INVITED ORIGINAL ARTICLE

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Incubation Period of Ebola Hemorrhagic Virus Subtype Zaire

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Abstract
Objectives: Ebola hemorrhagic fever has killed over 1300 people, mostly in equatorial Africa. There is still uncertainty about the natural reservoir of the virus and about some of the factors involved in disease transmission. Until now, a maximum incubation period of 21 days has been assumed.
Methods: We analyzed data collected during the Ebola outbreak (subtype Zaire) in Kikwit, Democratic Republic of the Congo, in 1995 using maximum likelihood inference and assuming a log-normally distributed incubation period.
Results: The mean incubation period was estimated to be 12.7 days (standard deviation 4.31 days), indicating that about 4.1% of patients may have incubation periods longer than 21 days.
Conclusion: If the risk of new cases is to be reduced to 1% then 25 days should be used when investigating the source of an outbreak, when determining the duration of surveillance for contacts, and when declaring the end of an outbreak.

1. Introduction

Ebola hemorrhagic fever, caused by the Ebola virus (EBOV) is a severe and often fatal disease. The first known outbreak occurred in Zaire (now the Democratic Republic of Congo) in 1976. Since then, single cases and large epidemics have recurred in equatorial Africa. Apart from Zaire, Sudan, Gabon, Ivory Coast, Uganda, Kenya, Angola and the Republic of the Congo have all been affected [1]. Until now, more than 1850 cases and about 1300 deaths have been reported [2]; the latest are from an outbreak in Uganda. Marburg virus, a close relative of EBOV [3], also causes severe outbreaks of hemorrhagic fever in Africa [4]. There are four known subtypes of EBOV named after the place of their first appearance: Zaire, Sudan, Ivory Coast, and Reston in Virginia, USA. The former three cause severe forms of the disease in humans. Subtype Reston is believed not to be dangerous in humans, but it can be fatal in non-human primates [5−7] and pigs [8]. A new strain of EBOV with the proposed name Bundibugyo EBOV was discovered in a Ugandan epidemic in November 2007 [9]. The subtypes have different mortality rates (about 90% for Zaire and 50% for Sudan) and may also have different incubation periods [10,11].

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The natural reservoir of the virus remains unknown [12,13]; bats and fruit bats are suspected to be hosts [14,15], and monkeys could be a vector in transmission to humans [16−18]. The two most important routes for person-to-person transmission seem to be direct physical contact and contact with body fluids. It is very likely that droplets or aerosols play little or no role [6,19,20]. When no symptoms are visible, EBOV-infected persons do not seem to be contagious [21,22]. The incubation period is generally assumed to range from 2 to 21 days [10,23−25]; for EBOV most calculations put it at 6 to 10 days [23,26,27]. Knowledge of the incubation period will aid research on EBOV transmission and is fundamental in determining for how long people should be monitored after having had contact with cases. This knowledge is also essential when investigating the animal source that might have triggered an epidemic. In this paper, we investigate the incubation period of EBOV using data collected during an outbreak in Kikwit, Democratic Republic of the Congo, in 1995 [22]. This data set is especially useful because it contains precise information on the contact behavior of 173 persons. We apply an estimation method that was used to study smallpox [28], but has never been used for Ebola hemorrhagic fever.

2. Materials and Methods

2.1. Description of the epidemic

In 1995, when the outbreak took place, Kikwit had about 200,000 inhabitants and only two hospitals that lacked electricity and running water [29]; protective equipment like gloves was in short supply [30]. On 6 January 1995, a forest worker fell ill with hemorrhagic symptoms and died some days later. In mid-April, a nosocomial outbreak began, leading to the spread of the disease within and between families in Kikwit and nearby areas. On 3 May, containment measures were implemented. One week later, EBOV was confirmed by the Centers for Disease Control and Prevention (Atlanta, USA) [31,32]. Having killed 81% of 315 infected people, the epidemic stopped on 16th July 1995 [5,19,33].

2.2. Data set

The data that we used were collected between 17 May and 3 June 1995 [22]. All members of every household from which a primary case had died or was discharged from hospital between 1 January and 7 May 1995 were interviewed. The resultant data set contains information on 27 households with a total of 23 primary cases and 173 contacts persons. It also contains detailed exposure information for each household member. We used this data to classify the contacts into strong contacts (contact with body fluids), weak contacts (direct physical contact but no contact with body fluids) and non-relevant contacts (only non-physical contact).

2.3. Model description and methods

Because the exact time of infection is not known, the duration of the incubation period could only be derived from the duration and intensity of contact with the index case. We were also forced to estimate the rates at which contacts were infected during the different periods of the disease in the index cases. Because the available information was not enough to allow us to estimate separate rates for the case's stay in the hospital and for the funeral, we used a common rate of infection for both periods. To distinguish between the effects of strong and weak contact and between contact at home and contact in hospital and during the funeral, and to keep the number of parameters to a minimum, we proposed three different contact models (Table 1). In Model 1, the infection rates caused by weak contact are the same at home and in hospital, whereas the infection rates produced by strong contact differ in the two places. In Model 2, the infection rates for weak contact differ at home and in hospital, and the infection rates for strong contact are obtained by adding a constant to the infection rate of the corresponding weak contact. In Model 3, the infection rates produced by strong contact differ in the two settings, and the infection rates produced by weak contact are obtained by multiplying the infection rate of the corresponding strong contact with a constant.

The incubation period is assumed to be lognormally distributed with mean μ and standard deviation (SD) σ. This type of distribution is frequently used to describe incubation periods of acute infectious diseases [34,35]. Having parameterized the distribution with mean μ and SD σ, the density function of the lognormal density is given by

$$f(x) = \frac{1}{\sqrt{2\pi}\sigma x} \exp\left(-\frac{(\ln x - \mu)^2}{2\sigma^2}\right),$$

where x is the duration of the incubation period.

Table 1. Infection rates $\lambda(t)$ for different contact types and disease periods for the Ebola outbreak in Kikwit 1995. *a, b* and *c* are parameters which are used to construct the infection rate for each day on which a contact between a case and a household member is reported

	At home		During hospitalization and funeral	
	Weak contact[a]	Strong contact[b]	Weak contact[a]	Strong contact[b]
Model 1	*a*	*a + b*	*a*	*a + c*
Model 2	*a*	*a + b*	*c*	*c + b*
Model 3	*a × b*	*b*	*a × c*	*c*

[a]Weak contact refers to direct physical contact; [b]Strong contact additionally involves contact with body fluids. For a detailed explanation, see the text.

We used maximum likelihood inference to estimate the parameters. This method is similar to the procedure proposed by Eichner and Dietz for smallpox incubation periods [25]. The likelihood contribution of each household member who escaped infection, is

$$e^{-\int_{t_0}^{\infty} \lambda(\tau)d\tau}$$

and the likelihood contribution of each household member who developed the disease, is

$$\int_{t_0}^{t_1} \lambda(t) * e^{-\int_{t_0}^{t} \lambda(\tau)d\tau} * f(t_1 - t)dt,$$

where t_0 is the time when the index case first developed symptoms and t_1 is the time when the household contact became ill. $\lambda(t)$ is the infection rate at time t (which depends on the type of contact and the stage of the disease in the index case; see Table 1). $f(t_1 - t)$ is the density of the incubation period (see $f(x)$ above) for the delay $t_1 - t$ between the household member becoming infected (unknown time t) and the onset of the disease (time t_1). Because of lack of information, we assumed that all household members who contracted the infection were infected by the index case in that household.

3. Results

Of the three models examined, the best was Model 1 with $c = 0$ (Table 1). The results of the maximum likelihood estimates and the supported ranges are given in Table 2.

The mean incubation period μ was estimated to be 12.7 days with a SD σ of 4.31 days. The force of infection a for all types of contact during hospitalization and the funeral, and for weak contact at home, was 0.0254 per day. This indicates that the probability of escaping infection for one week was 0.84 in spite of

Table 2. Maximum likelihood estimates and supported ranges for mean μ and standard deviation σ of the incubation period and for transmission possibilities of Ebola hemorrhagic fever in Kikwit in 1995

Parameter	MLE	Lower supported range	Upper supported range
μ	12.7 d	10.1 d	16.1 d
σ	4.31 d	2.56 d	8.60 d
a	0.0254/d	0.0118/d	0.0466/d
b	0.136/d	0.00550/d	0.370/d

having weak contact with the index case at home or at the hospital. The force of infection for strong contacts at home is $a + b = 0.161$ per day, indicating that the probability of escaping infection for 1 week is, in this case, only 0.32.

4. Discussion

In this study, the mean incubation period for EBOV hemorrhagic fever was estimated to be 12.7 days (SD 4.31 days). Earlier estimates for EBOV mostly indicated shorter incubation periods ranging from 6 days to 10 days [23,26,27]. Bwaka et al reported a longer incubation period for human-to-human transmission compared to the incubation period for infection caused by a needle prick [26]. In our study, infections were transmitted directly and not by needle prick [36], which may partly explain the longer incubation period estimate. A similar longer mean incubation period of 11.7 days was detected in EBOV infected gorillas [37]. For the Ebola-Sudan strain epidemic in Uganda in 2001, the biggest Ebola epidemic ever reported, the mean incubation period was calculated to range from 6.3 days to 12 days [38]. Bwaka et al and Breman et al estimated a mean incubation period for EBOV of 6.2 and 6.3 days, respectively, but both estimates were based on small data sets [23,26]. Lekone et al calculated a mean incubation period of 10.1 days for the same Kikwit 1995 epidemic [27] for which our data were collected; however, their estimate is not based on the same data set as ours and the authors assumed an exponential distribution for the incubation period. If our parameter estimations were also based on an exponentially distributed incubation period instead of a lognormal one, the estimated mean incubation period would drop to 12.0 days. A more empirical computation that distinguished between a minimum and a maximum incubation period was carried out by Dowell et al [22]. The minimum incubation period was calculated from the death of the index case to the onset of fever in the secondary case. The mean came out to be 7 days, and the range was from 1 day to 15 days. The maximum incubation period was calculated from the onset of fever in the index case to the onset of fever in the secondary case and the mean came out to be 17 days with the range going from 9 days to 25 days. Because infections occur between the onset of fever and the day of death, the mean incubation period should lie between 7 and 17 days.

Our study had a number of limitations: (i) when estimating the parameters, 31 of the 200 household members had to be excluded from the data set because important contact information was missing (two households with four and 19 members, respectively, that included seven secondary cases) or because the possibility that tertiary cases had occurred could not be ruled out (one household with eight members that included

three secondary cases); (ii) because we had to estimate the time of infection, there may be more variability in our estimate than if the time of infection had been known precisely; and (iii) different strains of the EBOV behave differently and the number of cases exposed via needles and via direct contact varies in different epidemics, making it difficult to extrapolate from historical data to future outbreaks.

However, our results suggest that a longer incubation period than previously assumed should be taken into consideration when exploring Ebola transmission possibilities and when searching for the original source of the infection that triggered an epidemic. In the past, EBOV transmission occurred when hunters [39] or scientists [40] came into contact with monkeys that were retrospectively assumed to be infected with EBOV. EBOV was propagated in human populations when needles and syringes were reused [19] or when health care workers or family members cared for Ebola cases [22,41]. Important tools in Ebola hemorrhagic fever containment are the surveillance of persons who have been in contact with Ebola-infected patients and the monitoring of convalescent patients before and after they have been discharged from hospital. The length of time these two measures should be carried out and the announcement of the end of an epidemic depend on the length of the incubation period. For all three scenarios that we modeled, a maximum incubation period of 21 days is normally assumed [19,33,36,38,42−44], meaning that in practice surveillance usually stops three weeks after the last contact. Epidemiologists have suggested that two consecutive incubation periods (a total of 42 days) must elapse before an outbreak is declared to be controlled [45,46]. By combining a rigorously collected data set with a new approach for calculating infectious disease incubation periods, our results suggest that the standard assumption of a maximum incubation period of 21 days may be incorrect. Our density function supports durations longer than 21 days. The risk of developing the disease after the 21st day is 4.1 %. To reduce the risk to 1%, 25 days should be taken as the incubation period. A surveillance duration of this length could be an acceptable compromise because: (i) the direct costs (surveillance) and indirect costs (economic cost of quarantines and lost livelihoods) for each day of observation are relatively small; (ii) there is minimal risk of further cases after the 25th day, and (iii) vigilance for Ebola infections by the population and the public health system should be encouraged so that new cases are detected early enough.

Acknowledgements

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