



## Pertussis in Lao PDR: Seroprevalence and disease

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### ARTICLE INFO

#### Article history:

Received 2 February 2020

Received in revised form 24 March 2020

Accepted 25 March 2020

#### Keywords:

*Bordetella pertussis*

Seroprevalence

Lao PDR

Vaccine immunogenicity

Vaccine-preventable infection

### ABSTRACT

**Objectives:** Pertussis is a debilitating vaccine-preventable infection. The aim of this study was to determine susceptibility and exposure to pertussis in Lao PDR in different age groups and subpopulations.

**Methods:** A total 3072 serum samples were obtained from different cohorts: children with documented vaccination, pre-schoolers, schoolchildren, blood donors, healthcare workers (HCWs), and pregnant women and paired cord blood. Samples were tested for anti-pertussis toxin IgG antibodies. A history of *Bordetella pertussis* exposure was defined according to antibody titres. Four hundred and seventy-five throat swabs and nasopharyngeal aspirates were analysed by PCR for the presence of *B. pertussis* in symptomatic children at the Children's Hospital in Vientiane.

**Results:** Overall pertussis seroprevalence was 57.5%. The prevalence of titres indicating acute infection or recent vaccination or infection/vaccination within the last 12 months ranged from 7.4% (100/1356) in adults to 21.4% (25/117) in pre-schoolers (age 1–5 years). *B. pertussis* was detected in 1.05% (5/475) of children with respiratory symptoms in Vientiane Capital.

**Conclusions:** It is suggested that routine childhood vaccination, in particular outreach, as well as vaccination of HCWs should be strengthened. A childhood booster and vaccination of pregnant mothers should be considered. There is also a need to improve reporting and to introduce pertussis testing in at least one central facility.

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### Introduction

Whooping cough or pertussis, a highly infectious disease that mainly occurs in children, is caused by the gram-negative bacterium *Bordetella pertussis*. Symptoms such as fever, runny nose, and cough occur within 7–10 days after exposure and develop into a paroxysmal cough followed by whooping (Guio et al., 2011; World Health Organization, 2015). Symptoms are often uncharacteristic, including apnoea, cyanosis, and lymphocytosis, and may not be recognized early on. Complications include

pneumonia, otitis media, encephalitis, or convulsions. Pertussis is more severe in infants and children than in adults, and may later include paroxysms of rapid coughs and whooping, vomiting, and exhaustion (Vittucci et al., 2016). Adults who have never been vaccinated or who have lost their immunity can be a reservoir for *B. pertussis* and a potential source of infection for children (Gonik et al., 2005). If the mother has sufficient antibodies against pertussis toxin (anti-PT), transplacentally acquired antibodies may protect newborns during the first months of life (Gonik et al., 2005; Hoang et al., 2015; World Health Organization, 2015).

Lao PDR has the highest mortality rate in under 5-year-olds within the Association of Southeast Asian Nations, with 63.9 deaths per 1000 live births (Thisyakorn et al., 2019). The diphtheria–tetanus–pertussis (DTP) vaccine was first introduced in two provinces, Vientiane and Savannakhet, in 1979. Nationwide DTP vaccination was implemented in 1989, but coverage had

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reached only 60% by 2008, 89% in 2015, and 82% in 2016 (Gavi The Vaccine Alliance, 2019). In 1990, pertussis caused 2.7% of premature deaths in Lao PDR, but pertussis had decreased from rank 22 to rank 33 as a cause of premature death by 2010 (Institute for Health Metrics and Evaluation, 2010). In 2009, the country switched from the DTP vaccine to the pentavalent vaccine (containing additional hepatitis B and *Haemophilus influenzae* components) given at 6, 10, and 14 weeks of age. Both vaccines contain a whole-cell pertussis emulsion (wP).

In a previous study, it was shown that the low seroprevalence of other components of the pentavalent vaccine was due not only to low vaccination coverage, but also to a low response to the vaccine (Evdokimov et al., 2017).

Outbreaks of pertussis were reported in 11 of 17 provinces throughout Lao PDR between 2011 and 2017. The World Health Organization (WHO) has described disease surveillance for *B. pertussis* in Lao PDR as inadequate (World Health Organization, 2012). The aim of this study was to investigate the current epidemiological situation in Lao PDR in order to estimate disease susceptibility and age-dependent incidence, and to develop strategies to mitigate pertussis outbreaks and reduce child mortality.

## Methods

### Study participants

This study included seven cohorts ( $N=3547$ ). Samples were collected at different times during previous studies and were included in this study retrospectively. Informed consent was obtained from all participants or their parents/guardians.

In the cohort descriptions, vaccination coverage refers to three vaccine doses during the first year of life. Outbreak data were obtained from Bouaphan Khamphongphone, National Centre for Laboratory and Epidemiology (NCLE). Data on vaccine coverage were received from Dr Anonh Xeuatvongsa, National Immunization Programme – Maternal and Child Health (NIP-MHCH), Lao PDR and the Gavi Vaccine Alliance (Gavi The Vaccine Alliance, 2019). The vaccine coverage data must be interpreted with caution, given the discrepancies in coverage data and birth rates identified by an international EPI review (World Health Organization, 2012).

### Children with documented vaccination

Children aged 3 months to 5 years with three documented doses of pentavalent vaccine were recruited from Vientiane Capital, Bolikhamxay and Khammouane provinces in 2013–2014, as described previously ( $n=958$ ) (Evdokimov et al., 2017) (ethics approval number NECHR 860/2013). Antibodies against tetanus (Evdokimov et al., 2017) were used as a marker for vaccination. Antibodies against diphtheria and hepatitis B surface antigen were used for comparison of immunogenicity.

### Pre-school children

One hundred and seventeen pre-school children aged 1 to 5 years were recruited in 2013 from Xamtai and Kuan districts of Houaphanh Province, as described previously (Nanthavong et al., 2015) (ethics approval number NECHR 732/2013). Immunisation status was known for 101 participants, including 66 fully immunised children. Vaccination coverage of the children with unknown immunisation status was estimated at 44% to 95%.

### School children

Schoolchildren aged 6 to 19 years ( $n=671$ ) were recruited from the provinces of Luang Prabang, Savannakhet, and Bolikhamxay. The recruitment was school-based as part of a study on measles/rubella vaccine coverage in 2011–2012 (ethics approval number

NEHCR 001/2011). Their individual immunisation status was unknown, but vaccination coverage was estimated to be up to 54% in Luang Prabang, 74% in Savannakhet, and 56% in Bolikhamxay.

### Blood donors

Adult blood donors 19 to 65 years of age ( $n=517$ ) were recruited between 2013 and 2015 from Xayaboury, Houaphanh, Luang Namtha, Khammouane, Attapeu, and Luang Prabang provinces and Vientiane Capital (ethics approval number NEHCR 059/2014). Their immunisation status was unknown. Vaccination coverage was estimated as 54% for those between 20 and 23 years of age. Those older than 38 years in Vientiane and older than 25 years in the other provinces were born before DTP introduction.

### Healthcare workers (HCWs)

Adult HCWs ( $n=469$ ) were recruited from three central, two provincial, and eight district hospitals within Vientiane Capital in 2013, as described previously (Black et al., 2015) (ethics approval number NEHCR 038/2013). Their immunisation status was unknown. Vaccination coverage was estimated to be up to 54% for those under 35 years of age, while those older than 35 years were born before DTP introduction.

### Pregnant women and paired cord blood

Serum of 170 pregnant women aged 15 to 42 years and paired cord blood samples were obtained from Savannakhet Province in 2017 as part of a study on maternal–neonatal tetanus. Women were recruited whilst attending district hospitals for delivery (ethics approval number NEHCR 046/2017). Their immunisation status was unknown. There was an estimated provincial vaccination coverage of up to 74% for those younger than 38 years of age, while those older were born before vaccine introduction in Savannakhet.

### Symptomatic children

In 2014–2015, throat swabs and nasopharyngeal aspirates were collected from 475 children aged 0–15 years, presenting to the Children's Hospital in Vientiane with an acute respiratory infection, i.e., fever ( $n=411$ ), cough ( $n=429$ ), and/or rhinorrhoea/nasal congestion ( $n=443$ ). The average duration of the illness was 3 days (range 0–59 days). Samples were stored at  $-80^{\circ}\text{C}$  in viral transport media until use. All participants had an unknown immunisation status (027/NIOPH/NECHR/2015).

### Serology

All serum samples ( $n=3072$ ) were analysed by commercial ELISA (EUROIMMUN, Lübeck, Germany) for anti-PT IgG. According to the manufacturer's instructions and previous studies, titres are indicative of vaccination and infection histories (Giammanco et al., 2003; Pebody et al., 2005; Guiso et al., 2011). Thus, a titre of  $<5$  IU/ml corresponded to seronegativity, a titre of 5–40 IU/ml corresponded to infection or vaccination more than 12 months before, a titre of 40–100 IU/ml indicated infection or vaccination within the past 12 months, and a titre  $\geq 100$  IU/ml indicated an acute infection or recent vaccination. Although a high antibody level of anti-PT IgG is associated with protection against *B. pertussis*, a protective titre has not been defined (Zepp et al., 1996; García-Corbeira et al., 2000; Van Der Wielen et al., 2003; Gonik et al., 2005; World Health Organization, 2015). According to the manufacturer, the sensitivity and specificity of the ELISA test were 95–97.8% and 100%, respectively. All tests had internal positive and negative controls and passed the validity criteria. The quality of the samples was confirmed by testing for other markers, such as antibodies against measles and rubella (data not shown). External quality

assessments of the tests were not performed. Antibodies against tetanus toxin (anti-TT Ig) were measured by commercial ELISA (EUROIMMUN, Lübeck, Germany).

### PCR

For symptomatic children, nasal swabs and nasopharyngeal aspirates were analysed for the presence of *B. pertussis* by multiplex conventional PCR (McDonough et al., 2005). DNA was extracted from pooled paired samples using Trizol LS (Life Technologies, Thailand) and Pure Link Viral RNA/DNA Mini Kits (Life Technologies, Belgium). Multiplex PCR is not one of the WHO recommended tests for the diagnosis of pertussis infection, but is appropriate for research purposes. Nevertheless, some cases of pertussis that would have been picked up by conventional PCR may have been missed.

### Statistical analysis

Data were entered into Excel and analysed using SPSS. Proportions of different groups were compared by Chi-square test. Logistic regression was used to estimate crude and adjusted odd ratios (OR) with 95% confidence intervals (CI). A *p*-value <0.05 was considered significant.

## Results

### Children with documented vaccination (age 3 months to 5 years)

Despite three documented doses of pentavalent vaccine, overall seronegativity was very high (38.2%; Figure 1). Even when using the anti-TT status as a marker for vaccination, 217/787 (27.6%) TT-positive children were seronegative for anti-PT IgG. Serology indicating vaccination (or infection) within the last 12 months was low (14.3%).

In children vaccinated within the last 12 months, there were significant differences in immunity levels between provinces and vaccine components (Figure 2, Supplementary Material Figure S1). While children from Khammouane had lower immunity against

diphtheria (50.0%) and hepatitis B (46.2%) than children from Vientiane (64.9%, *p* = 0.027; 52.6%, *p* > 0.05, respectively) (Evdokimov et al., 2017), their anti-PT IgG seroprevalence was significantly higher (74.2% and 57.8%, respectively, *p* = 0.018). This is suggestive of pertussis infections in Khammouane, for example during a reported outbreak there at the time of sample collection, rather than better vaccine immunogenicity.

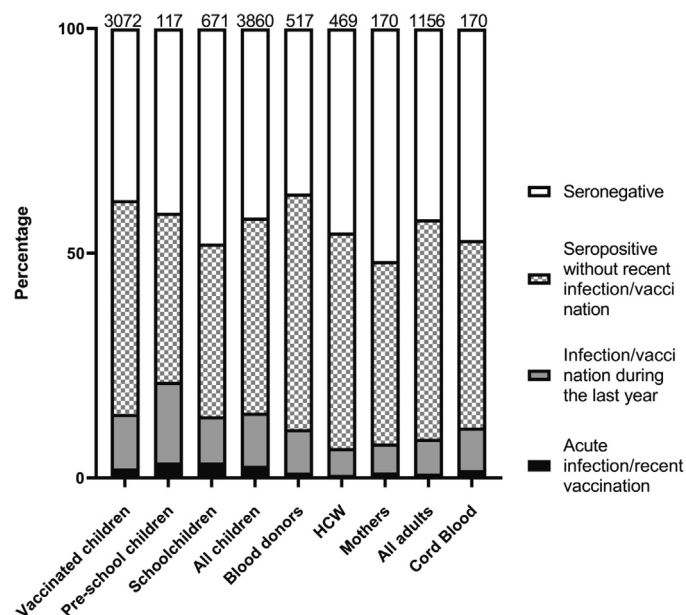
Overall, seronegativity was higher in those with documented vaccination >20 months ago (42.5%), compared with those with documented vaccination within the last 12 months (32.1%; *p* = 0.009) (Figure 2), suggestive of antibody waning. The prevalence of titres indicating vaccination/infection within the last 12 months did not differ significantly according to the time since vaccination (13.4% and 16.7%; *p* = 0.77; Figure 2), suggesting long-lived immunity once a sufficient titre was reached, while those with lower titres gradually become seronegative. Additional pertussis infections are also a possible explanation. It is possible that a small proportion of the seronegative group had been vaccinated too recently to have mounted an adequate immune response, although we do not expect this to impact significantly on the data.

### Pre-school children in Houaphanh Province (age 1–5 years)

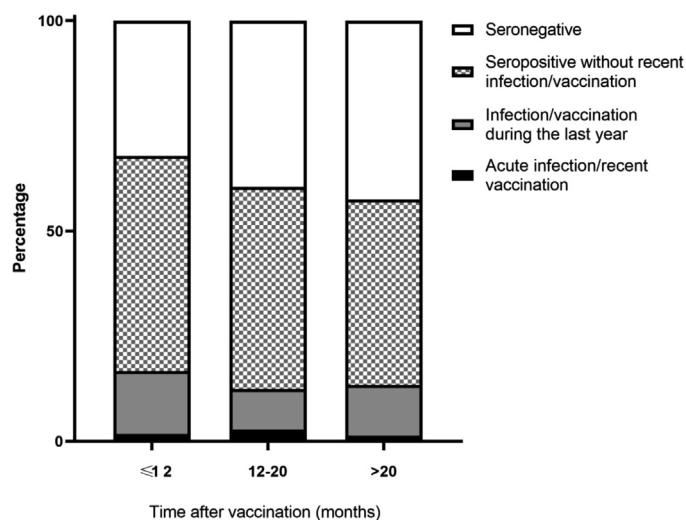
The highest rates of serology indicative of recent infection/vaccination (3.4%) and <12 months infection/vaccination (18.0%) were found in this cohort. Also, 37.6% had antibody titres suggesting infection/vaccination >12 months before and 41% were seronegative (Figure 1). In Xamtai District, closer to the nearest health facility, there was significantly higher seropositivity (38/53, 71.1%) compared to Kuan District (30/63, 47.6%; *p* = 0.009). A longer distance to the health facility (*p* = 0.007) and living in a household with less than seven members (*p* = 0.037) was associated with a higher rate of titres showing infection/vaccination <12 months ago.

### School children (age 6–19 years)

In this cohort, vaccination status was unknown, making the distinction between former infection and vaccination in



**Figure 1.** Levels of antibodies against pertussis toxin (anti-PT IgG) by cohort. Levels of seronegativity, seropositivity without recent infection/vaccination, infection/vaccination during the last year, and acute infection/recent vaccination were defined as described in the Methods section. Numbers above the columns indicate the number of participants per group.



**Figure 2.** Serology according to the time since last vaccination in children with documented vaccination. Numbers above the columns indicate the number of participants per group.

schoolchildren with low titres impossible. Titres above 40 IU/ml were interpreted as infections during the last year, since the vaccination was more than 5.5 years previously. Approximately half of the schoolchildren were seropositive (52.16%), including 10.28% with titres indicating infections <12 months ago and 3.34% indicating recent infections (Figure 1). A reported pertussis outbreak in Savannakhet in 2011–2012 was compatible with high seropositivity and recent infections. Seropositivity in Savannakhet (65.2%), as well as serology showing recent infections and infections <12 months ago (24.8%), were significantly higher than in Bolikhamxay (44.2% and 9.4%;  $p < 0.001$ ) and Luang Prabang (48.2% and 7.9%;  $p < 0.001$ ) (Supplementary Material Figure S2). In Bolikhamxay, the seropositivity rate was significantly higher in 13- to 19-year-olds (53%) than in 6- to 12-year-olds (37.6%;  $p = 0.018$ ), indicative of ongoing unreported outbreaks in these age groups. Thus, pertussis affects schoolchildren all through their adolescent life in all three provinces.

#### Adult blood donors

For the adult cohorts, a seropositive result was more likely to be due to a past infection, since the last vaccination was more than 14 years previously. Of the blood donors who were born before vaccine introduction in 1979 (Vientiane)/1989 (nationwide), 61.64% (188/305) were seropositive, suggesting a high incidence of pertussis during their childhood and more recently (11.5%). A reported outbreak in Khammouane in 2012–2013 was compatible with a significantly higher seropositivity in this province (85/115, 73.9%) than in Attapeu (69/118, 58.5%;  $p = 0.037$ ) or Vientiane (70/129, 54.3%;  $p = 0.026$ ).

#### Healthcare workers

The seroprevalence in HCWs was similar to that in the other adult cohorts (Figure 1). They had a relatively low prevalence of titres indicating recent infections (0.6%) and infections <12 months ago (6.0%). Notably, 45.4% were seronegative and susceptible to disease.

#### Mother and cord blood

There was a highly significant correlation between the mother's titre and the paired cord blood ( $r = 0.8148$ ,  $p < 0.001$ ;

Supplementary Material Figure S3). As there is no pertussis vaccination during pregnancy in Lao PDR, the high titres in 3/170 women (1.2%) were indicative of acute infections. Half of the mothers were seronegative (51.7%; Figure 1), also leaving their babies unprotected. A reported outbreak in Savannakhet 5 years before sample collection suggests that circulation in this province is high, increasing the risk of infection for newborns.

#### Symptomatic children

*B. pertussis* was detected in 1.05% (5/475) of children who presented with respiratory symptoms. The *B. pertussis*-positive children were between 3 and 13 years old (mean 7 years). Two cases were detected in March 2015 and the three other cases occurred in June 2015.

#### Discussion

This study is novel in addressing the epidemiology of pertussis in Lao PDR, the country with the highest disease-specific mortality in Southeast Asia.

Despite three documented doses of the pentavalent vaccine, only two-thirds of vaccinees aged 3 months to 5 years were seropositive. This is similar to the low immunogenicity against other components of the vaccine in this cohort (Evdokimov et al., 2017). Due to waning antibodies, the percentage of children protected by the vaccine will decrease further. In the other child cohorts, including the pre-schoolers, vaccination coverage was low. Nevertheless, these cohorts had higher incidences of titres  $\geq 40$  and  $\geq 100$  IU/ml. This serology, paired with many seronegatives, is suggestive of poor vaccine response/coverage followed by wild-type infection. Low vaccine response/coverage was particularly problematic for children living further away from the health care facility. Vaccination of pre-schoolers living in more remote areas is conducted by outreach teams, who transport the vaccine from the nearest health care facility to the villages; this could explain why vaccination may be less robust and the disease burden higher.

The high seronegativity in schoolchildren aged 6 to 19 years indicates incomplete vaccine response and coverage, and waning of immunity. Since this cohort was vaccinated at least 5 to 6 years earlier, the high prevalence of titres  $\geq 40$  IU/ml indicates high pertussis incidence. In Savannakhet, the high titres in



schoolchildren and also in older children and adults are compatible with the outbreak reported before or during the sample collection.

Adult blood donors had a lower rate of titres indicative of acute infections (1.16%) and infections <12 months ago (9.7%) than the pre-school children and schoolchildren, but this was higher than in Thailand (6.3%) (Wanlapakorn et al., 2016) and Europe (1–6%) (Kretzschmar et al., 2010) during the 1990s. There was a high percentage of blood donors with titres indicating infection >12 months earlier (52.42%) and a lower proportion of seronegatives (36.8%). Seropositivity in this age group is comparable to that in other countries such as Mexico with 50% and 47.6% seropositive (Hashemi et al., 2009; Conde-Glez et al., 2014).

It was found that a significant proportion of HCWs were susceptible to pertussis and at potential risk of infection from their patients. This is in line with previous reports of a high susceptibility of HCWs in Lao PDR to other vaccine-preventable infections (Black et al., 2015). This result is comparable to those of other studies addressing the susceptibility of HCWs to pertussis (De Juanes et al., 2004; Urbiztondo et al., 2015; Rodríguez de la Pinta et al., 2016).

Finally, a significant correlation in antibody levels between pregnant women and cord blood was observed, confirming transplacental transfer of protective antibodies (Gonik et al., 2005; Hoang et al., 2015; World Health Organization, 2015). Approximately half of the women and cord blood were seronegative and susceptible to infection. For this reason, some countries recommend antenatal vaccination with pertussis-containing vaccine (Gkentzi et al., 2017). So far, there has been no such recommendation in Lao PDR, but there are no previous data about pertussis in newborns and infants. A prospective clinical trial was recently conducted in Thailand in which pregnant women were vaccinated with Tdap vaccine, independent of their serological immune status. This booster vaccination was free of severe side effects and induced antibodies against pertussis toxin in all pregnant women (Sompagdee et al., 2020).

In a study in Thailand, 6.3% of children over 11 years of age were infected during the 12 months (Wanlapakorn et al., 2016). Using their cut-off of 40 IU/ml, we estimate infections in 10.3% (186/1799) of over 7-year-olds in Lao PDR. This corresponds to an estimated incidence of 10 339/100 000 in this population, similar to the estimated 9395/100 000 in Guangdong Province in China (Zhang et al., 2012). In the present study, the detection of *B. pertussis* in symptomatic children from Vientiane (1.05%) confirmed the active circulation of *B. pertussis* at least in this age group.

Despite serological evidence of high pertussis incidence, less than 50 annual cases have been reported from 2016 to 2018. While 1726 pertussis cases have been notified from 11 provinces throughout Lao PDR since 2011, none were reported from Vientiane Capital (NCLE). As in other countries, there seems to be a considerable underreporting of pertussis cases in Lao PDR (Barkoff et al., 2015).

This study has several limitations. First, the samples were tested retrospectively and were not randomised. Therefore, they may not be representative of the general population. This also prevented a more detailed age stratification in the young age groups, which would have allowed a clearer distinction between vaccination and acute infection in this vulnerable cohort. Individuals in Lao PDR rarely keep vaccination records beyond infancy, and hence we had to rely on provincial and nationwide coverage data to aid the interpretation of the results. Furthermore, the coverage data have been questioned for reliability and must be interpreted with caution (World Health Organization, 2012).

In conclusion, serology suggests that the incidence of pertussis is high in children under 5 years of age and infection continues in teenagers and even in adults. This seems to be due to a low vaccine response, especially after outreach vaccination, as well as to rapid

antibody waning. Vaccination management needs strengthening in order to increase coverage and immunogenicity, especially in rural areas. Follow-up studies are needed to confirm the trend of waning antibodies indicated by rising seronegativity at different time-points after vaccination in children. In adults born before the introduction of pertussis-containing vaccines, the seroprevalence could be as high as 75%, but less than 50% in HCWs. Thus, the systematic vaccination of HCWs, especially those involved in neonatal care, should be considered. More importantly, a booster for pregnant women during antenatal care and for children before the age of 5 years is recommended. There is also a need to strengthen the reporting of this highly prevalent notifiable disease. Pertussis diagnostics should be introduced in at least one central facility.

## Funding

This work was supported by the Ministry of Foreign and European Affairs, Luxembourg (project “Lao Luxembourg Partnership for Research and Capacity Building in Infectious Disease Surveillance II”, PARECIDS II).

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

The authors wish to thank all collaborators involved in logistical support, sample collection, laboratory analysis, and reviewing of the manuscript. These include Phonepaseuth Khampanisong<sup>1</sup>, Chantal Snoeck, Bounthome Samouny, Prapan Jutavijittum, Chanthasone Souvannaso, Keoudomphone Vilivong, and Phoumsavath Ounnavong. We are thankful to our collaborators at Luxembourg Development Cooperation, the Lao Red Cross, and provincial and district hospitals and health centres. We wish to acknowledge Dr Paul Brey and the staff at the Institut Pasteur du Laos for their support. We are especially grateful to all the study participants.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <https://doi.org/10.1016/j.ijid.2020.03.074>.

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