



UNIVERSITY OF
OXFORD



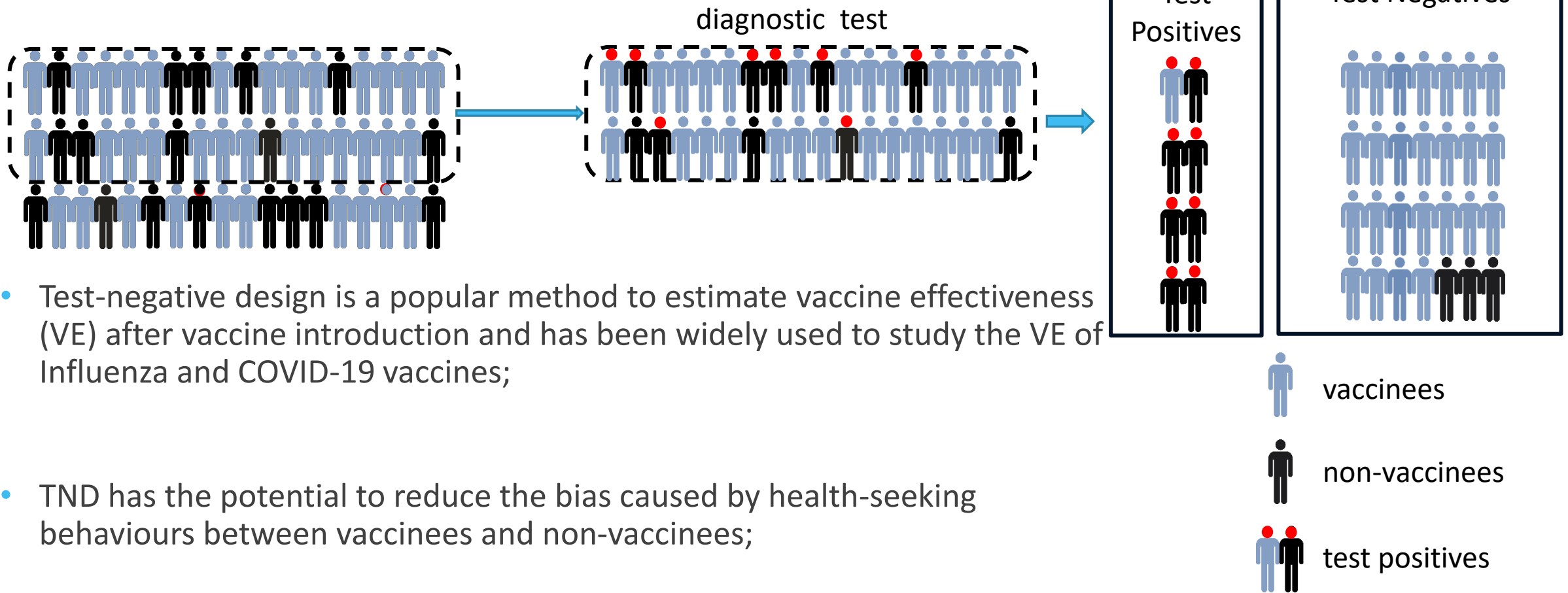
DEPARTMENT OF
PAEDIATRICS

The validity of Test-Negative Design

Assoc Prof Xinxue Liu



OXFORD
VACCINE GROUP



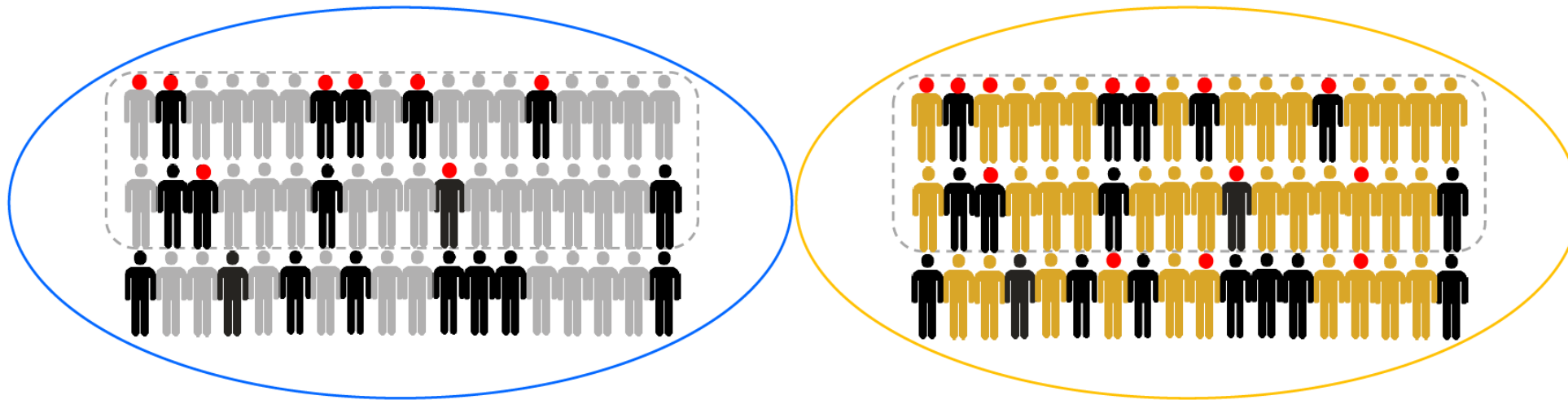
- Test-negative design is a popular method to estimate vaccine effectiveness (VE) after vaccine introduction and has been widely used to study the VE of Influenza and COVID-19 vaccines;
- TND has the potential to reduce the bias caused by health-seeking behaviours between vaccinees and non-vaccinees;

TND simulation study using IRCT and CRCT

- The validity of TND design has been tested several individual randomised trials, where participants in the control were considered as non-vaccinees in the community;
- However, the **health-care seeking behaviours is expected to be similar between intervention arm and control arm** due to randomisation

	Individual RCT	Cluster RCT
Vaccinee distribution	Vaccinees in both arms live in the same area	Vaccinees in two arms live in separate clusters
Study follow-up	Vaccinees only	Both vaccinees and non-vaccinees
Vaccination campaign	One campaign at the start of the study	Regular catch-up vaccination campaigns
Surveillance coverage	Vaccinees only	All residents

Methods – Vaccine protections



Analysis methods








Analysis population

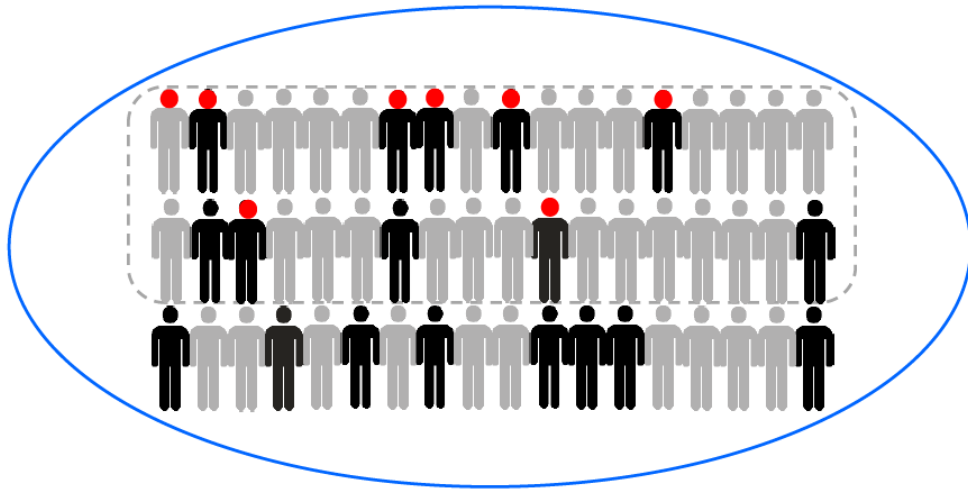
Comparisons

CRCT



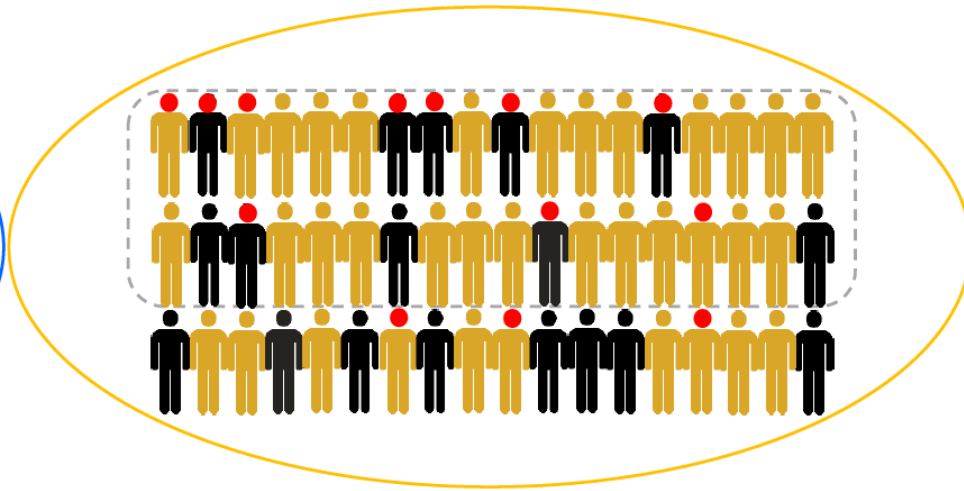
Vi-TT vaccinees vs. JE vaccinees in 150 clusters

-  Vi-TT vaccinee
-  JE vaccinee
-  non-vaccinee
-  Vi-TT cluster
-  JE cluster
-  seeking healthcare
-  Typhoid cases



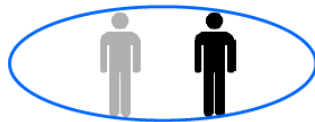
Analysis methods

Analysis population





Comparisons


Target Trial Emulation




Vi-TT vaccinees vs. non-vaccinees in 75 clusters randomised to Vi-TT


 Vi-TT vaccinee


 JE vaccinee

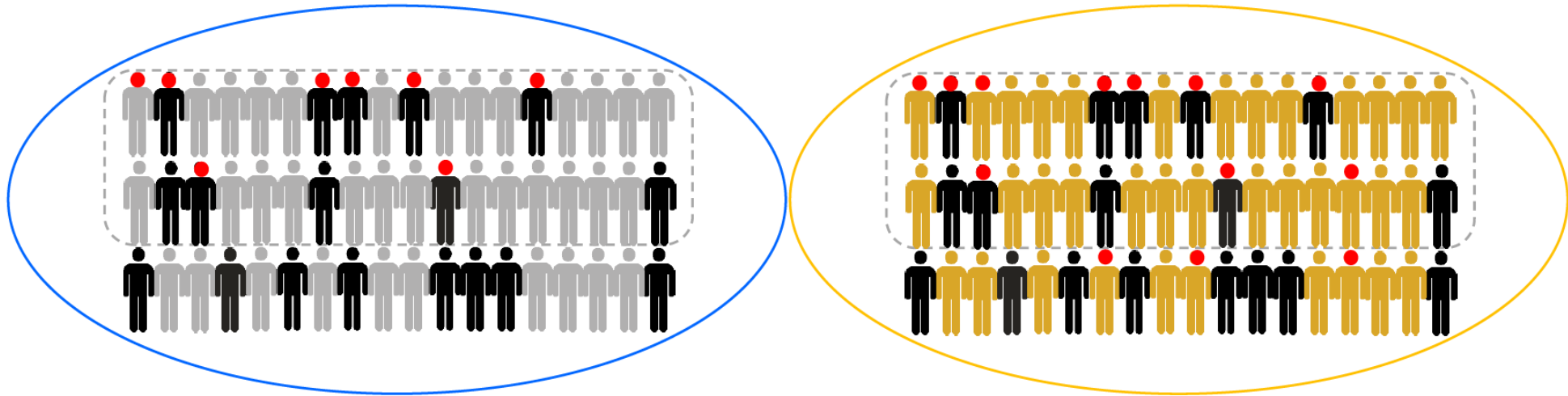
 non-vaccinee

 Vi-TT cluster

 JE cluster

 seeking healthcare

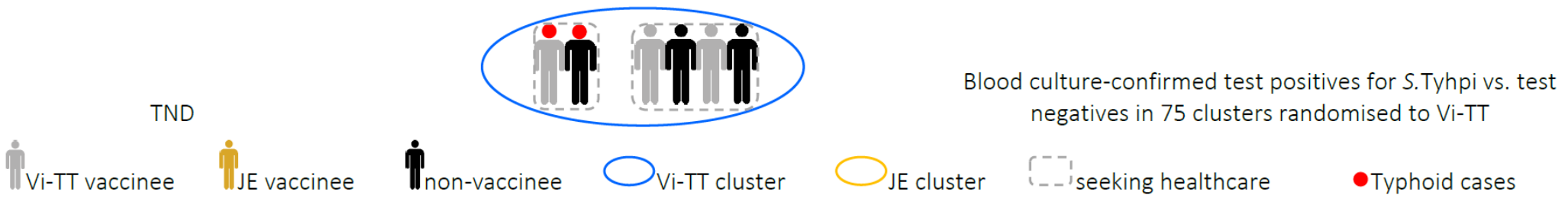
 Typhoid cases



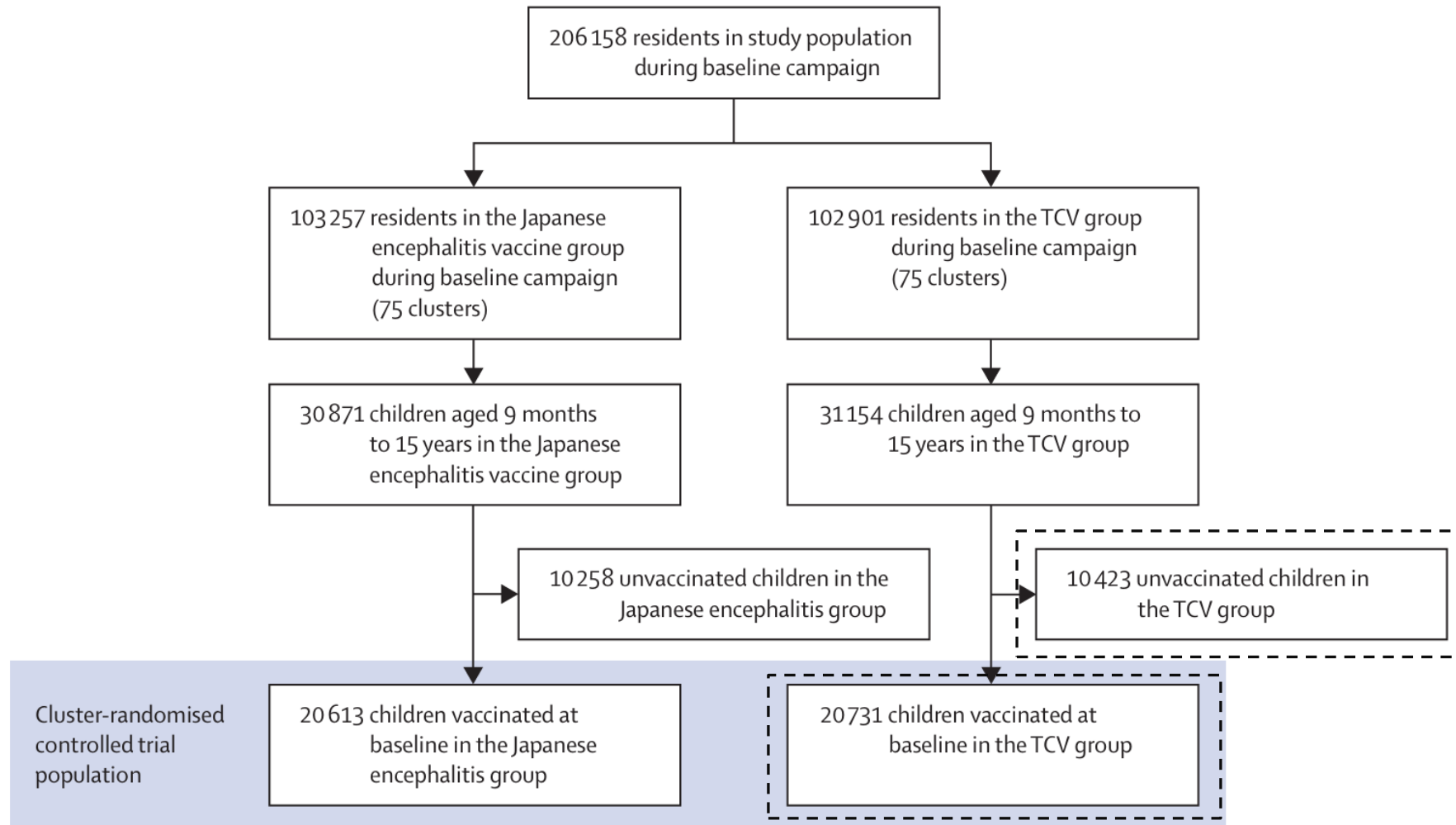
Analysis methods

Analysis population

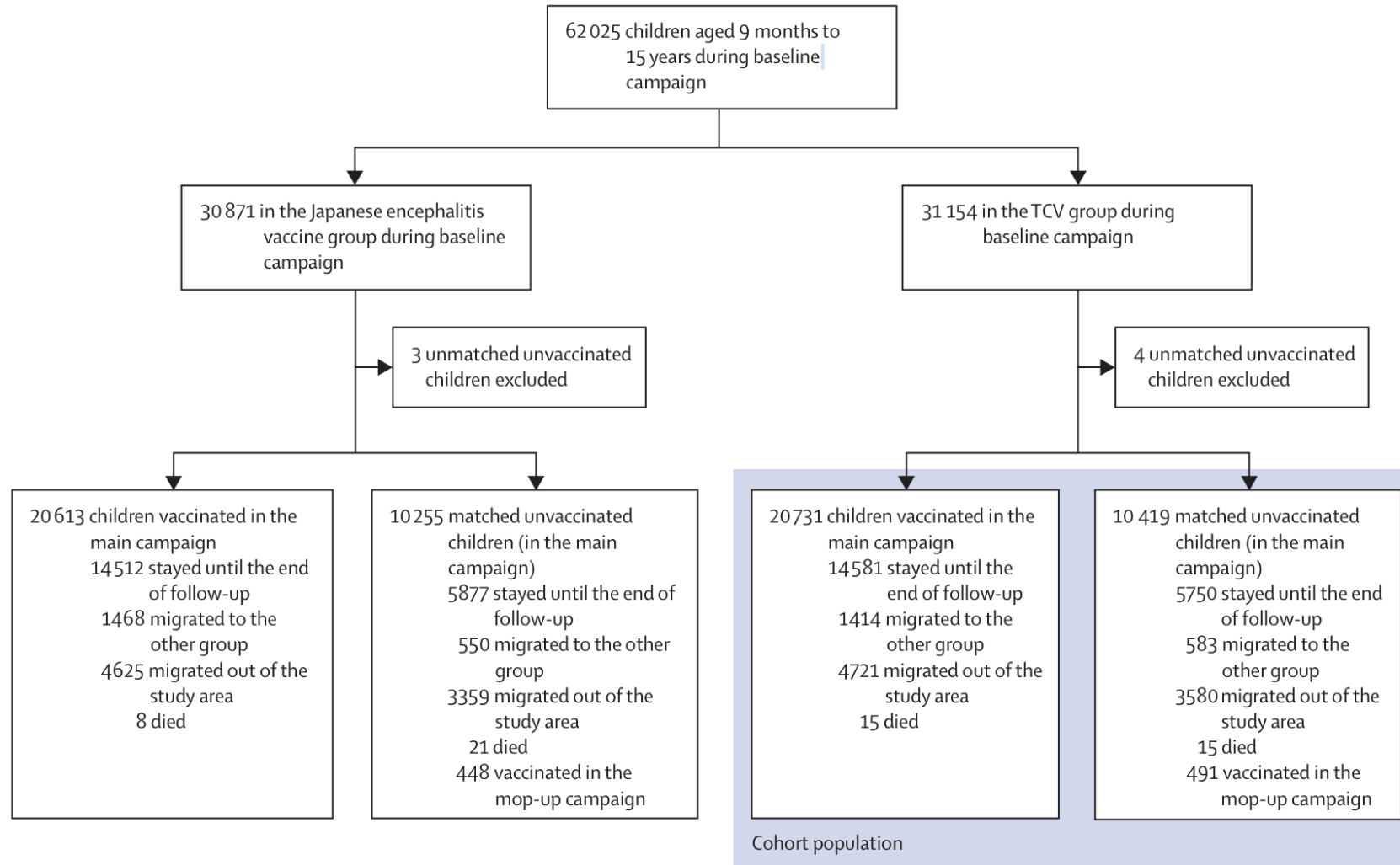
Comparisons



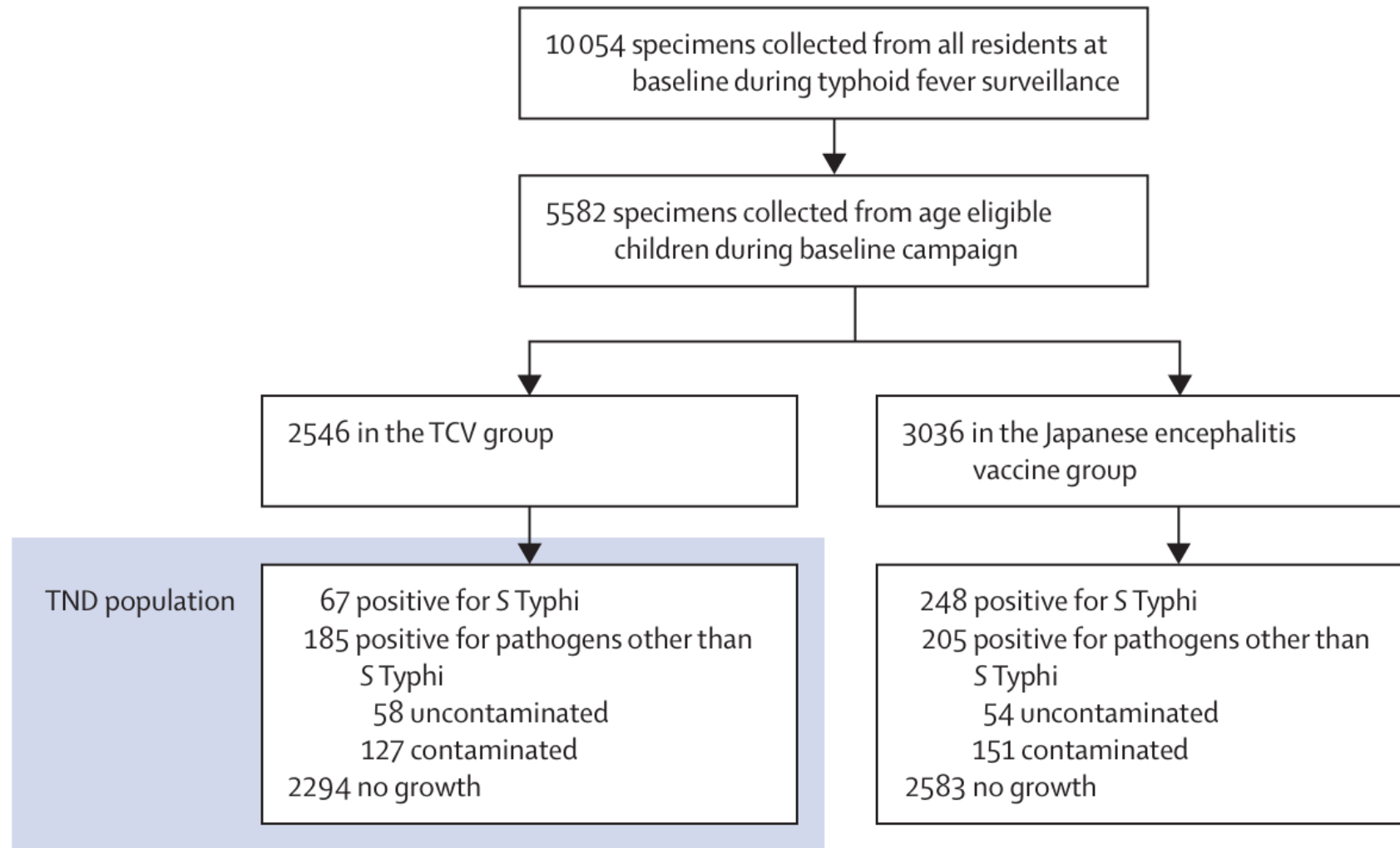
Consort Diagram- CRCT



Consort Diagram- Target Trial Emulation

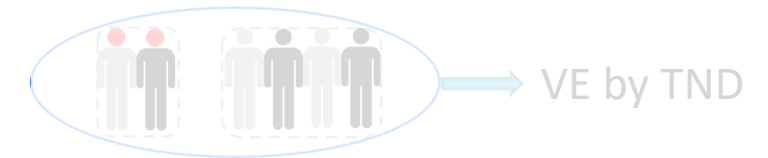
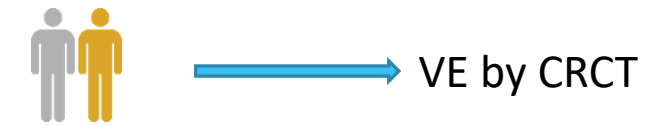


Consort Diagram- Test Negative Design

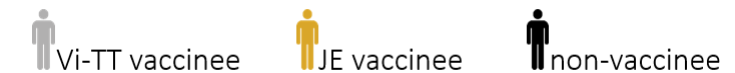


Vaccine protection by CRCT

CRCT (150 clusters)	JE-recipients	TCV-recipients	Adjusted IRR	VE (%)
	N=20 613	N=20 731		
Blood-culture confirmed typhoid fever (no.)/ Person-Years of follow-up	202/32 518	22/32 687	0.11 (0.06,0.19)	89%(81,93)
Incidence rate (per 100,000 PYs) (95% CI)	621(538,713)	67 (42,102)		



Adjusted for the cluster as a random effect, and randomisation stratification variables at the cluster level (geographical ward, distance to study clinics, number of eligible children at baseline), demographic factors (age and sex) and household hygiene factors as fixed effects

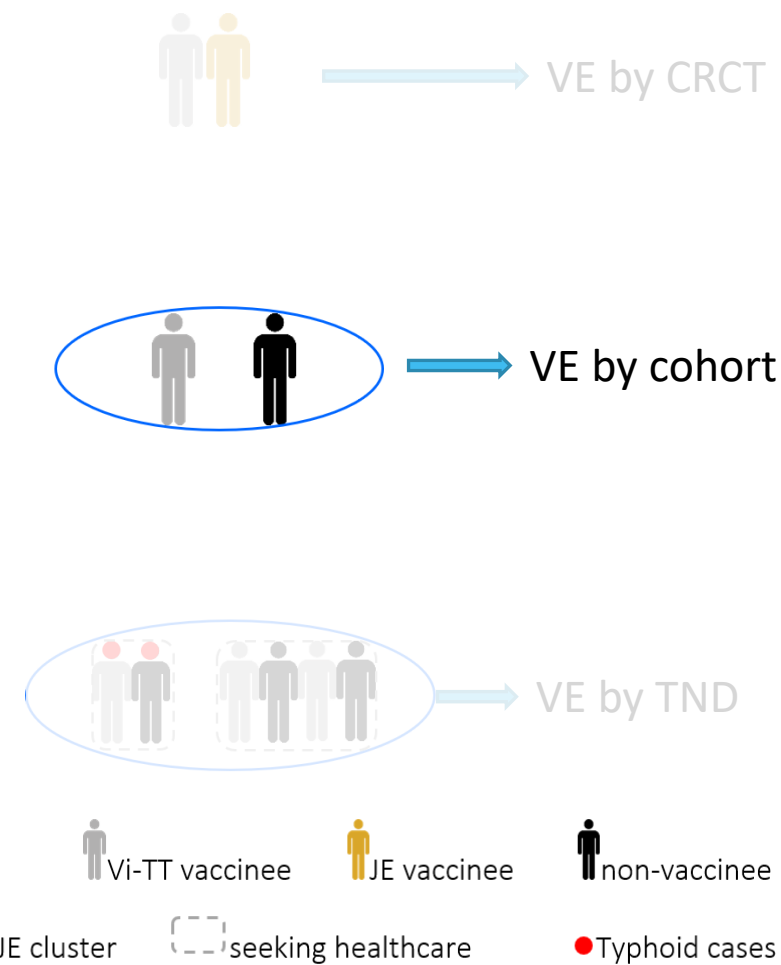


Vaccine protection by Cohort

CRCT (150 clusters)	JE-recipients	TCV-recipients	Adjusted IRR	Adjusted VE
	N=20 613	N=20 731		
Incidence rate (per 100,000 PYs) (95% CI)	621(538,713)	67 (42,102)	0.11 (0.06,0.19)	89%(81,93)

Cohort (75 TCV clusters)	Non-vaccinees	TCV-recipients	Adjusted IRR	Adjusted VE
	N=10 419	N=20 731		
Blood-culture confirmed typhoid fever (no.)/ Person-Years of follow-up	42/13 870	22/32 687	0.22 (0.15,0.31)	79% (70,86)
Incidence rate (per 100,000 PYs) (95% CI)	303 (218,409)	67 (42,102)		

Adjusted for the cluster as a random effect, and randomisation stratification variables at the cluster level (geographical ward, distance to study clinics, number of eligible children at baseline), demographic factors (age and sex) and household hygiene factors as fixed effects

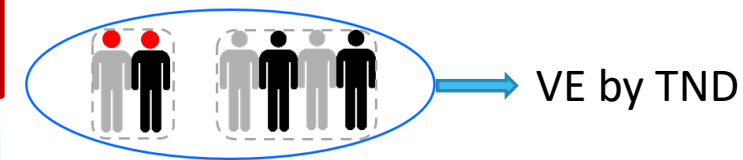


Vaccine protection by TND

CRCT (150 clusters)	<u>JE-recipients</u>	<u>TCV-recipients</u>	<u>Adjusted IRR</u>	<u>Adjusted VE</u>
	N=20 613	N=20 731		
Incidence rate (per 100,000 PYs) (95% CI)	621(538,713)	67 (42,102)	0.11 (0.06,0.19)	89%(81,93)



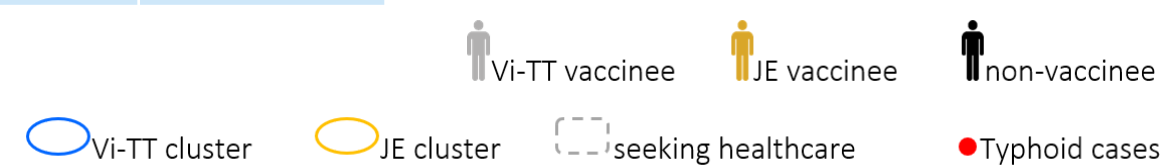
Test negative design (75 TCV clusters)#	Test negatives#	Test positives	Adjusted OR	<u>Adjusted VE</u>
Non-vaccinees	10 (17%)	45 (67%)	0.10 (0.04,0.25)	90% (75,96)
TCV-recipients	48 (83%)	22 (33%)		
	Test negatives*	Test positives		
Non-vaccinees	456 (20%)	45 (67%)	0.12 (0.07,0.21)	88% (79,93)
TCV-recipients	1838 (80%)	22 (33%)		



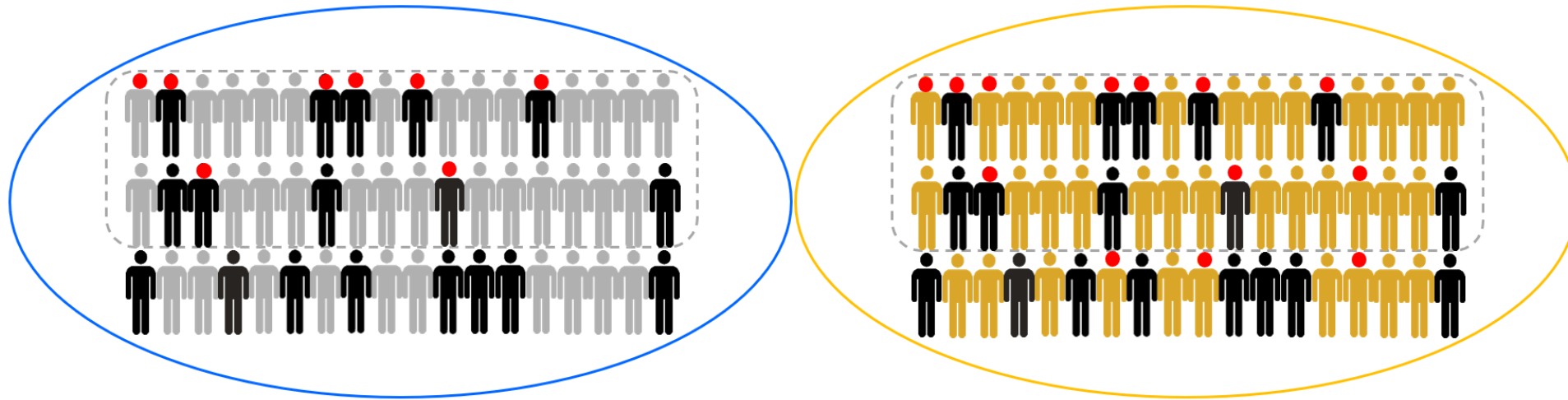
Defined as specimens that were positive for pathogens other than *S.Typhi*;

* Defined as specimens with no growth;

Adjusted for the same sets of covariates.



Methods-Negative Control Exposure (Bias indicator)

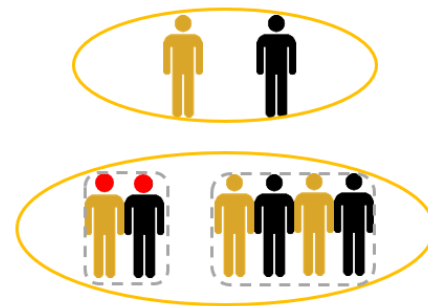


Analysis methods

Analysis population

Comparisons

Target Trial Emulation

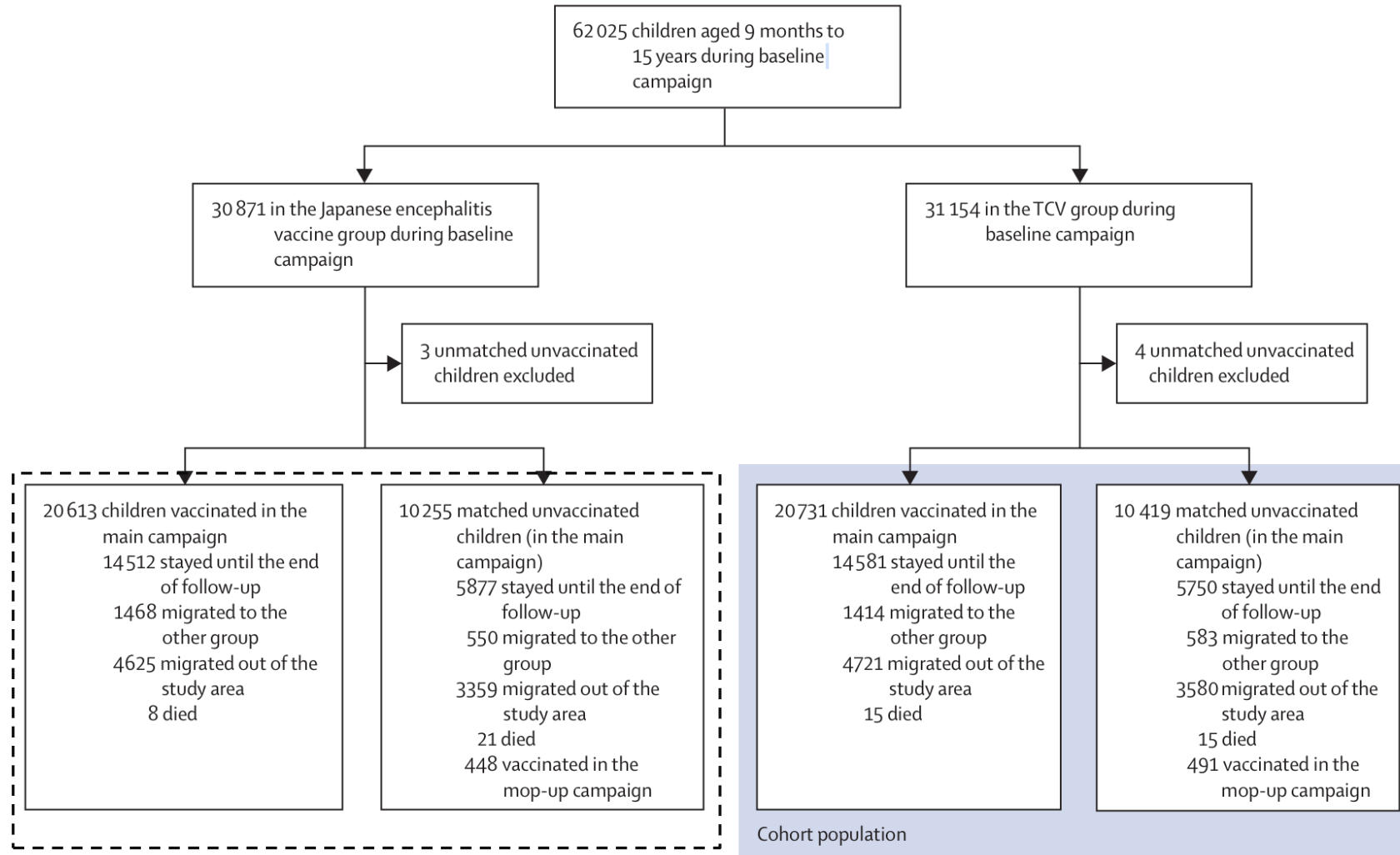


JE vaccinees vs. non-vaccinees in 75 clusters randomised to JE

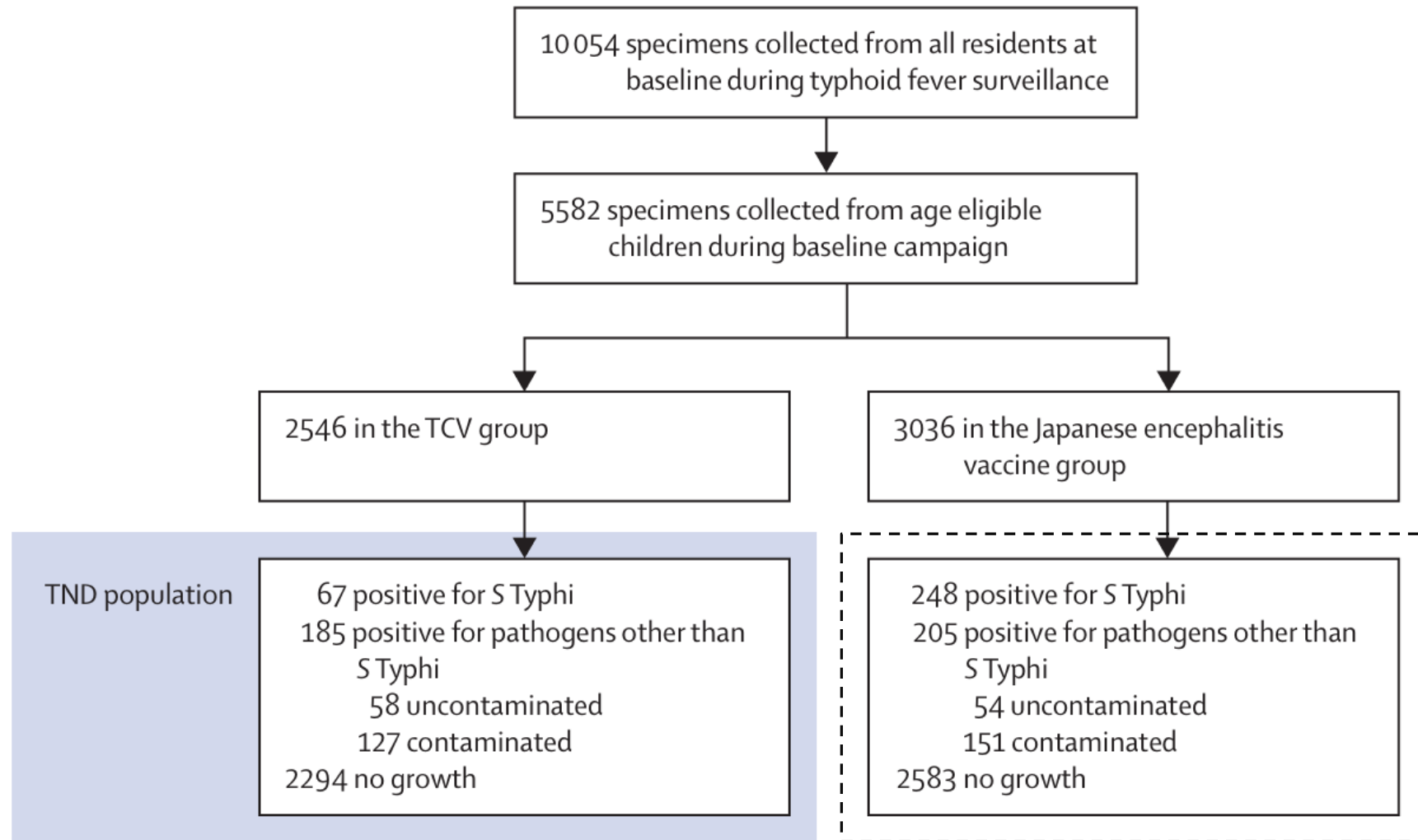
Blood culture-confirmed test positives for *S.Typhi* vs. test negatives in 75 clusters randomised to JE

 TCV vaccinee
  JE vaccinee
  non-vaccinee
  TCV cluster
  JE cluster
  seeking healthcare
  Typhoid cases

Consort Diagram- Target Trial Emulation

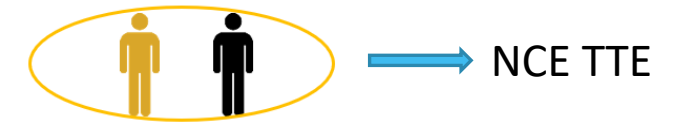


Consort Diagram- Test Negative Design

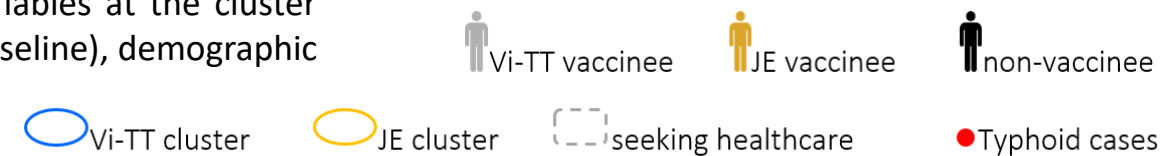


Negative Control Exposure Analysis (Association between typhoid fever and JE)

Cohort (75 JE clusters)	Non-vaccinees	JE-recipients	Adjusted IRR
	N=10 255	N=20 613	
Blood-culture confirmed typhoid fever (no.)/ Person-Years of follow-up	42/13 955	202/32 518	1.98 (1.56,2.52)
Incidence rate (per 100,000 PYs) (95% CI)	301(217,407)	621(538,713)	

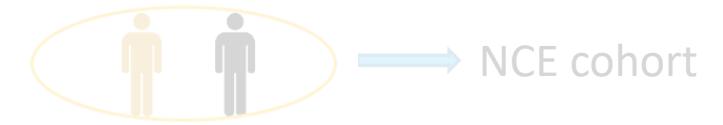


Adjusted for the cluster as a random effect, and randomisation stratification variables at the cluster level (geographical ward, distance to study clinics, number of eligible children at baseline), demographic factors (age and sex) and household hygiene factors as fixed effects



Negative Control Exposure Analysis (Association between typhoid and JE)

Cohort (75 JE clusters)	Non-vaccinees	JE-recipients	Adjusted IRR
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Blood-culture confirmed typhoid fever (no.)/ Person-Years of follow-up	42/13 955	202/32 518	1.98 (1.56,2.52)
Incidence rate (per 100,000 PYs) (95% CI)	301(217,407)	621(538,713)	



Test negative design (75 JE clusters)	Test negatives [#]	Test positives	Adjusted OR
Non-vaccinees	10 (19%)	46 (19%)	0.84 (0.37,1.92)
JE-recipients	44 (81%)	202 (81%)	
	Test negatives [*]	Test positives	
Non-vaccinees	483 (19%)	46 (19%)	1.04 (0.74,1.46)
JE-recipients	2100 (81%)	202 (81%)	



[#] Defined as specimens that were positive for pathogens other than *S.Typhi*;

^{*} Defined as specimens with no growth;




Adjusted for the same sets of covariates.

 Vi-TT cluster

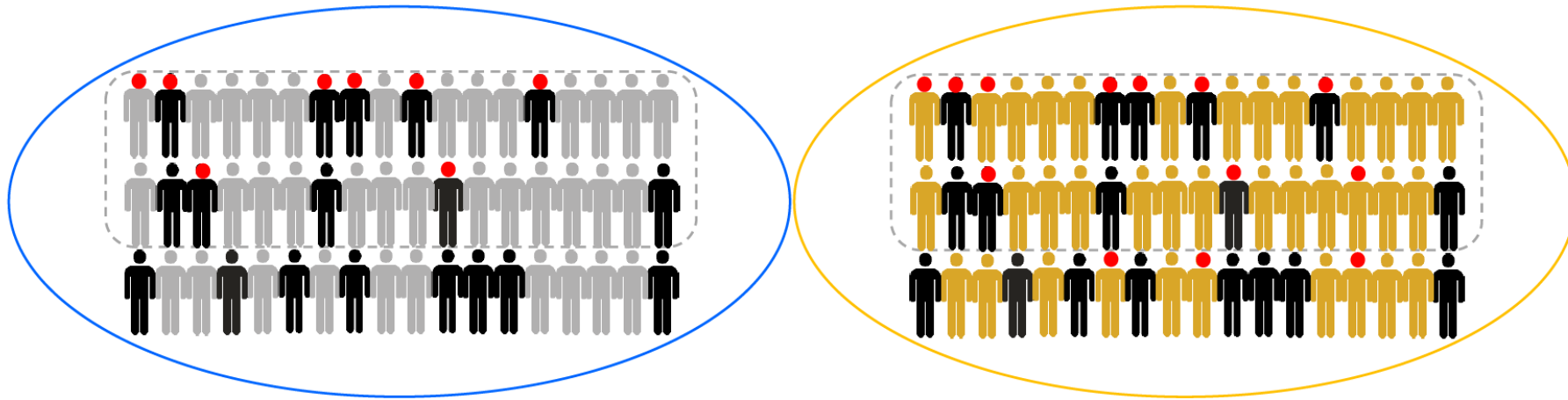
 JE cluster

 seeking healthcare

 Typhoid cases

 Vi-TT vaccinee  JE vaccinee  non-vaccinee

Methods – Negative Control Outcome (Bias indicator)



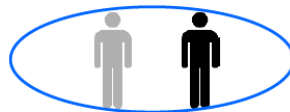
Analysis methods

Analysis population

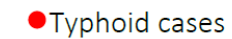
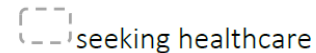
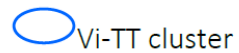
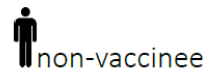
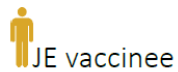
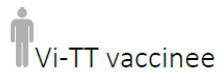
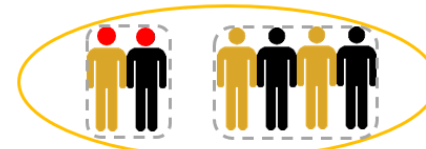
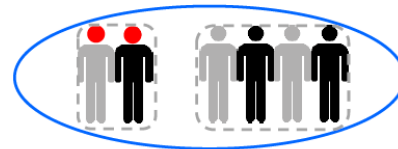
CRCT



Cohort design



TND



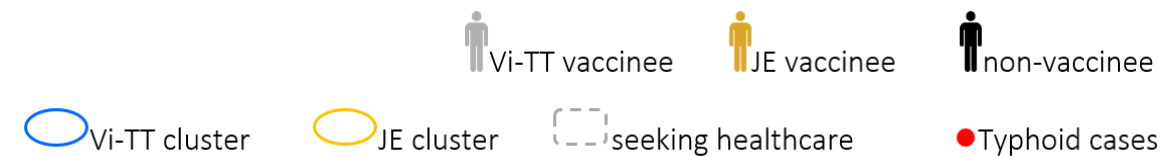
Blood culture-confirmed test positives other than *S. Tyhpi*

Negative Control Outcome Analysis (Association between non-typhoid fever and TCV/JE)

CRCT (150 clusters)	<u>JE-recipients</u>	<u>TCV-recipients</u>	<u>Adjusted IRR</u>
	N=20 613	N=20 731	
Blood-culture confirmed typhoid fever (no.)/ Person-Years of follow-up	45/32 518	47/32 687	1.08 (0.70,1.69)
Incidence rate (per 100,000 PYs) (95% CI)	138 (101,185)	144 (106,191)	



Adjusted for the cluster as a random effect, and randomisation stratification variables at the cluster level (geographical ward, distance to study clinics, number of eligible children at baseline), demographic factors (age and sex) and household hygiene factors as fixed effects

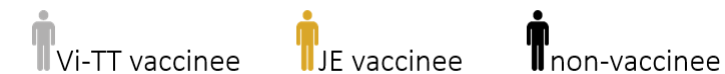
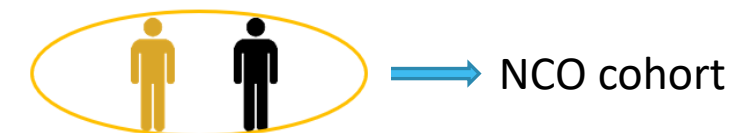
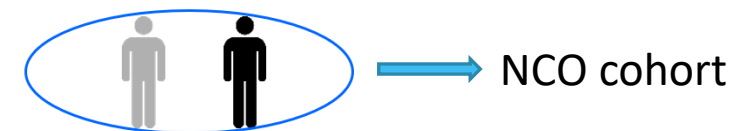


Negative Control Outcome Analysis (Association between non-typhoid and TCV/JE)

CRCT (150 clusters)	JE-recipients	TCV-recipients	Adjusted IRR
	N=20 613	N=20 731	
Incidence rate (per 100,000 PYs) (95% CI)	138 (101,185)	144 (106,191)	1.08 (0.70,1.69)

Cohort analysis (in TCV clusters)	Non-vaccinees	TCV-recipients	Adjusted IRR
	N=10 419	N=20 731	
Blood-culture confirmed typhoid fever (no.)/ Person-Years of follow-up	6/13 870	47/32 687	2.88 (1.58,5.26)
Incidence rate (per 100,000 PYs) (95% CI)	43 (16,94)	144 (106,191)	

Cohort analysis (in JE clusters)	Non-vaccinees	JE-recipients	Adjusted IRR
	N=13736	N=20 613	
Blood-culture confirmed typhoid fever (no.)/ Person-Years of follow-up	7/13 955	45/32 518	2.45 (1.38,4.35)
Incidence rate (per 100,000 PYs) (95% CI)	50 (20,103)	138 (101,185)	

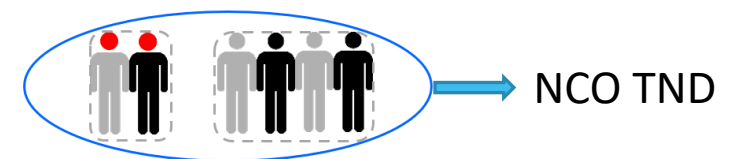


Negative Control Outcome Analysis (Association between non-typhoid and TCV/JE)

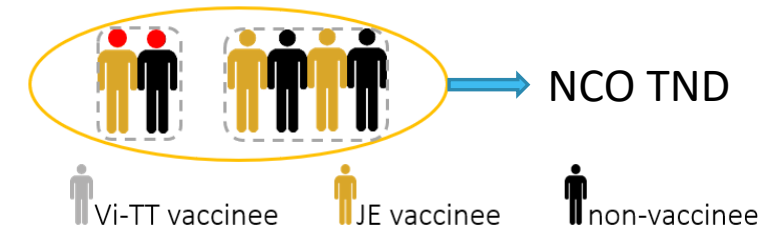
CRCT (150 clusters)	JE-recipients	TCV-recipients	Adjusted IRR
	N=33398	N=33822	
Incidence rate (per 100,000 PYs) (95% CI)	129(97,167)	144(111,184)	1.14 (0.73,1.78)



Test negative design (75 TCV clusters)	Test negatives	Test positives	Adjusted OR
Non-vaccinees	456 (20%)	10 (17%)	1.26 (0.63,2.53)
TCV-recipients	1838 (80%)	48 (83%)	



Test negative design (75 JE clusters)	Test negatives	Test positives	Adjusted OR
Non-vaccinees	483 (19%)	10 (19%)	0.99 (0.49,2.01)
JE-recipients	2100 (81%)	44 (81%)	



○ Vi-TT cluster
 ○ JE cluster
 seeking healthcare
 ● Typhoid cases

Interpretations

- Our findings underscore the validity of TND as an efficient method to monitor effectiveness
- We recommend the adoption of TND alongside bias identification techniques such as NCE and NCO to support the TND results
- TTE design can bias VE, possible due to unmeasured confounding effects, such as health-care-seeking behaviours.

The validity of test-negative design for assessment of typhoid conjugate vaccine protection: comparison of estimates by different study designs using data from a cluster-randomised controlled trial

Shuo Feng, Yiyuan Zhang*, Farhana Khanam, Merryn Voysey, Virginia E Pitzer, Firdausi Qadri, John D Clemens, Andrew J Pollard, Xinxue Liu*

DOI: [10.1016/S2214-109X\(25\)00056-7](https://doi.org/10.1016/S2214-109X(25)00056-7)

Typhoid Vaccine Acceleration Consortium (TyVAC)

Reduce the global burden of typhoid by accelerating the introduction of typhoid conjugate vaccines (TCVs) in low-resource countries.



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Gates Foundation

