Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplementary Material

Transmission of Nipah virus: 14 years of investigations in Bangladesh

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1. Previous studies reporting Nipah virus patients in Bangladesh

Nipah virus cases included in our analysis were identified during 14 years of outbreak investigations and routine surveillance (Table S1). Previous reports on single outbreaks and a study combining 122 Nipah virus cases identified during 2001-2007[1] generated hypotheses about mechanisms for person-to-person transmission of Nipah virus. These studies however lacked the power or generality necessary to test and validate these hypotheses and evaluate their relative importance. Here we used data on all 248 Nipah virus cases identified during 2001 to 2014 and their 2,606 contacts to perform the most comprehensive characterization of Nipah virus person-to-person transmission to date.

A few other studies combined Nipah virus cases across multiple transmission seasons to address research questions unrelated to person-to-person transmission (Table S1). These studies investigated characteristics of cases (2001-2004)[2], spillover infections through drinking palm-sap (2010-2014)[3], and rare risk factors of infections (2004-2012)[4], and assessed surveillance strategies for Nipah virus (2001- 2011)[5].

2. Reported Nipah virus outbreaks outside of Bangladesh

Bangladesh is the only country reporting Nipah virus outbreaks regularly with 79 reported spillover events during 2001-2014, compared to only four reported outside the country (one in Malaysia/Singapore[6] and three in India, including a very recent one in Kerala[7-9]). Since person-to-person transmission of Nipah virus was not a prominent feature during the outbreak in Malaysia, our study includes all but three known outbreaks of Nipah virus with person-to-person transmission.

3. Identification of Nipah virus cases in Bangladesh (2001-2014)

Nipah virus cases were identified through hospital-based surveillance and outbreak investigations conducted by the icddr,b in partnership with the Institute of Epidemiology, Disease Control and Research. Serum samples of suspected Nipah virus cases (defined as individuals with history of fever or documented fever with axillary temperature >38.5°C and either altered mental status, new onset of seizures, or new neurological deficit) were tested using an IgM capture enzyme-linked immunosorbent assay (ELISA) as previously described in Daniels et al..[10] A confirmed Nipah virus case was defined as an individual with detectable Nipah virus IgM in serum or detectable DNA in serum, throat swabs or cerebrospinal fluid and a probable Nipah virus case as an individual who fulfilled the definition of a suspected Nipah virus case but died before sample collection and had an epidemiological link to a confirmed Nipah virus case. For retrospectively identified outbreaks, Nipah virus cases who survived and had Nipah virus IgG in serum (detected through ELISA as previously described in Daniels et al.[10]) were also considered as confirmed cases. Follow-up outbreak investigations were conducted in the communities of confirmed and probable cases to detect additional cases who did not present to surveillance hospitals. A detailed case report was created for each probable or confirmed Nipah virus case, which included demographic information, the timeline of disease, symptoms, disease outcome, and the potential source of infection.

Nipah virus outbreak investigations

Before the establishment of hospital-based surveillance for Nipah virus in Bangladesh, outbreaks were reported through the healthcare system to central authorities. Nipah virus cases were subsequently identified through outbreak investigations conducted by the icddr,b in partnership with the IEDCR.

In 2001 and 2003, clusters of febrile neurologic illness were reported in Meherpur and Naogaon districts; both outbreaks were investigated retrospectively in 2003.[11] In 2004, two Nipah virus outbreaks were recognized in Rajbari and Faridpur districts, in 2005 an outbreak was recognized in Tangail district; all triggered immediate outbreak investigations.[12-14]

Hospital-based surveillance for Nipah virus

Bangladesh established a sentinel surveillance system for Nipah virus in 2006 that included three tertiary and seven district-level hospitals; four of these district hospitals were converted into passive surveillance sites in 2007. At sentinel surveillance hospitals, any admitted case with suspected meningo-encephalitis was investigated for Nipah virus infection, while passive surveillance hospitals only reported to the icddr,b and IEDCR if an unusual number of meningo-encephalitis cases was observed. Once a Nipah virus case was identified in a surveillance hospital, additional Nipah virus cases were identified through outbreak investigations in the community of the Nipah virus case.

4. Comparison of confirmed and probable Nipah virus cases

Laboratory confirmation was not possible for 43% (107/248) of Nipah virus cases due to their death before a suitable sample could be obtained. For the main analysis, we aggregated confirmed and probable Nipah virus cases. Here we demonstrate the validity of the inclusion of probable cases by comparing their characteristics to confirmed cases (Table S2). Consistent with the case definition, probable cases generally showed a more severe disease form than confirmed cases with a shorter time from symptom onset to death (median of 5 days vs. 7 days) and more prevalent respiratory symptoms (difficulty breathing 76% vs. 53%, cough 63% vs. 42%). Probable cases infected on average more individuals (reproduction number 0.7 vs. 0.04, $p<0.001$), indicating a good specificity of the identification of probable Nipah virus cases.

5. Estimating the serial interval distribution and the incubation period distribution in secondary Nipah virus cases

We calculated the serial interval of epidemiologically-linked transmission pairs as days between symptom onset in the infector to symptom onset in the secondary case. The mean and median serial interval was 13 days (IQR 12 to 14) and 95% of secondary cases developed symptoms within 16 days of symptom onset in their infector (corresponding to a gamma distribution with mean 12.7 and standard-deviation 3.0).

The incubation period can be calculated as the time between date of infection and date of symptom onset. Eleven secondary cases were exposed to their infector on a single day and hence had a known infection date, for other secondary cases the precise date of infection was unobserved. The possible timing of infection for these cases can however be narrowed down using (i) reported exposure time windows (Figure S1) or (ii) the dates of symptom onset and death in their infectors. Here we implemented a statistical model that uses such exposure window information together with the dates of symptom onset in secondary cases to estimate the most likely duration of the incubation period. We assumed that the incubation period followed a discretized gamma distribution with mean M_{inc} and standard-deviation SD_{inc}. We used Bayesian data augmentation techniques to incorporate unobserved infection dates.[15]

At every iteration, we implemented the following sampling scheme:

i) Metropolis-Hastings update for the parameters of the incubation period distribution. At every iteration, M_{inc} and SD_{inc} were updated once. Updates were performed on the log-scale.

ii) Independence sampler for the day of infection in secondary cases. Candidate infection dates were drawn from the incubation period distribution. An observational model ensured the compatibility of sampled infection dates with reported exposure timing. The update was performed in random case order.

For a secondary case i with symptom onset on day s_i and augmented infection date t_i the contribution to the likelihood was

$$P_i = h(s_i - t_i)$$

where h represents a discretized gamma distribution h with mean M_{inc} and standard-deviation SD_{inc}.

We estimated model parameters based on a Markov chain Monte Carlo (MCMC) Bayesian framework. Parameters were updated using a Metropolis-Hastings algorithm that runs for 60,000 iterations with a burn-in of 10,000 iterations and every 10^{th} sampled value is stored. Convergence was visually assessed. We provided parameter estimates as the posterior median and a 95% credible interval.

Based on the model and data from all 82 secondary cases, we estimated an incubation period distribution with M_{inc} of 9.7 days (95% credible interval 9.1 to 10.4) and SD_{inc} of 2.2 days (95% credible interval 1.7 to 2.8). This corresponds to a median incubation period of 9 days (interquartile range (IQR) 8 to 11), where 95% of secondary cases developed symptoms within 13 days of infection (Figure S2*).* In a simpler analysis restricted to eleven secondary cases with single day exposure, the median incubation period was estimated at 9 days (IQR 8 to 11.5, range 6 to 14 days). Our model estimate using information from all 82 secondary cases also agrees with the median incubation period of 9 days previously estimated by Luby et al.[1] based on 14 Nipah virus cases with ≤2 days exposure.

6. Assumptions about the incubation and infectious periods for identification of secondary cases and contact tracing

Observations from case and contact data support the assumptions about the incubation and infectious period. Based on the contact tracing data, assuming a wider incubation period window would not have led to major changes in case classification, identification of infectors, or the estimated incubation period distribution. Only one Nipah virus case developed symptoms 3 days after contact to a Nipah virus case, however this case also reported previous consumption of palm sap, which was considered as the more likely source of infection. Although we cannot exclude that a few cases develop symptoms earlier or later than the assumed incubation period window, these would have been likely picked up during contact tracing activities and would have led to a reconsideration of the assumed incubation period window.

Although contacts were assessed for up to 15 days after illness onset, the majority of secondary cases seem to have acquired infections within a week of illness onset in their infectors. No secondary case had contact to the infector later than 9 days after illness onset, and 83% of secondary cases only had contact within the first 5 days (Figure S3). This is consistent with the observation that only fatal Nipah virus cases transmitted, and a median time to death of Nipah virus cases of 6 days (96% of fatal cases died by day 15).

7. Cases and contacts included in risk factor analyses

The analysis of risk factors associated with the reproduction number (risk factor analysis I) was based on 248 Nipah virus cases identified in Bangladesh during 2001-2014 (Figure S4). Three secondary cases with uncertain infectors were excluded from the baseline analysis: two secondary cases each had two potential infectors, the third secondary case was part of a cluster but without reported exposure to cases other than the index case (who was excluded as infector due to the long delay between onset dates).

The analysis of risk factors associated with acquiring Nipah virus infection (risk factor analysis II) was based on 2,600 contacts of 140 Nipah virus cases identified during 2007-2014 (Figure S4). Six of 2,606 case contact pairs were excluded from the analysis: two individuals who had acquired Nipah virus but were each in contact with two potential infectors (four contacts) and one individual who had acquired Nipah virus from the reservoir prior to contact to a Nipah virus case were completely excluded; one individual who acquired the infection from an identified source before exposure to a second Nipah virus case was excluded from the contacts of this second case.

8. Fit of the negative binomial model

To choose a suitable model to investigate case characteristics associated with the reproduction number, we assessed the fit of a negative binomial distribution and the fit of a zero-inflated negative binomial distribution (allowing for excessive zeros) to the observed number of secondary cases per case. We selected the negative binomial model based on

visual assessment (Figure S5) and a lower Akaike Information Criterion (AIC) (negative binomial 243; zero-inflated negative binomial 245).

9. Association between number of secondary cases and number of contacts per case

Based on 140 Nipah virus cases with available contact information, we investigated whether the number of caused secondary cases was associated with the number of contacts a case reported using negative binomial regression analysis. We did not find a significant association between the number of secondary cases per case and the number of contacts a case reported (relative infectivity 1.4, 95%CI 0.8 to 2.7 per increase of 10 contacts, P=0.36) (Figure S6). The number of contacts with >12 hours exposure (median of 5 contacts for cases who spread versus a median of 6 contacts for cases who did not spread, Wilcoxon rank sum test P=0.20), the number of contacts exposed to cases body fluids (median of 5 contacts for cases who spread versus a median of 6 contacts for cases who did not spread, P=0.29), and the number of contacts with >12 hours and body fluid exposure (median of 2.5 contacts for cases who spread versus a median of 4 contacts for cases who did not spread, P=0.09) did not differ significantly between cases who infected ≥1 individuals and cases who did not spread. This further supports the finding of the analysis of the contacts that the major determinant of Nipah virus transmission remained the specific case the contact was exposed to rather than the attributes of the contact.

10. Covariates in risk factor analyses

10.1. Categorization of variables

For continuous variables, we first visually assessed associations with the reproduction number (case age, time to death, time to hospitalisation) or the risk of acquiring infection (contact exposure duration) (Figure S7). We further compared the fit of models including each variable as a linear term or as a non-linear term (spline of second degree) based on the AIC, where a lower AIC generally indicates a better model fit. We considered a difference in AIC (ΔAIC) of ≥2 as substantial. Non-linear models had generally a lower AIC; for time to death, time to hospitalisation and exposure duration the difference in AIC was substantial. For the ease of interpretation and to take these non-linear associations into account, we categorized continuous variables as follows:

- Age (AIC linear 226 vs. non-linear 225, ΔAIC 1): ≤14, 15 to 29, 30 to 44, ≥45 years
- Illness outcome (AIC linear 229 vs. non-linear 207, ΔAIC 22): survived ≤7 days after symptom onset, survived >7 days after symptom onset
- Hospitalisation (AIC linear 210 vs. non-linear 207, ΔAIC 3): admitted ≤2 days, 3 to 5 days, ≥6 days after symptom onset, and not hospitalized
- Exposure duration (AIC linear 263 vs. non-linear 251, ΔAIC 12): ≤1, >1 to 6, >6 to 12, >12 to 24, >24 to 48 and >48 hours

10.2. Exposure type groupings

During contact tracing, detailed information was collected about the type of exposure to the Nipah virus case. Contacts were asked about 38 different exposure types: eat with the case; share food with the case from the same plate or bowl; share the same cup, glass, or plate without washing them; feed the person with a spoon or cup; feed the case with hands; talk to the case; touch the case; hold the case's hands; touch the case's face; hug the case; kiss the case; share a bed with the case; help the case walk, sit or stand; lift or carry the case; clean the case's hands with a cloth or clothing; clean the case's face with a cloth or clothing; wipe saliva from around the case's mouth; wipe mucus from the case's face; wipe the case's face with hands; wipe the case's nose or mouth with hands; clean vomit from the case's body; put lip gel on the case's lips; help the case change clothes; help the person use the toilet; clean faeces from the cases body; change the case's bed linens; wash the case's clothes; wash the case's bed linens; help the case bathe; have fluid from the case on skin; eat some of the food that the case started eating but did not finish; whisper religious verses in the case's ear; share a cigarette with the case; receive a cough from the case in face; receive a sneeze from the case in face; being spit on by the case; being in same room/ veranda/ vehicle when the case was vomiting; clean the case's mouth and nose after death.

We aggregated 32 of these 38 exposures into the following non-exclusive exposure type groupings to investigate support for the main transmission hypotheses.

Physical contact with a Nipah virus case. Previous studies based on animal models showed that physical contact is necessary for successful transmission of Nipah virus.[16] We therefore investigated whether physical contact with a case was also a significant risk factor for the transmission of Nipah virus among humans.

Touching the face of a Nipah virus case. It has been previously suggested that Nipah virus is transmitted through contact with respiratory secretions.[1,17,18] Such secretions are mainly located around the mouth and nose of a case and touching the face of a case may increase exposure risk to such secretions and potentially also the risk of acquiring Nipah virus infections.

Contact with respiratory secretions of a Nipah virus case. Contacts may also have exposure to respiratory secretions without touching the case's face, for example through direct exposure to cases' body fluids, but also through large droplets (e.g., when being coughed or sneezed on by a case). We investigated if exposure to cases' body fluids increased the risk of acquiring Nipah virus infections and included in this category all fluids that were potentially respiratory secretions. One included exposure (i.e., having case's body fluids on skin) may however also represent fluids not related to respiratory secretions such as urine or vomitus.

Contact with an item that was touched by a Nipah virus case. Previous studies found Nipah virus RNA on items in proximity to Nipah virus cases in hospitals (e.g., on the walls close to the bed).[12] We therefore investigated if the risk of acquiring Nipah virus infection increased by sharing items with a case.

Post-mortem exposure. Previous studies provided evidence for Nipah virus transmission after the death of a case.[19,20] Drying out the mouth and nose of deceased individuals is a specific post-mortem procedure carried out during funeral preparations. We therefore additionally investigated whether this specific exposure type constituted a risk factor for acquiring Nipah virus infection among spreader contacts.

The components of each exposure type group are provided in Table S3.

11. Sensitivity analysis

11.1. Reproduction number

To assess the epidemic potential of Nipah virus we estimated the reproduction number among primary cases and assessed sensitivity of estimates to changes in surveillance or implementation of interventions (Table S4).

Potential bias of the estimated reproduction number due to changes in surveillance or implementation of interventions

The reproduction number may be higher than the baseline estimate if disease control interventions were implemented during later stages of the outbreaks. To assess potential effects of interventions we estimated the reproduction number for (i) only primary Nipah virus cases and (ii) only Nipah virus cases who were not hospitalized or hospitalized only after more than 7 days since symptom onset. The 166 primary cases infected 50 individuals, corresponding to a reproduction number among primary cases of 0.30 (95%CI 0.15 to 0.61). Luby et al. previously reported a reproduction number of 0.48 among 60 primary cases detected during 2001-2007.[1] Improved surveillance since 2007, and hence better capacity to detect small clusters, may explain the difference between these two estimates. Among 48 cases who were hospitalized late or were not hospitalized we estimated that the reproduction number was 0.60 (95%CI 0.07 to 4.97). In contrast, the reproduction number may be lower than the baseline estimate if single cases or smaller case clusters were less likely to be detected than larger transmission chains. Such surveillance bias may have occurred particularly before the implementation of sentinel surveillance in 2007. To assess potential effects of changes in the surveillance system, we estimated the reproduction number only for Nipah virus cases identified during 2007-2014 after the implementation of the sentinel surveillance system. Among 146 Nipah virus cases identified during 2007-2014, we estimated that a Nipah virus case infected on average 0.23 individuals (95%CI 0.11 to 0.46).

Although estimates of the reproduction number may be slightly biased by surveillance or interventions, this does not affect the conclusion that the transmission potential is currently lower than what is required for self-sustaining transmission.

11.2. Risk factors associated with the reproduction number

11.2.1. Secondary cases with multiple potential infectors

Here we evaluated the sensitivity of risk factor estimates to the inclusion of the three secondary cases with multiple potential infectors by assigning the secondary cases to each of their potential infectors (Table S5). Comparable to the baseline analysis, age and difficulty breathing were significantly associated with the reproduction number in each of the 44 infector scenarios in the multivariable analysis. The effect of age varied only slightly depending on the infector scenario. All scenarios resulted in a lower point estimate of the relative infectivity of cases with difficulty breathing, the estimates were however within the 95%CI of the baseline analysis.

11.2.2. Effect of individual cases on risk factor estimates

To investigate whether risk factors associated with the reproduction number were driven by any specific case, in particular superspreaders who infected a larger number of persons, we performed leave-one-out negative binomial regression (Figure S8). The age profile was influenced by a male 60 year-old Nipah virus case with difficulty breathing who died five days after illness onset and infected 21 individuals. Excluding this case, the reproduction number peaked in 30 to 44 year-old cases instead of ≥45 year-olds; associations with other case characteristics were however not strongly dependent on this case (Figure S9).

11.3. Risk factors associated with infection among contacts

11.3.1. Association between infection among contacts and relationship to the case

We excluded the relationship to the case from the multivariable analysis as the aim of the analysis was to identify underlying mechanisms of transmission while relationship likely represents a combination of measured and unmeasured risk factors. Indeed, we observed strong associations between being the spouse of a case and exposure duration (median 81 hours vs. 5 hours; Wilcoxon rank sum test $P<0.001$) or type (Table S6).

11.3.2. Infected contacts with multiple potential infectors

Six case-contact pairs were excluded from the baseline analysis as explained in Section 5. Here we evaluated the sensitivity of risk factor estimates to inclusion of the contacts with two potential infectors by assuming the four possible infector scenarios (Table S7). Consistent with the baseline analysis, the risk of acquiring Nipah virus was associated with duration of exposure and contact to cases' body fluids in all four scenarios in the multivariable analysis. Although the effect estimates varied slightly dependent on the scenario, point estimates were within the 95%CI of the baseline analysis.

11.3.3. Missing exposure types

We classified exposure type groups as positive if any of the underlying exposures was positive, including in the case where other underlying exposures were missing. However, in the case where an underlying exposure was missing and no other underlying exposure was positive, the overall exposure type group was classified as missing. To assess any potential bias introduced by this classification algorithm, we imputed all missing exposure type

groups by either positive or negative values. Both imputations led to consistent results with the baseline analysis, where exposure to body fluids and duration of exposure were associated with the risk of acquiring infection in multivariable analysis (Table S8). Effect estimates varied only minimally.

12. Case fatality among Nipah virus cases by age

We estimated the case fatality among age-groups of Nipah virus cases (≤14, 15 to 29, 30 to 44, ≥45 years) with 95% confidence intervals (95% CIs) based on the Pearson-Klopper exact method (Figure S10).

13. Using case characteristics to identify Nipah virus spreaders

Nipah virus cases represent a small proportion of meningoencephalitis patients in Bangladesh.[5] In the absence of rapid diagnostic tests, Nipah virus cases are generally identified too late for effective precautionary measures and, therefore, all meningoencephalitis cases would need to receive an intervention to prevent Nipah virus person-to-person transmission. In a resource limited setting as Bangladesh, the scale of such an intervention is unrealistic and targeting a subset of meningoencephalitis cases, which include those Nipah virus cases that are most likely to transmit, may provide an opportunity to reduce the strain on the healthcare setting.

Overall, 63% of all Nipah virus cases had difficulty breathing. Focusing targeted interventions only on identifying and isolating these individuals would result in the identification of 91% of the cases who infected ≥1 individuals (Figure S11A) and 100% of cases who infected ≥2 individuals (Figure S11B). To assess a combined criterion based on age and difficulty breathing, we first identified a suitable age cut-off based on a receiver operating characteristics (ROC) curve (Figure S11C). An age cut-off of >25 years, representing 46% of all cases, would allow the identification of 95% of cases who infected ≥1 individuals and 100% of cases who infected ≥2 individuals. A combined criterion based on age AND difficulty breathing, representing 33% of all cases, would detect 86% of cases who infected ≥1 individuals and 100% of cases who infected ≥2 individuals. Selecting cases based on age>25 OR difficulty breathing would allow detecting 100% of cases who infected ≥1 individuals, however the selected cases would also represent 77% of all cases. Specificities for the identification of cases who infected ≥1 individuals (≥2 individuals) were 58% (57%) for age alone, 40% (40%) for difficulty breathing alone, 73% (71%) for the age AND difficulty breathing criterion, and 25% (24%) for the age OR difficulty breathing criterion.

14. Viral loads in Nipah virus cases

We tested throat swab samples of 46 Nipah virus cases (including 3 Nipah virus spreaders) by real-time PCR as previously described in Lo et al..[21] Nipah virus RNA was detected in throat swabs of 22 Nipah virus cases (1 spreader) (Figure S12). The measured cycle threshold value was not lower (indicative for a higher viral load) for the spreader than for other cases.

15. Sequenced Nipah virus strains

Diversity in virus strains may have contributed to variations in infectivity. Investigating the contribution of genetic diversity to the transmission heterogeneities observed in Bangladesh is however currently not possible due to the limited number of sequenced virus strains. Isolating and sequencing Nipah virus strains from patients has been extremely difficult. Lo et al.[21] for example attempted virus isolations from 21 Nipah virus patients, resulting in only 2 successful isolations. Moreover, Nipah virus cases who transmitted often died before a sample for biological analysis could be obtained (in our dataset, biological samples were obtained for 5 of the 22 Nipah virus spreaders, only 3 of them had samples taken during the first week of the illness). As a consequence of these difficulties, only 3 full genome sequences from human patients in Bangladesh are currently available (1 from 2004[22], and 2 from 2008[21]).

Supplementary Figures

Figure S1. Reported exposure of 53 secondary cases to their 19 infectors by day of illness in the infector. The figure shows exposure days of five infectors (#1 to 5) with their 20 secondary cases from an outbreak in 2004 where detailed outbreak investigations were done and 14 infectors (#6 to 19) with their 33 secondary cases included in routine contact tracing procedures during 2007-2014. Each block represents an infector (first row) and the caused secondary infections (each secondary case is a subsequent row in the block). Not shown are the exposure windows of two further secondary cases with multiple potential infectors. The other 27 secondary cases without reported exposure windows were included in the model based on the dates of symptom onset and death of their infectors.

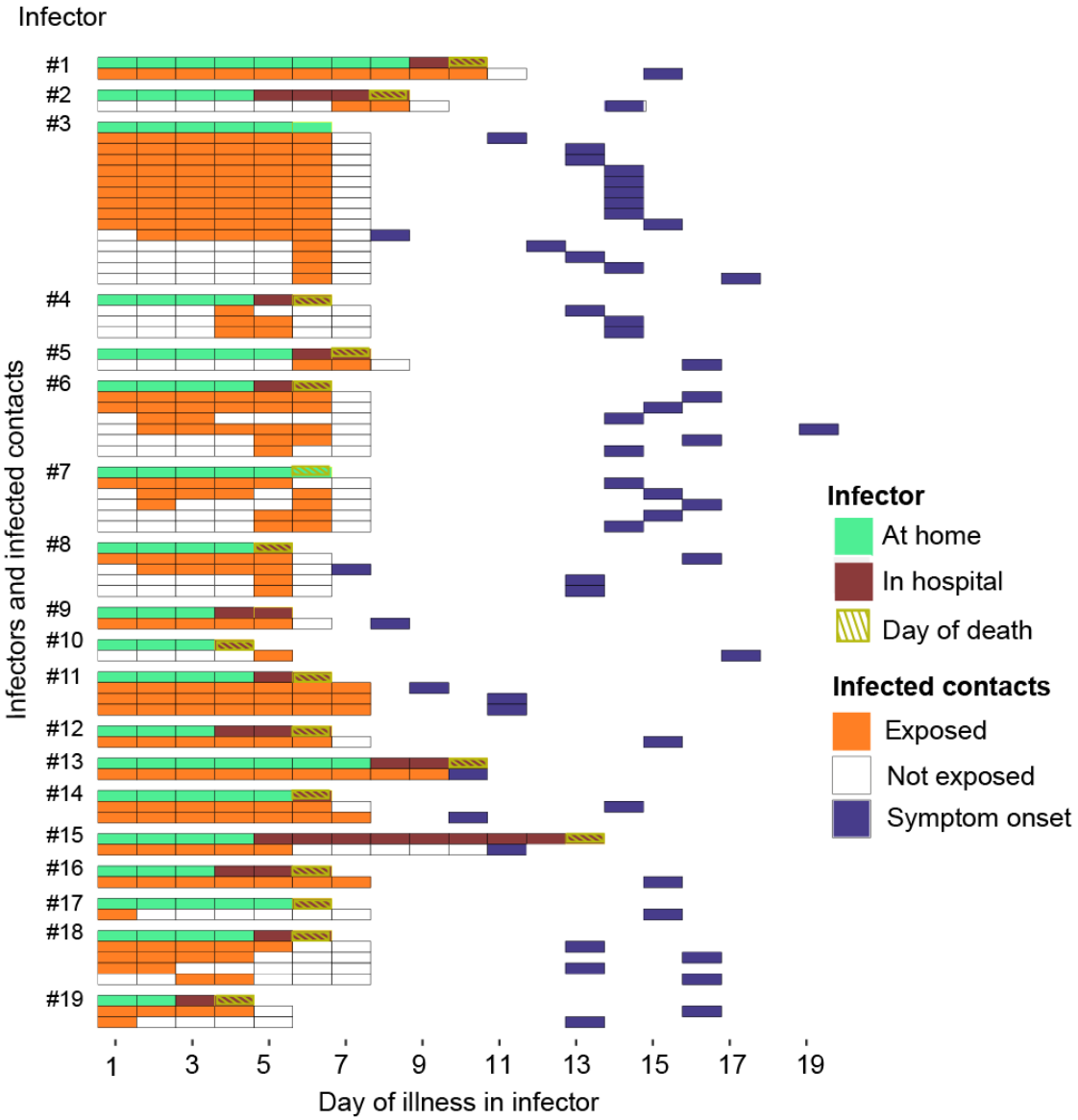

Infector
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
#11
#12
#13
#14
#15
#16
#17
#18
#19
Infectors and infected contacts
1
3
5
7
9
11
13
15
17
19
Day of illness in infector
Infector
At home
In hospital
Day of death
Infected contacts
Exposed
Not exposed
Symptom onset

Figure S2. Incubation period distribution in secondary Nipah virus cases. The probability of developing symptoms by day since infection was estimated based on a statistical model using information on exposure timing and symptom onset dates. The observed number of cases developing symptoms by day since infection was based on 11 secondary cases with single day exposure.

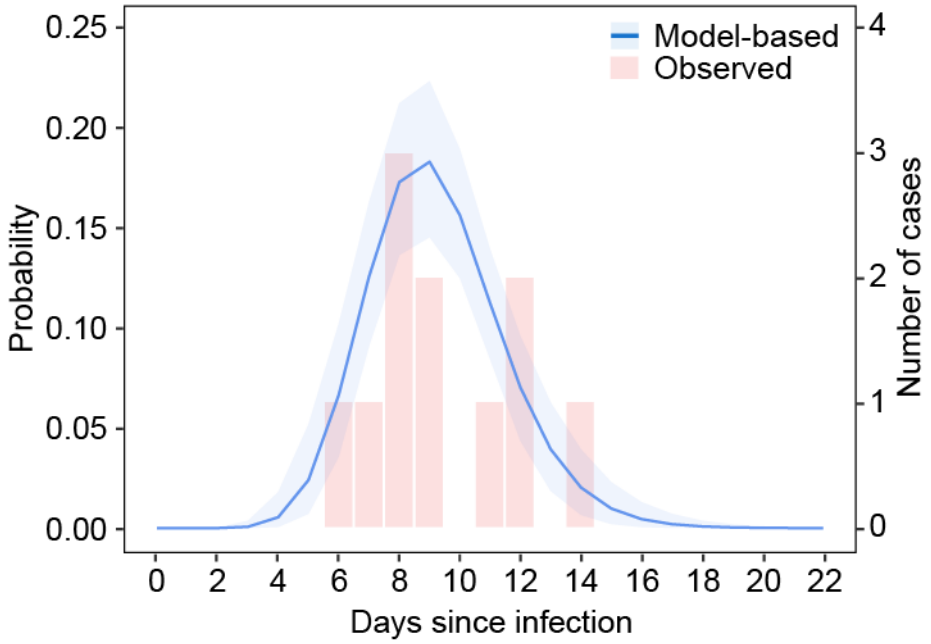


Figure S3. Cumulative distribution of latest day of contact to the case. The cumulative distribution is based on 53 secondary cases with exposure time information.

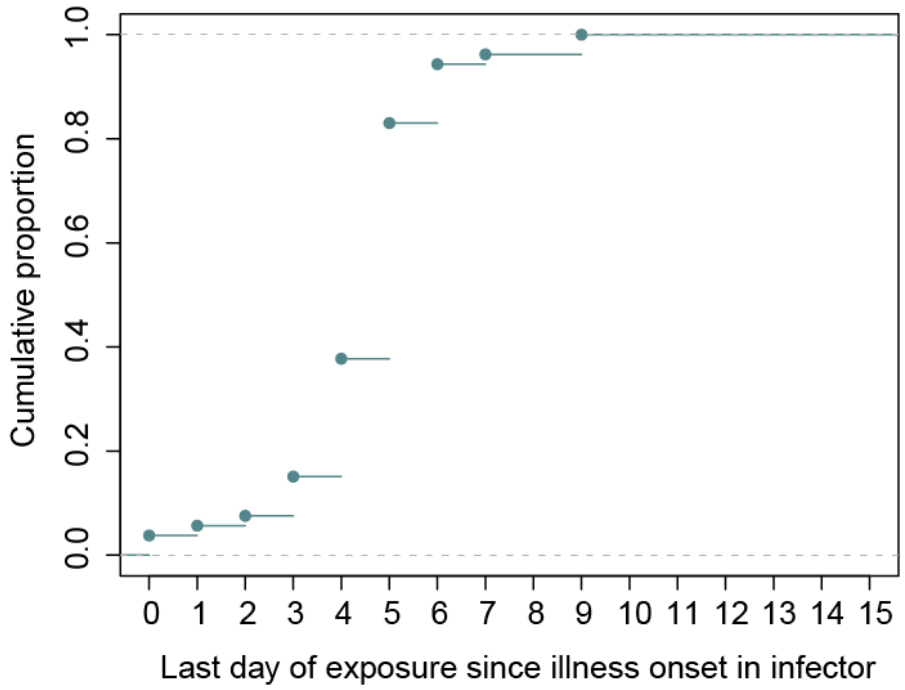
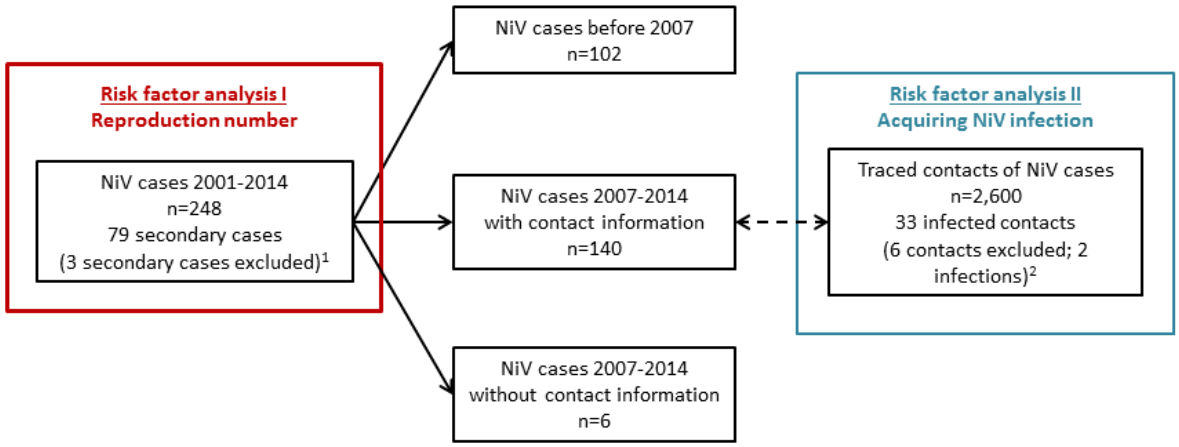


Figure S4. Data from Nipah virus cases and their contacts included in the two risk factor analyses.



[1]Three secondary cases with uncertain infector.

[2]Four contacts with uncertain infector, one contact infected by reservoir and one contact infected by another NiV case.

Figure S5. Fit of a negative binomial model to the distribution of observed number of secondary cases per case.

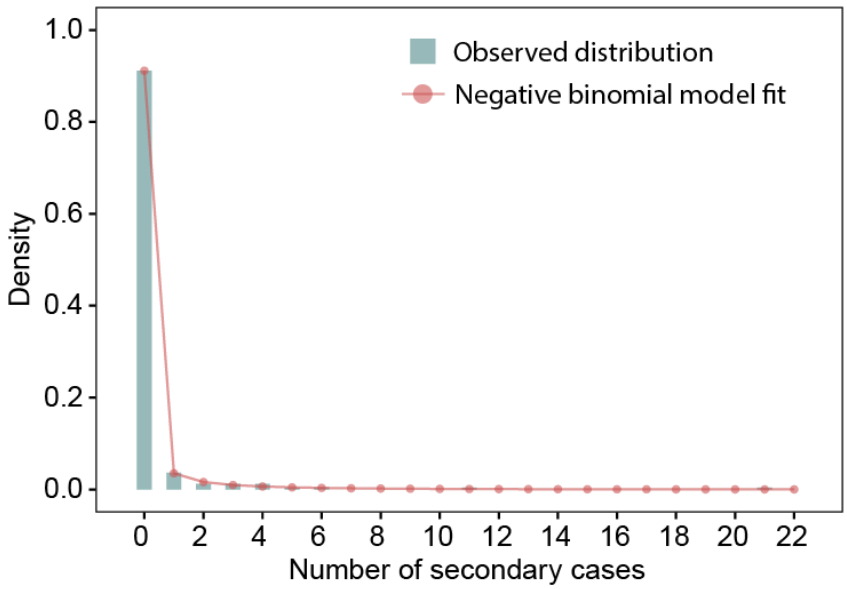


Figure S6. Number of secondary cases per case by number of reported contacts per case.

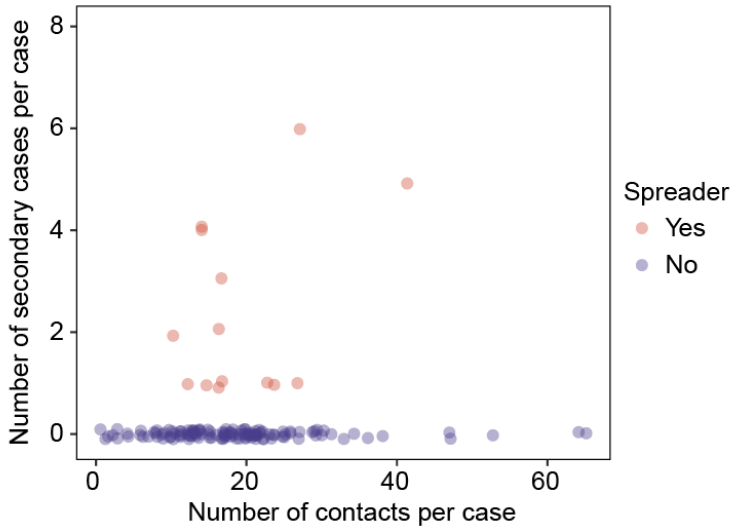


Figure S7. Case and contact characteristics associated with onward transmission and infection. (A) Number of secondary cases by case age. Category boundaries are indicated as dashed lines. (B) Number of secondary cases by time to death. (C) Number of secondary cases by time to hospitalisation. (D) Proportion infected by duration of exposure. Proportions were calculated for groups of 50 contacts ordered by their exposure duration.

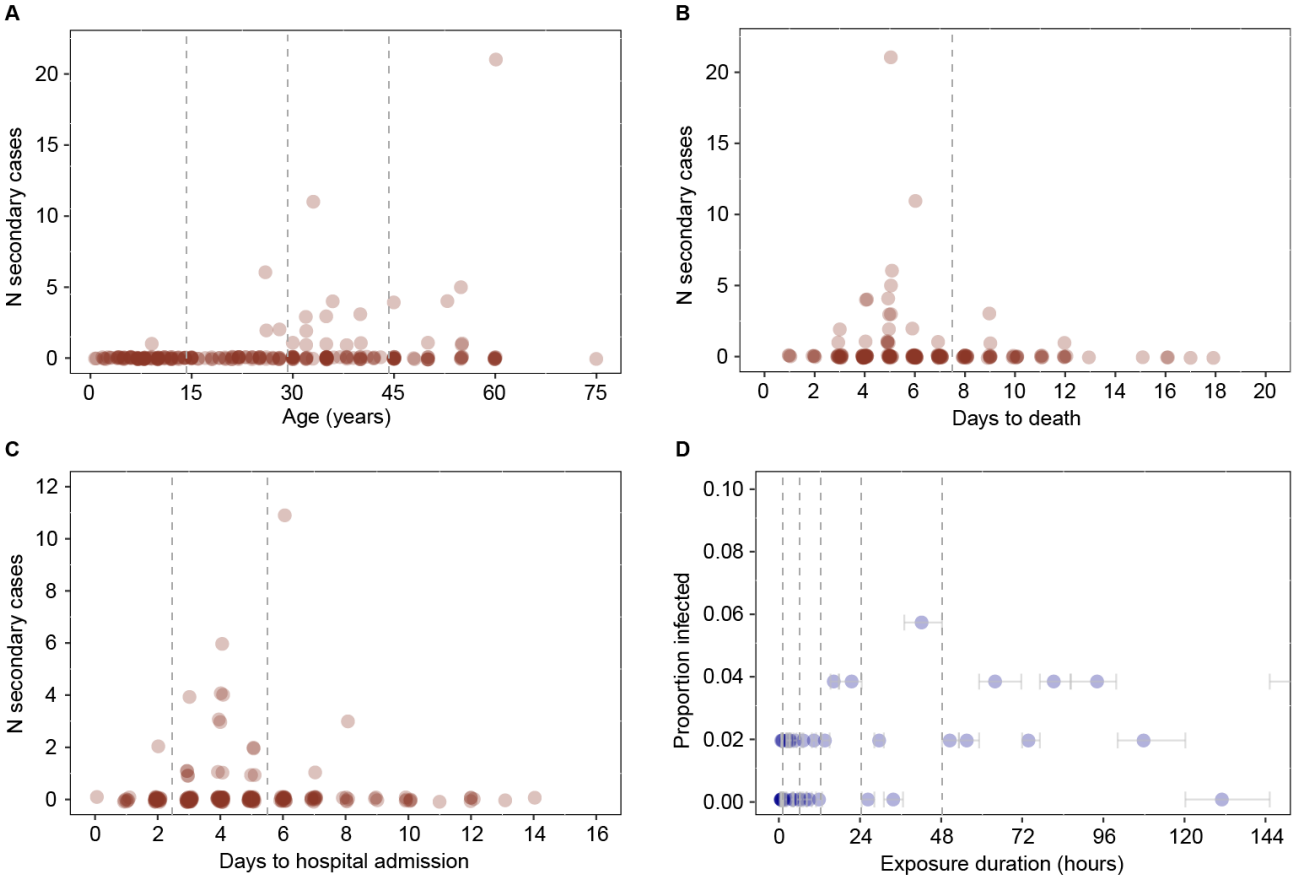


Figure S8. Sensitivity of unadjusted estimates to exclusion of a superspreader who infected 21 individuals. Panel A shows the reproduction number by age groups and panel B shows relative infectivity of cases depending on case characteristics.

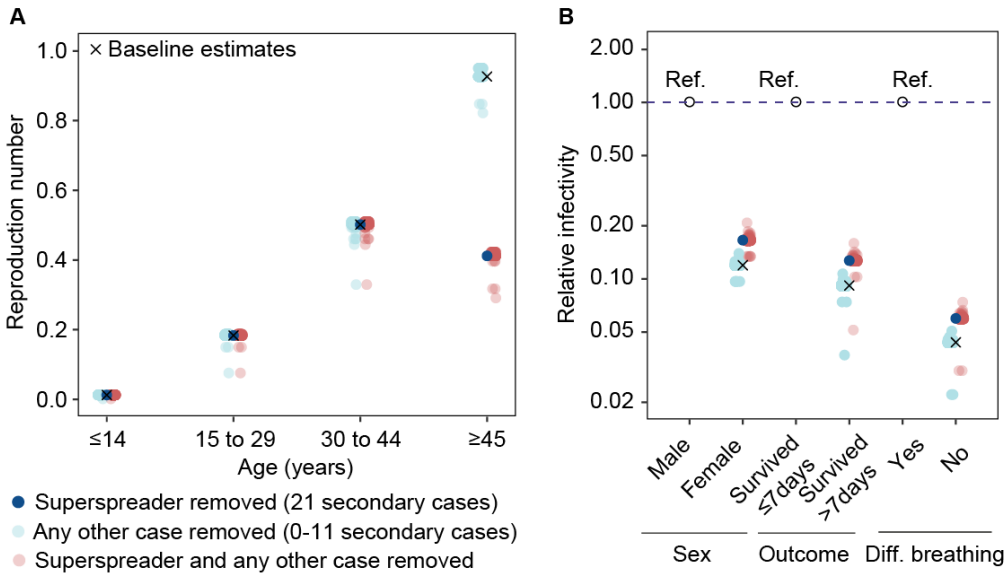


Figure S9. Sensitivity of adjusted estimates to exclusion of a super spreader (s.s) who infected 21 individuals.

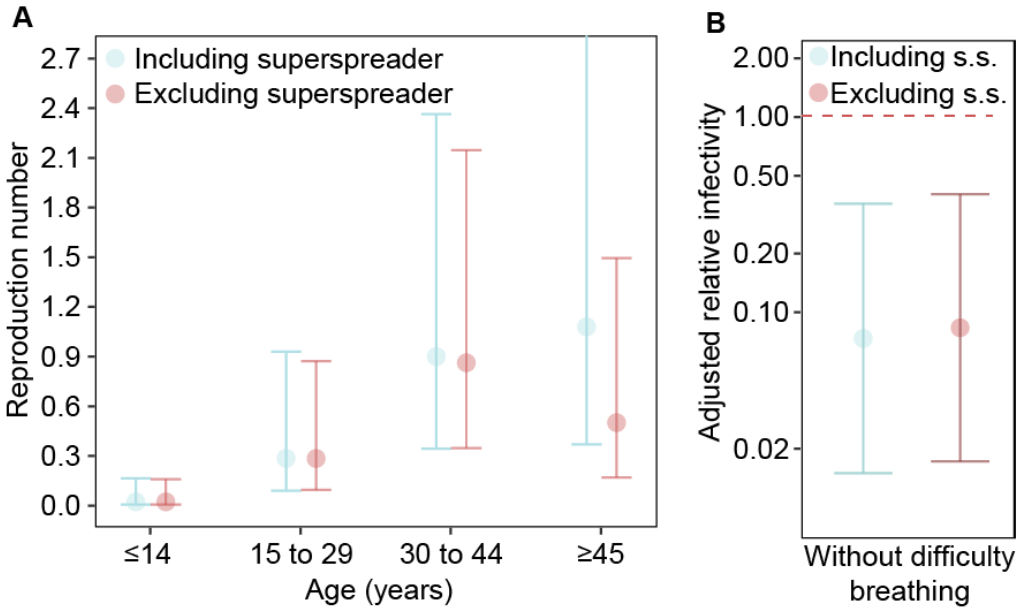


Figure S10. Case fatality among Nipah virus cases by age.

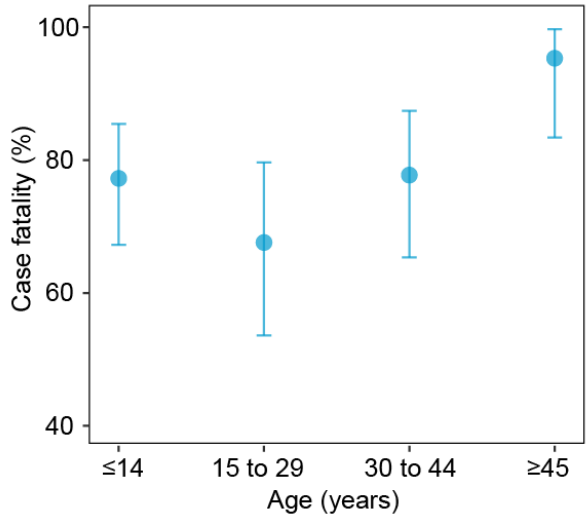


Figure S11. Identification of Nipah virus spreaders who infected ≥1 individuals (A) or ≥2 individuals (B) based on case characteristics. The graph shows the proportion of Nipah virus spreaders who infected ≥ 1 or ≥ 2 individuals against the proportion of all Nipah virus cases that were selected based on different case criteria. We assessed the following case characteristics: Nipah virus cases (i) with difficulty breathing, (ii) aged >25 years, (iii) aged >25 years with difficulty breathing, and (iv) aged >25 years or with difficulty breathing. **(C) ROC analysis to find an age-cut off with reasonably high sensitivity and specificity to identify Nipah virus spreaders.** The cross indicates sensitivity and specificity at the chosen cut-off of >25 years.

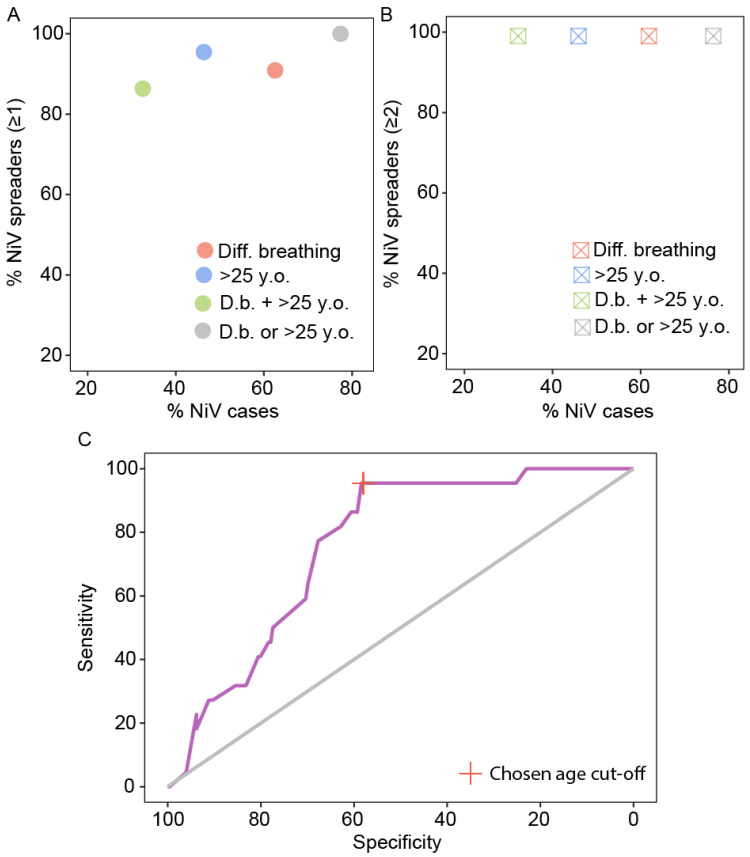
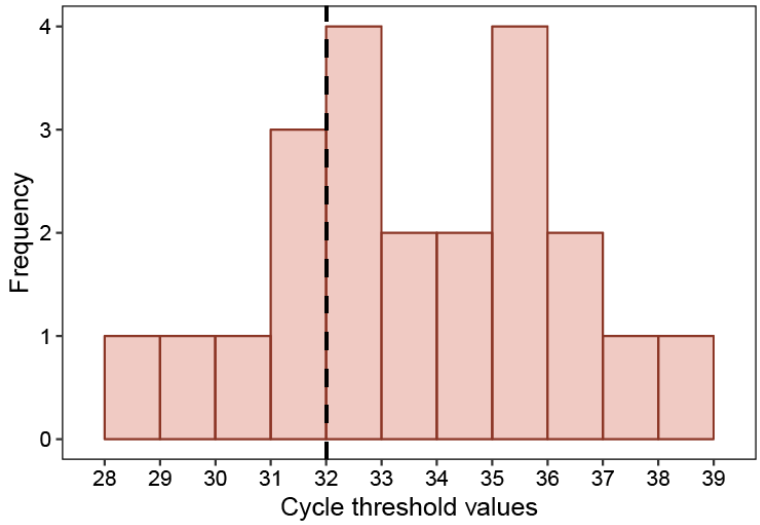


Figure S12. Cycle threshold (CT) values in throat swab samples of Nipah virus cases. Lower cycle threshold values are indicative of higher viral loads. The dashed line indicates the CT value of the case who transmitted Nipah virus to another person.



Supplementary Tables

Table S1. Nipah virus cases from Bangladesh included in previous reports by year.

Year	Number of Nipah virus cases	Partially or fully reported in
2001	13	1,2,5,11
2003	12	1,2,5,11
2004	65	1,2,4,5,12,13
2005	12	1,4,5
2007	18	1,4,5,23,24
2008	10	4,5,21,25
2009	2	4,5,21
2010	19	3-5,19-21
2011	41	3-5,19
2012	16	3,4,26
2013	26	3,26,27
2014	14	3,27

Table S2. Characteristics of confirmed and probable Nipah virus cases.

	Confirmed cases (N=141)		Probable cases (N=107)		
	n/N[1]	% (95%CI)[2]	n/N[1]	% (95%CI)[2]	P value[3]
Male	83/141	59 (50; 67)	75/107	70 (60; 79)	0.08
Age (years)					
≤14	60/141	43 (34; 51)	31/107	29 (21; 39)	<0.001
15 to 29	37/141	26 (19; 34)	18/107	17 (10; 25)	
30 to 44	32/141	23 (16; 31)	30/107	28 (20; 38)	
≥45	12/141	9 (4; 14)	28/107	26 (18; 36)	
Illness outcome					
Survived ≤7 days	54/140	39 (30; 47)	86/104	83 (74; 89)	<0.001
Survived >7days	86/140	61 (53; 70)	18/104	17 (11; 26)	
Hospitalised					
Within ≤2 days	16/141	11 (7; 18)	20/107	19 (12; 27)	0.11
Within 3 to 5 days	70/141	50 (41; 58)	58/107	54 (44; 64)	
Within ≥6 days	40/141	28 (21; 37)	18/107	17 (10; 25)	
Not hospitalized	15/141	11 (6; 17)	11/107	10 (5; 18)	
Infected by reservoir	91/141	65 (56; 72)	75/107	70 (60; 79)	0.41
Symptoms					
Difficulty breathing	73/139	53 (44; 61)	79/104	76 (67; 84)	<0.001
Cough	58/138	42 (34; 51)	66/104	63 (53; 73)	<0.001
Vomiting	75/139	54 (45; 62)	59/105	56 (46; 66)	0.79

[1]n number of cases with characteristics, N number of cases with available information

[2]exact 95% confidence interval

[3]Fisher's exact test

Table S3. Exposure types. Exposure types were combined into groups to investigate support for the main transmission hypotheses.

Transmission hypothesis	Included exposure types
Physical contact with a case	Touch case; hold case's hands; touch case's face; hug case; kiss the case; clean case's hands; clean case's face; wipe saliva around case's mouth; wipe mucus from case's face; wipe case's face with hands; wipe case's nose or mouth with hands; clean vomit from case's body; clean faeces from case's body; help case bathe; feed case with hands; put lip gel on lips of case; share a bed with the case; help case walk, sit, or stand; help case change clothes; help case use toilet; lift or carry the case
Touching the face of a case	Feed case with hands; touch case's face; kiss the case; clean the case's face; wipe case's face with hands; wipe case's nose or mouth with hands; put lip gel on lips of case
Contact with respiratory secretions of a case	Have case's fluid on skin[1]; wipe saliva around case's mouth; wipe mucus from case's face; receiving a sneeze in face from case; receiving a cough in face from case; being spit on by case
Contact with an item that was touched by a case	Share food with case from same bowl; share cup with case; change bed linens of case's bed; wash clothes of case; wash bed linens of case's bed; share cigarette with case
Post-mortem exposure	Drying out the nose or mouth of the case after death

[1]For Nipah virus cases, such body fluids are often respiratory secretions; discrimination between respiratory secretions and other fluid types was however not possible.

Table S4. Sensitivity of the estimated reproduction number.

Sensitivity analysis	N cases	N secondary cases	Reproduction number[1]
Cases identified 2007-2014	146	33	0.23 (0.11; 0.46)
Primary cases	166	50	0.30 (0.15; 0.61)
Cases hospitalized >7 days since symptom onset or not hospitalized	48	29	0.60 (0.07; 4.97)
Baseline analysis	248	82	0.33 (0.19; 0.59)

[1]Point estimate ranges for infector scenarios or point estimates with 95%CIs

Table S5. Sensitivity of adjusted relative infectivity estimates to inclusion of three secondary Nipah virus cases with two potential infectors (resulting in 44 possible infector scenarios). Age and difficulty breathing were significantly associated with the reproduction number in all 44 scenarios.

	Infector scenarios (N=44)		Baseline analysis	
	Adj. relative infectivity point estimate range	**N scenarios with P value <0.05**	**Adj. relative infectivity (95%CI)**	**Adj. P value**
Age (years)				
≤14	0.01 - 0.04	44	0.02 (0.00; 0.2)	<0.001
15 to 29	0.2 - 0.3		0.3 (0.05; 1.3)	
30 to 44	0.6 - 1.0		0.8 (0.2; 3.4)	
≥45	Ref.		Ref.	
Difficulty breathing (vs. no difficulty breathing)	8 – 11	44	19 (3.2; 114)	<0.001

Table S6. Associations between exposure types and being the spouse of a case. The odds ratio compares the odds of a specific exposure type among spouses to other contacts.

	Exposure type OR (95%CI)	**P value**
Touching case's face	13 (1.8; 97)	<0.001
Contact with items touched by case	14 (7.2; 28)	<0.001
Contact with case's body fluids	7.5 (3.9; 15)	<0.001
Clean out mouth and nose of case after death	2.9 (1.6; 5.2)	<0.001

Table S7. Sensitivity of adjusted risk factor estimates for acquiring infection to inclusion of two secondary cases with multiple potential infectors (resulting in 4 scenarios).

	Infector scenarios (N=4)		Baseline analysis	
	adj. OR point estimate range	**N scenarios adj. P value <0.05**	**adj. OR (95%CI)**	**adj. P value**
Exposure duration (hours)				
≤1	Ref.	4	Ref.	0.005
>1 to 6	2.8 - 3.0		2.2 (0.5; 10)	
>6 to 12	1.8 - 1.9		1.8 (0.3; 13)	
>12 to 24	11 - 12		13 (2.0; 86)	
>24 to 48	7.1 - 8.9		9.3 (1.4; 62)	
>48	9.9 - 11		13 (2.6; 62)	
Contact with case's body fluids (vs. no contact with body fluids)	3.7 - 5.1	4	4.3 (1.6; 11)	0.003

(adj. OR) adjusted odds ratio; (adj. P value) adjusted P value

Table S8. Sensitivity of adjusted risk factor estimates for acquiring infection to missing exposure types in uNipah virusariable and multivariable analysis. Missing exposure types were once all imputed as positive exposures and another time as negative exposures.

	N	Baseline		Imputed as positive		Imputed as negative	
	imputed	OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)	P value
Contact with case's body fluids (vs. no contact with body fluids)	4	7.6 (3.1; 19)	<0.001	7.6 (3.1; 19)	<0.001	7.6 (3.1; 19)	<0.001
Touching face (vs. not touching face)	84	1.2 (0.4; 4.2)	0.74	1.2 (0.4; 4.2)	0.75	1.5 (0.5; 4.3)	0.45
Contact with items touched by a case (vs. not contact with items)	4	2.3 (0.9; 5.8)	0.10	2.5 (1.0; 6.4)	0.06	2.1 (0.8; 5.5)	0.12
Drying out mouth (vs. not drying out mouth)	1	1.9 (0.7; 5.3)	0.26	2.1 (0.8; 5.9)	0.15	1.8 (0.6; 5.1)	0.29
		adj. OR (95%CI)	adj. P value	adj. OR (95%CI)	adj. P value	adj. OR (95%CI)	adj. P value
Contact with case's body fluids (vs. no contact with body fluids)	4	4.3 (1.6; 11)	0.003	4.3 (1.6; 11.3)	0.003	4.3 (1.6; 11)	0.003
Exposure duration (hours)							
≤1	NA	Ref.	0.005	Ref.	0.005	Ref.	0.005
>1 to 6	NA	2.2 (0.5; 10)		2.2 (0.5; 10)		2.2 (0.5; 10)	
>6 to 12	NA	1.8 (0.3; 13)		1.8 (0.3; 13)		1.8 (0.3; 13)	
>12 to 24	NA	13 (2.0; 86)		13 (2.0; 86)		13 (2.0; 86)	
>24 to 48	NA	9.3 (1.4; 62)		9.4 (1.4; 62)		9.3 (1.4; 62)	
>48	NA	13 (2.6; 62)		13 (2.6; 62)		13 (2.6; 62)	

(OR) odds ratio; (adj. OR) adjusted odds ratio; (adj. P value) adjusted P value

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