As maternal immunisation for RSV and other pathogens is implemented globally, factors that affect transplacental antibody transfer must continue to be assessed, and whether sufficient antibody concentrations can be induced to overcome potential deficits in transport must be determined. As Saso and Kampmann indicate, the need is great: in our study population, a third of infants were born with RSV antibody concentrations below a putative protective threshold.[2]

We declare no competing interests.

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Zika virus and microcephaly

The recent case-control study by Thalia de Araújo and colleagues[1] presents important new data with critical implications for the ongoing Zika virus public health emergency. However, there are several issues that require closer consideration to optimise the use of these results to inform ongoing health policy and response.

Although the authors are explicit about this being a preliminary and interim analysis, the publication of effect sizes before reaching the predefined sample size could undermine these power calculations with the potential to consequently produce misleading effect sizes.[2]

The reported primary outcome (effect of Zika virus on risk of microcephaly) is an odds ratio (OR) of 55·5 (95% CI 8·6 to +∞). The authors rightly use methods for small cell counts (so-called exact logistic); however, other methods exist and produce complementary estimates that are potentially less biased.[3] Effect size estimates from these models (table)[4] have 95% CIs that are considerably narrower, but retain the large effect sizes and confidence intervals, suggesting that there is inherent instability in estimating these odds ratios.

A second major advantage of these models over exact logistical methods is that they provide for full covariate adjustment, which would allow the effect of arboviral co-infections (59% of 91 mothers had evidence of multiple flaviviral infections) to be fully explored as both interactions and as covariates, enabling improved clarity on the poorly understood effect of pre-existing antigenic responses or arboviral co-infections on Zika virus pathogenicity.[5] Moreover, the omission of covariates has the potential to artificially inflate effect size measures if the covariate is acting as a confounder.[6]

Finally and most importantly, the authors report that 24% of approached controls refused participation in the study; the potential impact of seropositivity in these unavoidably missing controls on the sparse data structure is examined (table), and shows dramatic changes in effect size. Although these results remain statistically significant, the odds ratios are now closely aligned with those from other TORCH agents (eg, *Toxoplasma gondii*).[7]

	Data	Firth logistic
I	n=94, from de Araújo[1]	86·5 (4·9–1523·4; p=0·002)
II	n=114 (20 controls, with 5 ZIKV+)	9·8 (3·2–29·6; p<0·0001)
III	n=114 (20 controls, with 10 ZIKV+)	4·8 (1·9–12·3; p=0·001)

Models II and III are hypothetical scenarios with five and ten Zika virus-positive controls from 20 refusals total, respectively. Firth logistic has been given as odds ratio (95% CI). All analyses used Stata 14.1 (College Station, TX, USA; all tests were two-tailed, with α=0.05; bias reduction logistic from the –firthlogit- package).[4] ZIKV=Zika virus.

Table: **Complementary measures of association between Zika virus positivity and risk of microcephaly with three models, Brazil 2016**

Evidence exists to suggest that people who decline to participate in epidemiological studies generally have lower socioeconomic status and education levels,[8] which has been specifically found to be associated with greater arboviral seropositivity in Recife in northeastern Brazil.[9]

In summary, these preliminary measures of the association between Zika virus and microcephaly represent unstable estimates from case-control trials, with several important potential sources of bias that should be taken into account before definitive statements about association can be made.

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See **Articles** page 1356

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Authors' reply

We would like to thank Andrew Lover for using our case-control study[1] (of the association between Zika virus infection in neonates and microcephaly) to discuss an alternative analytical approach, penalised logistic regression.[2] In that approach the odds ratio would have been higher (OR 86·5, 95%CI 4·9–1523·4) than the one estimate using exact logistical regression (OR 55·5, 95% CI 8·6–+∞). We note that the inferior limit is lower, but the association is still highly statistically significant.

Lover also did a sensitivity analysis (again using penalise logistical regression) to explore the potential effect of the refusal rate among control participants of about 25%. The observed laboratory positivity (the exposure under study) in cases was 41%. None of the 62 controls were laboratory confirmed for Zika virus. In his sensitivity analysis, Lover assumed that 10–50% of controls refusing to participate in the study were laboratory confirmed. Even in the clearly unrealistic assumption of 50% laboratory confirmation among controls refusing study participation (a higher positivity than in cases), the association is still statistically significant. Whatever the analytical approach used, the conclusion is the same: congenital Zika virus infection is the cause of microcephaly.

The next public health question is not the magnitude of the odds ratio, but what is the risk of microcephaly and others manifestation of the congenital Zika syndrome in babies of women who have Zika infection during pregnancy. This estimation, and the effect of any cofactors of this risk, will not be established in a case-control study but in the ongoing cohort studies[3] of pregnant women with Zika virus infection.

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Crimean-Congo haemorrhagic fever virus and Eid-Ul-Adha festival in Pakistan

Crimean-Congo haemorrhagic fever (CCHF) has caused 20 deaths in Pakistan as of Aug 20, 2016.[1] These deaths might be attributable to Eid-ul-Adha, an annual religious festival observed by Muslims, during which nearly 8 million animals, including goats, sheep, cows, and camels are sacrificed.[2] Pakistan has experienced various nosocomial outbreaks of CCHF and Eid-Ul-Adha is regarded as a vulnerable period for these outbreaks. In routine butchery for food, animals are slaughtered at designated facilities in the presence of veterinarians to ensure the animal's health.[3] However, during Eid-Ul-Adha, the pattern of animal slaughter changes in accordance with religious beliefs. These changes include factors such as transport of animals for sale from endemic rural to urban areas, little regulation of animal sales, advanced purchase of animals, few health checks on purchased animals, freelance and non-professional butchers, slaughtering of animals in public areas, gathering of spectators around the butcher to watch the slaughter, absence of formal training among butchers, delayed disposal of blood and carcasses of sacrificed animals, and scarceness of appropriate methods for the disposal of the animal waste. These factors result in increased exposure of the general public to viraemic animals and enable animal-to-animal, animal-to-human, and human-to-human transmission of CCHF virus.

Despite efforts made by Government of Pakistan, the upsurge of CCHF remains uncontrolled.[4] Moreover, the death of a senior surgeon who became infected while operating on a patient with CCHF has also raised serious concerns over biosafety measures at health facilities.[5] The government has not taken a hard line and we believe that putting full effort into the control of the aforementioned factors could go a long way to combating CCHF in Pakistan.

In the next 10–15 years, Eid-Ul-Adha will occur in summer when CCHF is more prevalent, suggesting a dire need to implement policies on the slaughter of sacrificial animals to prevent a potential health catastrophe. We believe that cattle farmers, shepherds, and butchers are unaware of the health hazards posed by CCHF virus, especially via the infected blood of slaughtered animals. Provision of appropriate and comprehensible training will be of paramount importance for reducing CCHF transmission.

We suggest that the Government of Pakistan should focus its efforts on vertical programmes for the control