at weeks 10 and 17
  - Active community disease surveillance, birth-2 years
- Outcome measures
  - RV diarrhea diagnosed by stool EIA
  - Infant secretor phenotype determined by saliva dot-blot assay, EIA
  - Seropositivity = total serum RV-specific IgA >20 U/mL
  - Seroconversion = seronegative pre-vaccination converting to seropositive post-vaccination (week 18)
  - Secretor phenotyping on stored breast milk (collected ~week 6) inferred by Lewis antigen EIA[2]
    - Note: unable to accurately perform UEA-I EIA for secretor positivity on breast milk samples, therefore all specimens limited to Lewis-positive mothers

1. Colgate et al 2016. PMID 27217217
2. Reeck et al 2010. PMID 20815703

Methods

- Stratified analyses by vaccination status
  - Unadjusted Kaplan-Meier survival curves
  - Multivariable logistic regression to evaluate maternal non-secretor phenotype as predictor of RV diarrhea through years 1 and 2
    - Controlled for covariates previously implicated in RV risk in this cohort
    - Controlled effect vs total effect model to specifically test RV-IgA contribution
- Vaccine efficacy (VE) = $(risk_{unvaccinated} - risk_{vaccinated}) / risk_{unvaccinated}$

Results: Unvaccinated infants (N=227)



Maternal secretor N=156 (68.7%)

Maternal nonsecretor N= 71 (31.3%)

HR 0.730 (95% CI 0.498-1.070)

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Results: Unvaccinated infants

	Effect of maternal non-secretor phenotype		
Model including RV-IgA seroconversion	**OR**	**(95% CI)**	**P-value**
Through year 1	1.332	(0.699-2.540)	0.384
Through year 2	0.858	(0.465-1.583)	0.625

Controlling for: Infant secretor status; Household water treatment; Weeks of exclusive breastfeeding; Serum zinc (week 18); Week 10 length-for-age Z score

Results: Unvaccinated infants

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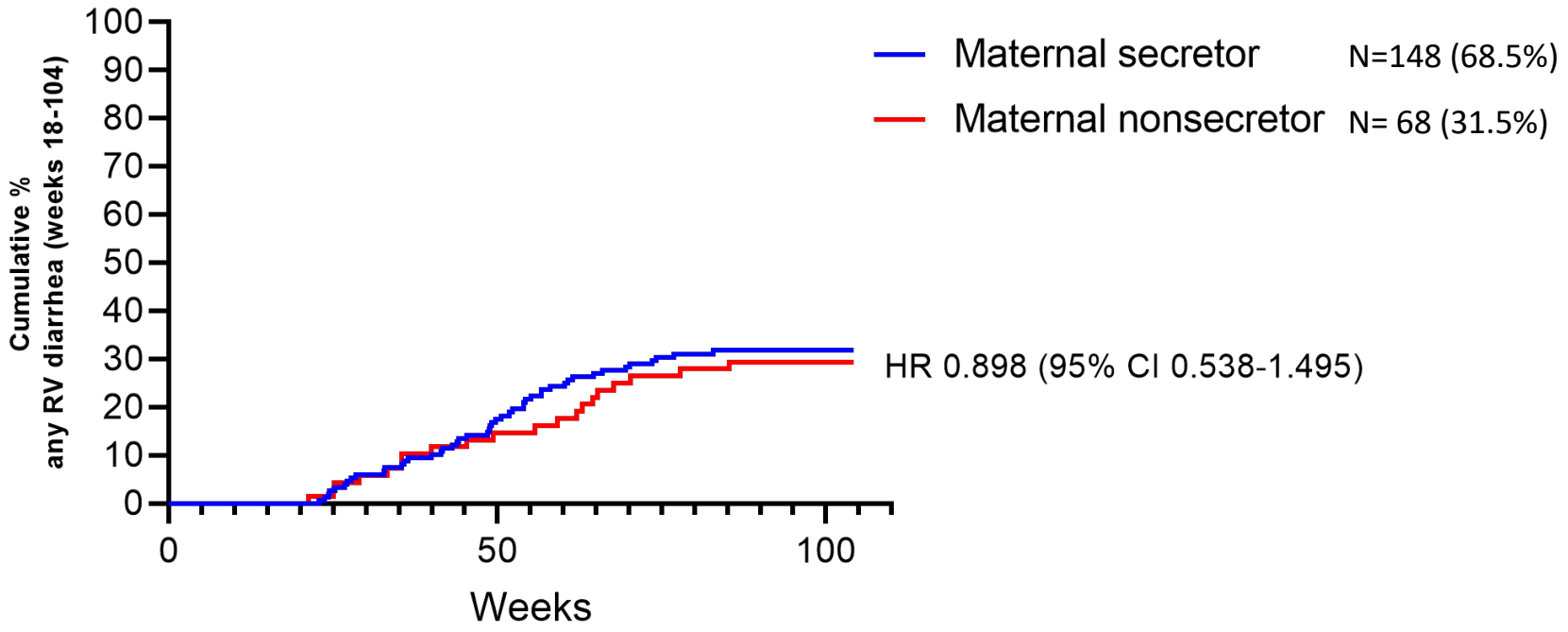
Through year 2	0.858	(0.465-1.583)	0.625

Model excluding RV-IgA seroconversion	**OR**	**(95% CI)**	**P-value**
Through year 1	1.337	(0.702-2.546)	0.378
Through year 2	0.865	(0.470-1.591)	0.640

Controlling for: Infant secretor status; Household water treatment; Weeks of exclusive breastfeeding; Serum zinc (week 18); Week 10 length-for-age Z score

Results: Vaccinated infants (N=216)



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Results: Vaccinated infants

	Effect of maternal non-secretor phenotype		
Model including RV-IgA seroconversion	**OR**	**(95% CI)**	**P-value**
Through year 1	0.943	(0.376-2.367)	0.901
Through year 2	1.406	(0.658-3.005)	0.380

Controlling for: Infant secretor status; Household water treatment; Weeks of exclusive breastfeeding; Serum zinc (week 18); Week 10 length-for-age Z score

Results: Vaccinated infants

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Through year 1	0.943	(0.376-2.367)	0.901
Through year 2	1.406	(0.658-3.005)	0.380

Model excluding RV-IgA seroconversion	**OR**	**(95% CI)**	**P-value**
Through year 1	0.818	(0.338-1.979)	0.656
Through year 2	1.115	(0.547-2.271)	0.765

Controlling for: Infant secretor status; Household water treatment; Weeks of exclusive breastfeeding; Serum zinc (week 18); Week 10 length-for-age Z score

Results: Vaccine efficacy against any rotavirus diarrhea

	VE through year 1 (95% CI)	VE through year 2 (95% CI)
Maternal secretors	44.8 (25.4-69.6)	37.6 (28.2-78.9)
Maternal non-secretors	53.0 (15.1-83.3)	25.2 (-28.3-67.5)

Limitations

- Sampling bias (retrospective sub-study based on specimen availability)
  - Year 2 efficacy slightly higher (48.2%) in parent cohort
  - Sample size limited, particularly for non-secretors
- Unable to examine effects in Lewis-negative mothers
- Unable to assess for contribution of maternal RV-IgG due to low numbers with maternal antibody data

Rogawski et al. 2018. PMID 29514306.

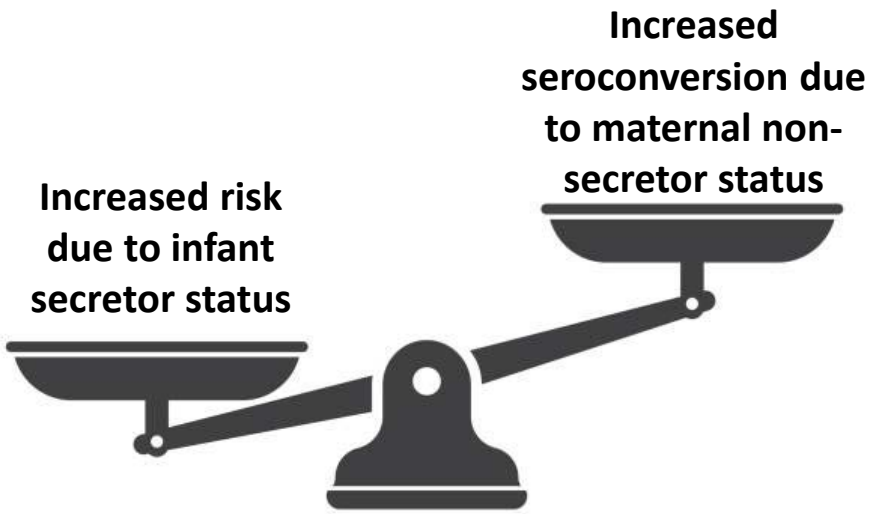
Conclusions

- Maternal breast milk non-secretor phenotype was not associated with increased protection from RV diarrhea
  - No evidence of mediation via RV-IgA, despite evidence of increased RV-IgA seroconversion in vaccinated infants of non-secretor mothers
- Findings are consistent with prior data from MAL-ED suggesting lack of impact of maternal status on rotavirus diarrhea risk
- These findings highlight the limitations of immunogenicity measurement alone in assessing vaccine response

Colston et al. 2019. PMID 30768135.

Potential explanations

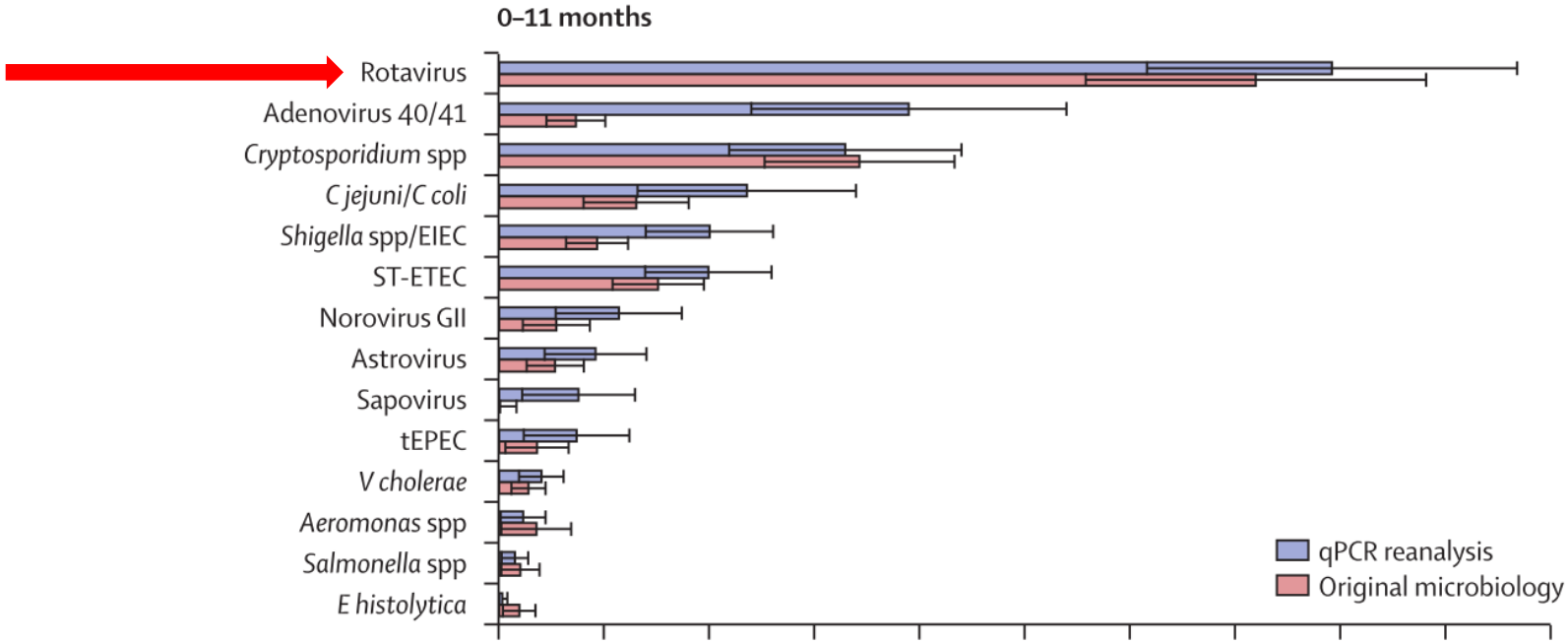
- The effect of infant status on diarrhea risk outweighs maternal
- Low population-level effect due to minority of individuals (including mothers) having non-secretor status
- RV-IgA is a non-mechanistic correlate of protection



Thank you very much

Extra slides

Globally, rotavirus (RV) is the #1 cause of diarrhea in infants



Liu et al 2016. PMID 27673470

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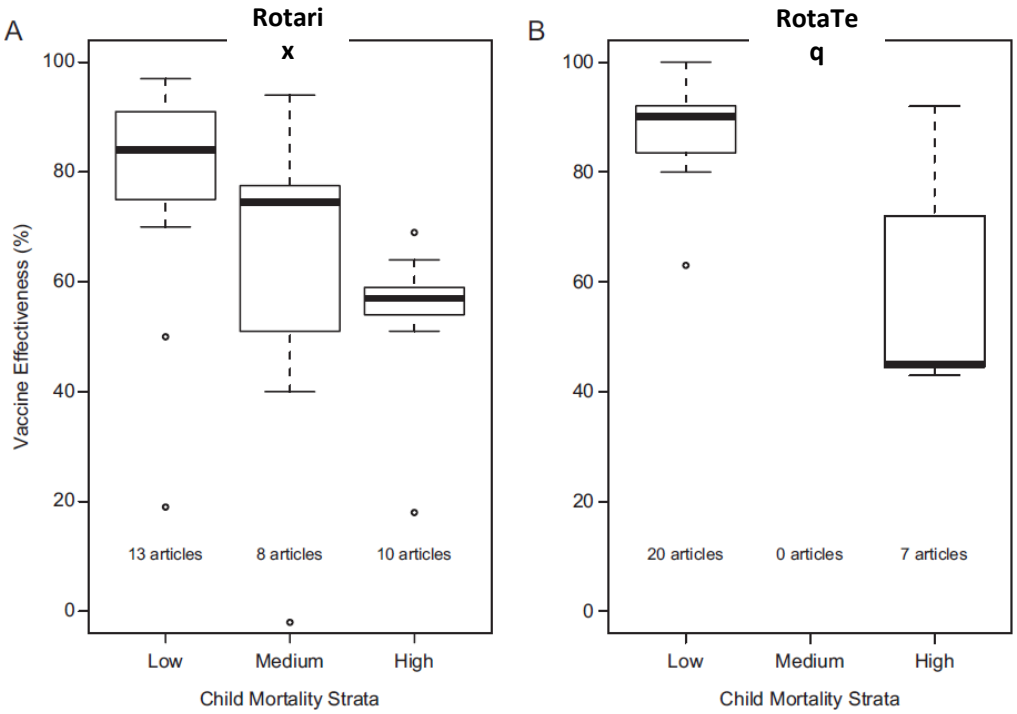
Oral RV vaccines underperform in high child-mortality settings

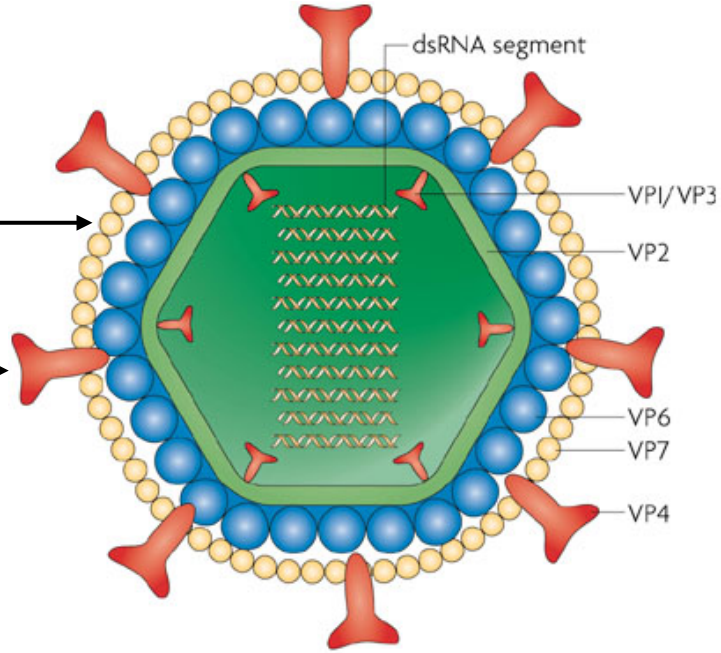


Jonesteller et al 2017. PMID28444323

RV: non-enveloped dsRNA virus

VP7: **G**lycoprotein shell
Defines **G** type

VP4: **P**rotease-sensitive spike
Defines **P** type



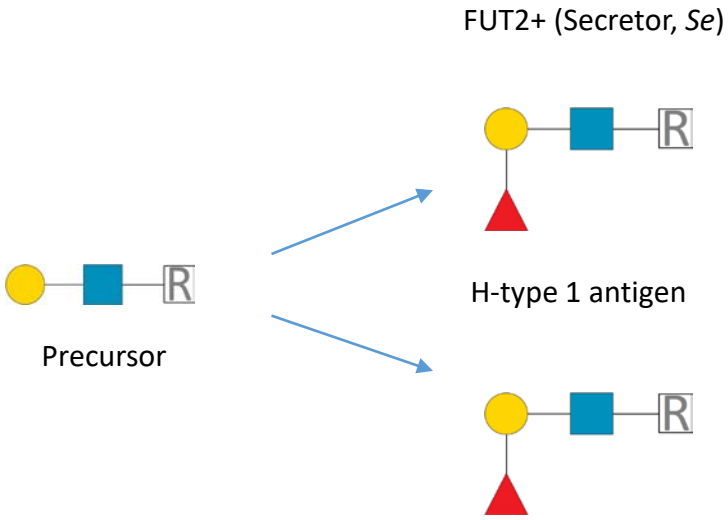
Nature Reviews | Microbiology

Angel et al 2007. PMID 17571094

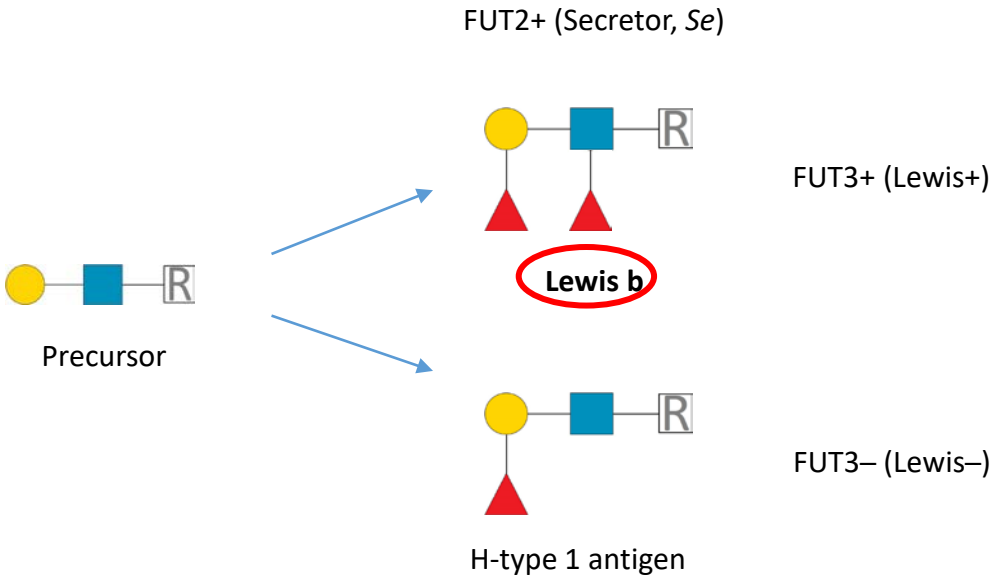
Overview of Lewis and secretor systems

Precursor

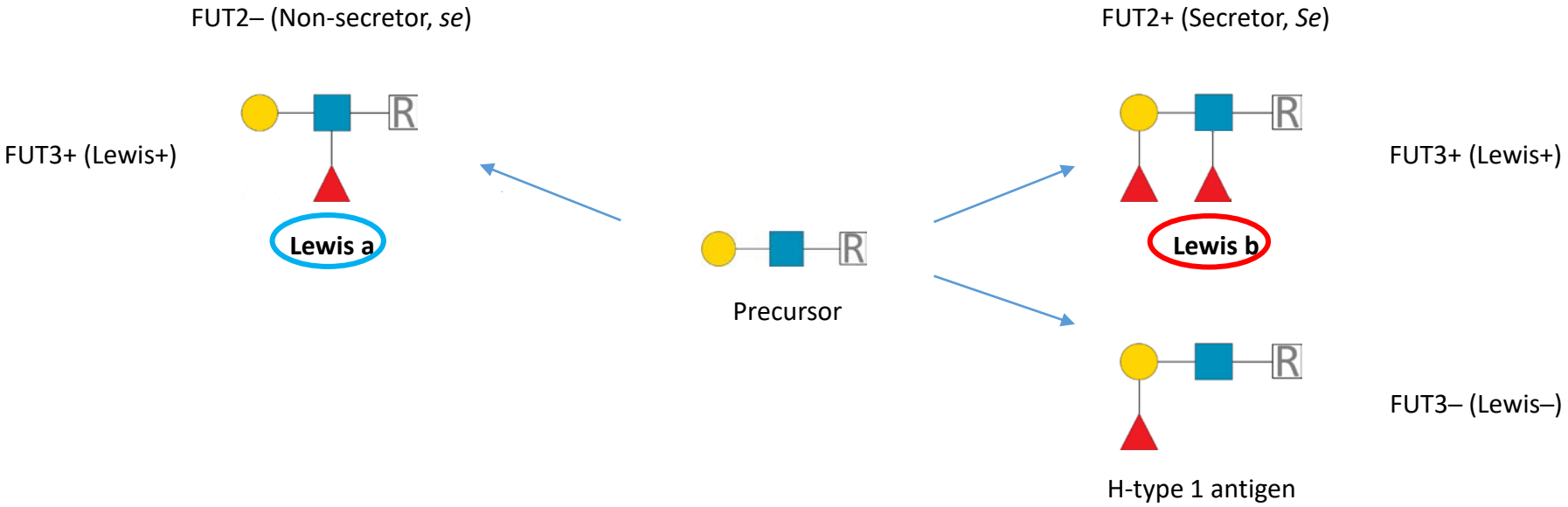
Overview of Lewis and secretor systems



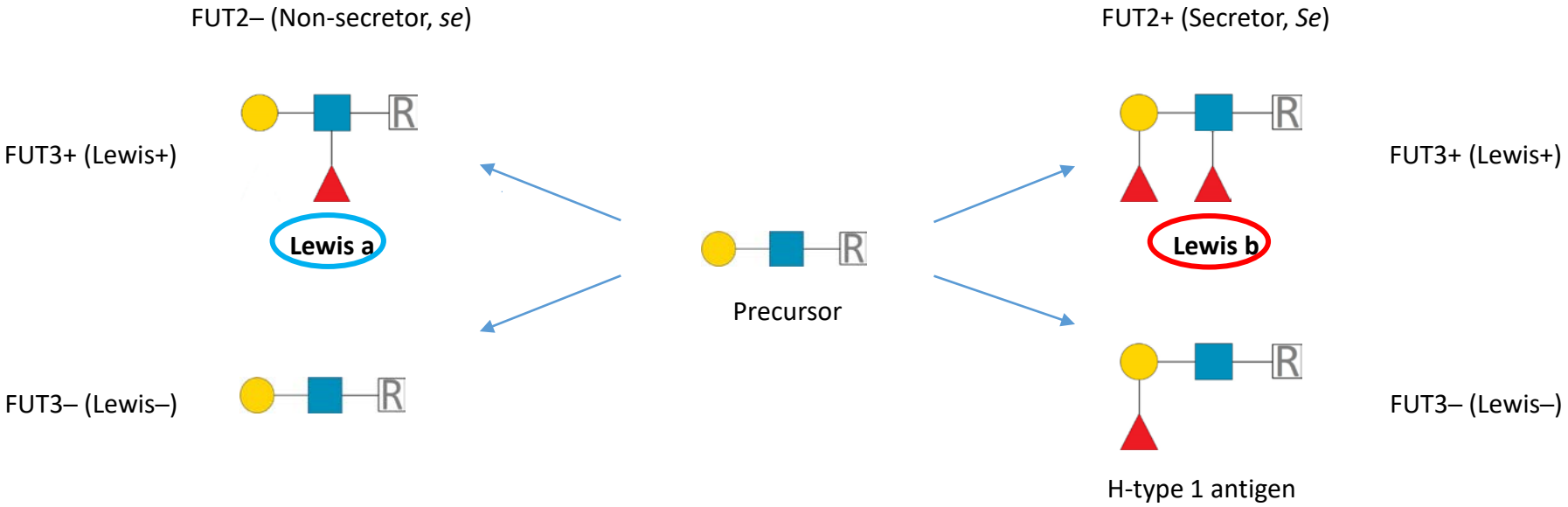
Overview of Lewis and secretor systems



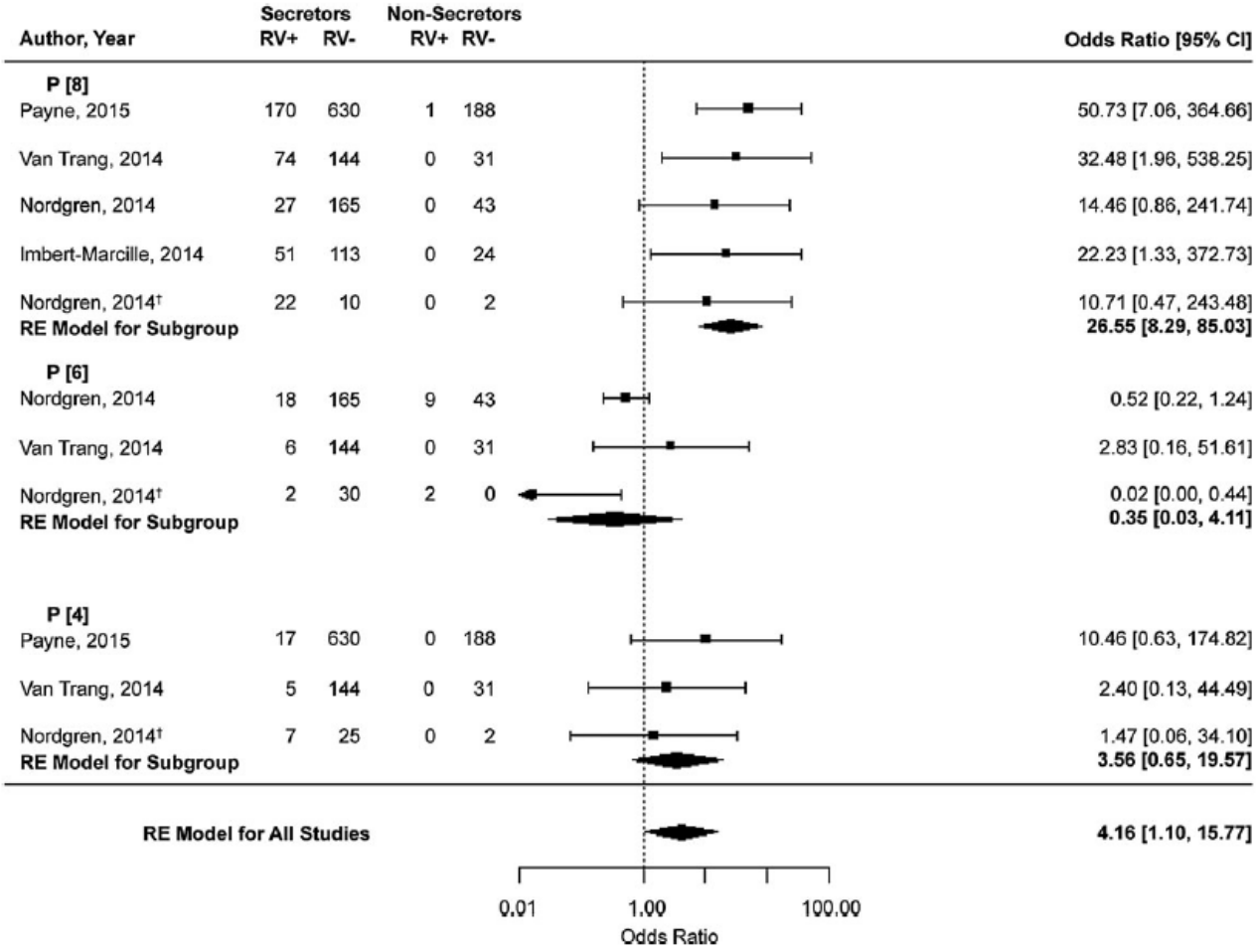
Overview of Lewis and secretor systems



Overview of Lewis and secretor systems



Secretor status affects susceptibility to rotavirus diarrhea in a P-genotype dependent manner



Kambhampati et al. 2016. PMID 26508510

Results

- 486 maternal-infant dyads identified with breast milk data (242 unvaccinated, 244 vaccinated)
  - 227 unvaccinated infants with complete data
    - 156 (68.7%) maternal secretor
    - 71 (31.3%) maternal non-secretor
  - 216 vaccinated infants with complete data
    - 148 (68.5%) maternal secretor
    - 68 (31.5%) maternal non-secretor

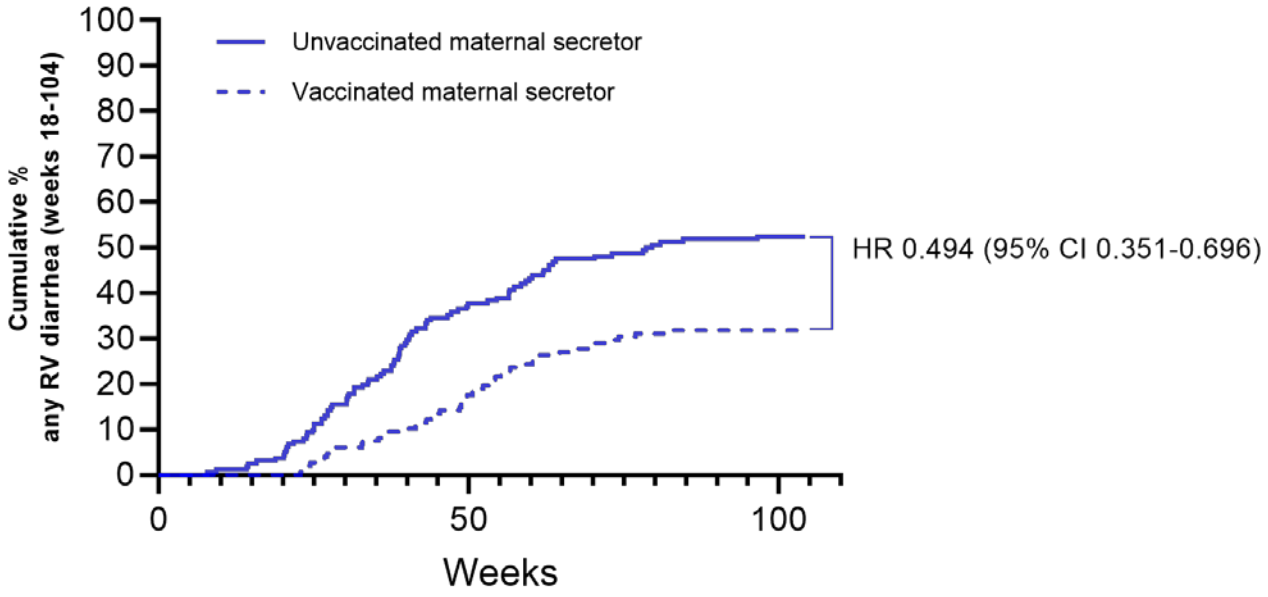
Results: Unvaccinated infants

	Variable	OR	(95% CI)	P-value
Through year 1	Infant non-secretor phenotype	0.335	(0.173-0.648)	0.001
	Lack of RV-IgA seroconversion	1.302	(0.611-2.773)	0.494
	Lack of water treatment	2.008	(1.104-3.651)	0.022
	HAZ at week 10	0.913	(0.668-1.248)	0.568
	Serum zinc level at week 18	1.018	(0.996-1.041)	0.104
	Weeks of exclusive breastfeeding	1.045	(1.009-1.083)	0.013
	Maternal non-secretor phenotype	1.332	(0.699-2.540)	0.384
Through year 2	**Infant non-secretor phenotype**	**0.406**	**(0.223-0.739)**	**0.003**
	Lack of RV-IgA seroconversion	1.567	(0.764-3.213)	0.220
	Lack of water treatment	1.503	(0.842-2.682)	0.168
	HAZ at week 10	0.978	(0.726-1.318)	0.885
	Serum zinc level at week 18	1.019	(0.998-1.040)	0.075
	Weeks of exclusive breastfeeding	**1.045**	**(1.011-1.082)**	**0.01**
	Maternal non-secretor phenotype	0.858	(0.465-1.583)	0.625

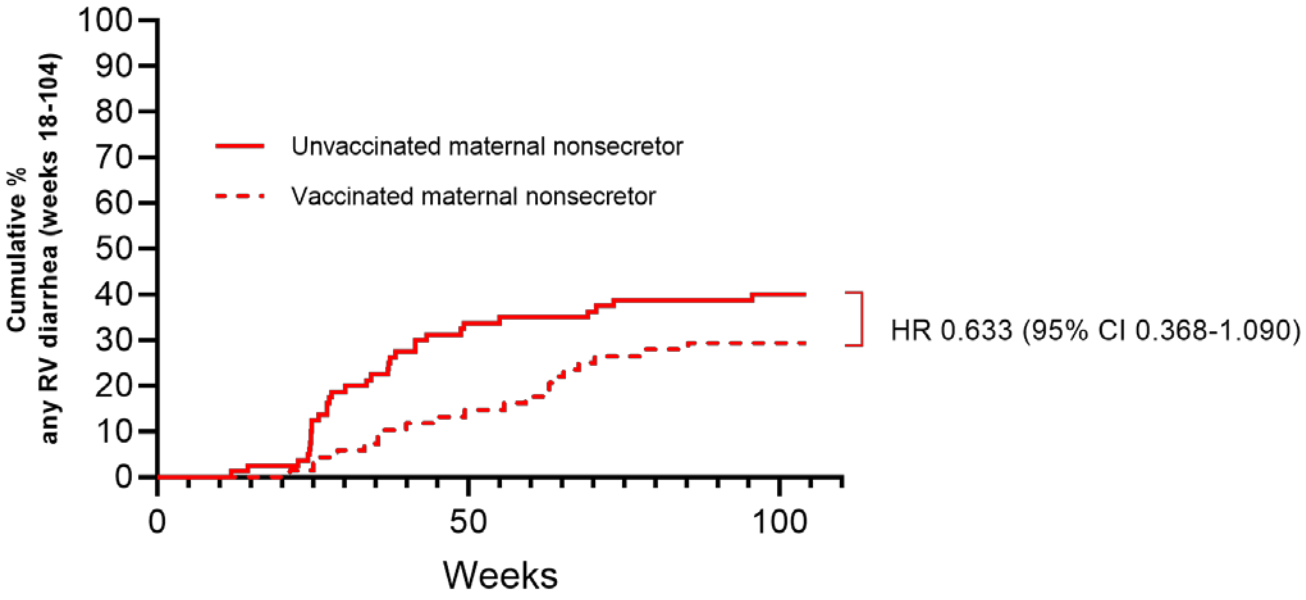
Results: Vaccinated infants

	Variable	OR	(95% CI)	P-value
Through year 1	Infant non-secretor phenotype	0.571	(0.223-1.460)	0.242
	Lack of RV-IgA seroconversion	2.73	(0.983-7.581)	0.054
	Lack of water treatment	1.06	(0.491-2.287)	0.883
	HAZ at week 10	1.058	(0.679-1.648)	0.804
	Serum zinc level at week 18	1.044	(1.012-1.078)	0.007
	Weeks of exclusive breastfeeding	0.999	(0.958-1.042)	0.966
	Maternal non-secretor phenotype	0.943	(0.376-2.367)	0.901
Through year 2	**Infant non-secretor phenotype**	**0.439**	**0.201-0.962**	**0.04**
	Lack of RV-IgA seroconversion	**3.366**	**1.484-7.634**	**0.004**
	Lack of water treatment	0.917	0.477-1.765	0.795
	HAZ at week 10	1.113	0.772-1.603	0.567
	Serum zinc level at week 18	**1.036**	**1.011-1.062**	**0.005**
	Weeks of exclusive breastfeeding	0.984	0.949-1.019	0.36
	Maternal non-secretor phenotype	1.406	0.658-3.005	0.38

Results: Vaccine effects



Results: Vaccine effects



Results: Vaccine effects