Epidemiol. Infect. (2002), **128**, 193–204. © 2002 Cambridge University Press
DOI: 10.1017/S0950268801006707 Printed in the United Kingdom

Quantification of transmission in one-to-one experiments

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(*Accepted 20 November 2001*)

SUMMARY

We study the statistical inference from data on transmission obtained from one-to-one experiments, and compare two algorithms by which the reproduction ratio can be quantified. The first algorithm, the transient state (TS) algorithm, takes the time course of the epidemic into account. The second algorithm, the final size (FS) algorithm, does not take time into account but is based on the assumption that the epidemic process has ended before the experiment is stopped. The FS algorithm is a limiting case of the TS algorithm for the situation where time tends to infinity. So far quantification of transmission has relied almost exclusively on the FS algorithm, even if the TS algorithm would have been more appropriate. Its practical use, however, is limited to experiments with only a few animals. Here, we quantify the error made when the FS algorithm is applied to data of one-to-one experiments not having reached the final size. We conclude that given the chosen tests, the FS algorithm underestimates the reproduction ratio R_0, is liberal when testing $H0: R_0 \geqslant 1$ against $H1: R_0 < 1$, is conservative when testing $H0: R_0 \leqslant 1$ against $H1: R_0 > 1$ and calculates the same probability as the TS algorithm when testing $H0: R_{0\text{-control}} = R_{0\text{-treatment}}$ against $H1: R_{0\text{-control}} > R_{0\text{-treatment}}$. We show how the power of the test depends on the duration of the experiments and on the number of replicates. The methods are illustrated by an application to porcine reproductive and respiratory syndrome virus (PRRSV).

INTRODUCTION

Laboratory experiments are an important tool in the epidemiology of infectious diseases to estimate transmission parameters and to determine the effect of an intervention of transmission. A transmission experiment consists of a number of trials. In each transmission trial a number of infectious and susceptible animals are housed together and sampled regularly to monitor the epidemic process. An advantage of transmission experiments over field studies is that they offer a controlled environment in which the influence of a single factor on the transmission can be investigated, while minimizing variation caused by other factors. This implies that more insight can be obtained into causative mechanisms underlying the transmission dynamics of the pathogen. Furthermore, transmission experiments are usually less expensive and less time-consuming than field studies, and make it possible to evaluate

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intervention measures that are not yet implemented or realizable in the field.

Data from transmission experiments may serve to construct and fit an epidemiological model. Once accepted as being appropriate, such a model can be used to estimate certain biologically interpretable parameters, and to test hypotheses. A model that is often used in the epidemiology is the *SIR* model, in which individuals are either susceptible, infectious, or recovered [1, 2]. An interesting transmission parameter of the *SIR* model is the reproduction ratio (R_0) that is defined as the average number of secondary infections that would be caused by one infectious individual during its infectious period in a large population of susceptible individuals. If R_0 exceeds 1 the pathogen can spread and may cause a major outbreak, while if R_0 is smaller than 1 the pathogen cannot spread or it will at most produce a minor outbreak.

Transmission experiments have already proved to be useful in studies on viral pathogens such as pseudorabies virus [3, 4], classical swine fever virus [5], porcine reproductive and respiratory syndrome virus [6] and bovine herpes virus [7]. The main aim of these studies was to quantify the effect of interventions like vaccination on R_0 using the traditional final size (FS) algorithm. The observed data in these experiments were the 'final sizes' of the local epidemics, ie, the total number of individuals ultimately infected in the experiment. Thus, it was assumed that either no infectious individuals or no susceptible individuals were left at the end of the transmission trial so that the epidemic process has ended before the trial was stopped.

For some pathogens the final size approach may be feasible, but for others it may not. Consider, for instance, the bacterial pathogen *Actinobacillus pleuropneumoniae* in pigs. The length of the infectious period induced by this pathogen is unknown, and its excretion pattern varies widely between individual pigs [8], making it difficult to determine whether the epidemic process has ended when the transmission trial is stopped. For those pathogens it would be better to use an estimation method that does not rely on a final size situation.

An algorithm for the calculation of state probabilities that is not based on the final size assumption is available from the stochastic *SIR* model [9]. In this paper we will call it the 'transient state' (TS) algorithm. The TS algorithm takes the time course of the experimental epidemic into account with no need for a final size situation. Although an explicit solution for any population size is theoretically available from the TS algorithm, its practical use is restricted to experiments with few individuals. This is because its high degree of recursiveness may cause numerical problems or long computation time [2, 10, 11]. The high degree of recursiveness in the TS algorithm disappears if time tends to infinity, turning the TS algorithm into the readily applicable FS algorithm.

As long as the TS algorithm cannot be used for experiments with larger numbers of individuals the FS algorithm will have to be used, even if the final size has not been reached. In this paper we investigate the error made when the FS algorithm is applied to experiments where a final size situation has not been reached. We focus on what we call one-to-one transmission experiments. One such experiment consists of replicated one-to-one trials in which a single infectious individual is housed with a single susceptible individual.

There are several reasons for preferring one-to-one experiments over experiments with more individuals. From a mathematical point of view, there is the advantage that a full analytic solution of the TS algorithm is within reach, and that the estimation methods can be based on binomial distributions, so that standard methods of estimation and testing are available. From a biological point of view, one-to-one trials have the advantage that there is no doubt as to which individual infected which other individual, and co-infection can be excluded. Furthermore, it is possible to estimate the probability of infection from one-to-one experiments without assuming an underlying model, so that the estimated parameter is robust. Therefore, one-to-one experiments are most appropriate compared to bigger experiments if the aim is to estimate R_0 or to test the effect of an intervention on R_0, knowing that R_0 in both treatment groups is larger than the threshold value 1 [6].

The outline of the paper is as follows: (i) the stochastic *SIR* model is described briefly; (ii) an explicit solution for a single one-to-one trial is obtained; (iii) these solutions are converted to a binary outcome; (iv) the statistical inference with both algorithms is investigated; and (v) the error made when using the FS algorithm instead of the TS algorithm is investigated. This error is investigated for three topics: estimating R_0 and the corresponding confidence interval; testing the size of R_0 in relation to its threshold value 1; and testing the reduction of R_0 due to an intervention. To illustrate the results we

have added an example of a particular one-to-one experiment with porcine reproductive and respiratory syndrome virus (PRRSV) among pigs.

STOCHASTIC *SIR* MODEL

The stochastic *SIR* model, also called the General Stochatic Epidemic, was proposed by Bartlett [9] and has been the subject of analysis by others [2, 10, 11]. In this model individuals are either susceptible, infectious or recovered. Let $S(t)$ be the number of susceptible individuals at time t, and let $I(t)$ be the number of infectious individuals at time t. The total population size is constant, i.e., $N(t) = N$, so that the number of recovered individuals $R(t)$ at time t is given by $R(t) = N - S(t) - I(t)$. Hence, the population state at time t is denoted by the pair $(S(t),I(t))$, and a particular realization by $(s(t), i(t))$ or simply (s,i).

Given that the population state is (s,i) at time t, it will be in state $(s-1,i+1)$ at some later moment if a susceptible individual becomes infectious upon an infection event. It will be in state $(s,i-1)$ if a infectious individual becomes immune upon a recovery event. The rate at which infection events occur is proportional to the number of susceptible individuals, the proportion of infectious individuals present, and the infection parameter β. This assumption is commonly referred to as the 'mass-action' assumption [12]. The rate at which recovery events occur is proportional to the number of infectious individuals and the recovery parameter α. Assuming that recovery events occur independently then the mean infectious period is given by $1/\alpha$.

Given the above assumptions, the dynamics of the model are governed by a Markov process. The one-step transition probabilities in a small time interval Δt are given by:

$$\begin{aligned}
&Pr\{(S(t+\Delta t),I(t+\Delta t)) = (s-1,i+1)\,|\,(S(t),I(t)) = (s,i)\} \\
&\quad = \beta\frac{si}{N}\Delta t + o(\Delta t) \\
&Pr\{(S(t+\Delta t),I(t+\Delta t)) = (s,i-1)\,|\,(S(t),I(t)) = (s,i)\} \\
&\quad = \alpha i\Delta t + o(\Delta t) \\
&Pr\{(S(t+\Delta t),I(t+\Delta t)) = (s,i)\,|\,(S(t),I(t)) = (s,i)\} \\
&\quad = 1 - \left(\beta\frac{si}{N} + \alpha i\right)\Delta t + o(\Delta t), \qquad (1)
\end{aligned}$$

where $o(\Delta t) \to 0$ when $\Delta t \to 0$. Denoting the initial state of the process by (s_0,i_0), the state probabilities can be written as:

$$p_{s,i}(t) = Pr\{(S(t), I(t)) = (s,i)\,|\,(S(0),I(0)) = (s_0,i_0)\}. \quad (2)$$

After rescaling time to units of the mean infectious period $1/\alpha$, the adjacent state probabilities satisfy the forward differential-difference equations:

$$\begin{aligned}
\frac{d}{dt}p_{s,i}(t) &= (i+1)p_{s,i+1}(t) \\
&\quad + \left(R_0\frac{(s+1)(i-1)}{N}\right)p_{s+1,i-1}(t) \\
&\quad - \left(R_0\frac{si}{N} + i\right)p_{s,i}(t), \qquad (3)
\end{aligned}$$

where

$$R_0 = \frac{\beta}{\alpha}$$

for $0 \leqslant s+i \leqslant s_0+i_0$, $0 \leqslant s \leqslant s_0$, and $0 \leqslant i \leqslant s_0+i_0$. Subject to the initial value $p_{s_0,i_0}(0) = 1$ this equation can be solved using standard methods. The solution that we call the transient state (TS) algorithm can be used to calculate a continuous-time state probability for each state in the epidemic process.

Despite the fact that the solution of equation (3) is formally available, an exact calculation of the continuous-time state probabilities for all states in the epidemic process is very laborious for all but the simplest cases. Attempts to find useful explicit solutions for the stochastic *SIR* model have been made [10, 13–17], but calculation of the state probabilities is still recursive, or involves a considerable number of multiple summations and products.

The probability distribution used in the TS algorithm is given by the set of all five time-dependent state probabilities (Fig. 1). The probability distribution of the FS algorithm is the limiting case of the TS algorithm where time tends to infinity. In fact, as $t \to \infty$ all state probabilities where $i \neq 0$ tend towards zero, so that the limiting probabilities of the states where $i = 0$ approach the final size distribution of the experimental epidemic.

When using methods based on the *SIR* model to quantify R_0, one should remember that the results will also depend on the assumptions underlying the model. Some of the assumptions are: all animals within the population have random contacts with each other; every class S, I and R consists of a homogeneous group of individuals; the infection rate is constant during the entire infectious period; the duration of the infectious period is exponentially distributed; and each recover ed animal is fully immune towards infection. Thus, application of both the TS and the FS algorithm requires these assumptions to be checked carefully.

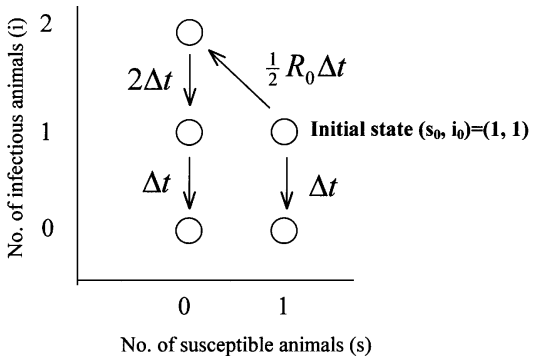

Fig. 1. A schematic structure of the epidemic process in a one-to-one trial. Each state is given by the number of susceptible and infectious individuals. In the long run, the population ends up in one of the absorbing states (0,0) or (1,0). The transition rates are given next to the arrows, where time is scaled in units of infectious periods.

ONE-TO-ONE TRIAL

In a one-to-one trial a single infectious individual is housed together with a single susceptible individual. Hence, $s_0 = 1$, $i_0 = 1$ and $N = 2$, at $t = 0$. The following states are distinguished: (1,1), (1,0), (0,2), (0,1) and (0,0). In the long run the pair will always end up in one of the absorbing states (1,0) or (0,0) (Fig. 1). The probability distribution belonging to the TS algorithm is:

$$
\begin{aligned}
p_{1,1}(t) &= \exp\left(-\frac{R_0+2}{2}t\right)\\
p_{1,0}(t) &= \frac{2}{R_0+2}\left(1-\exp\left(-\frac{R_0+2}{2}t\right)\right)\\
p_{0,2}(t) &= \frac{R_0}{R_0-2}\left(\exp(-2t)-\exp\left(-\frac{R_0+2}{2}t\right)\right)\\
p_{0,1}(t) &= -2\frac{R_0}{R_0-2}\exp(-2t)+\frac{4}{R_0-2}\exp\left(-\frac{R_0+2}{2}t\right)\\
&\quad +2\exp(-t)\\
p_{0,0}(t) &= \frac{R_0}{R_0-2}\exp(-2t)-\frac{8}{R_0^2-4}\exp\left(-\frac{R_0+2}{2}t\right)\\
&\quad -2\exp(-t)+\frac{R_0}{R_0+2}, \qquad (4)
\end{aligned}
$$

if $R_0 \neq 2$. If $R_0 = 2$ the state probabilities for (0,2), (0,1) and (0,0) are given by: $p_{0,2}(t) = t\exp(-2t)$, $p_{0,1}(t) = -2\exp(-2t)(1+t-e^t)$ and $p_{0,0}(t) = e^{-2t}(\frac{3}{2}+t)-2\exp(-t)+\frac{1}{2}$. Note that the probability distribution for the FS algorithm is a limiting case of the TS algorithm, and is given by the state probabilities of the two absorbing states:

$$
\begin{aligned}
p_{1,0} &= \lim_{t\to\infty} p_{1,0}(t) = \frac{2}{R_0+2} \text{ and}\\
p_{0,0} &= \lim_{t\to\infty} p_{0,0}(t) = \frac{R_0}{R_0+2}, \qquad (5)
\end{aligned}
$$

while all state probabilities of the transient states are zero: $p_{1,1} = p_{0,2} = p_{0,1} = 0$.

Figure 2 shows an example of the dynamics of the model (4) where $R_0 = 3$. The probability of being in the initial state (1,1) equals one at $t = 0$, and decreases in time. The state probabilities of the intermediate states (0,2) and (0,1) initially increase with time, and thereafter decrease asymptotically to zero. The state probabilities of the absorbing states (0,0) and (1,0) increase asymptotically to a non-zero value when t tends to infinity.

From the explicit solution, some interesting quantities can be determined, e.g., the mean time spent in state (s,i) and its variance. Assumptions (1) and the Markov property of the chain imply straightforwardly that the time spent in the transient states (1,1), (0,2) and (0,1) are independently exponentially distributed with probability densities $(\frac{1}{2}R_0+1)\exp(-(\frac{1}{2}R_0+1)t)$, $\exp(-2t)$ and $\exp(-t)$, respectively [18]. Hence, the respective sojourn times have means $2/(R_0+2)$, $\frac{1}{2}$ and 1 with variances $(2/(R_0+2))^2$, $\frac{1}{4}$ and 1, respectively.

The mean time before absorption takes place and its variance can be calculated as follows. There are two possible routes towards absorption, the first directly from (1,1) to (1,0), and the second from (1,1) via (0,2) and (0,1) to (0,0). The probability of the first route is equal to the probability that the waiting time for transition to (1,0) is less than the waiting time for transition to state (0,2). Hence this probability is $2/(R_0+2)$, and the probability of the second route is $R_0/(R_0+2)$. Now let $T_{s,i}$ denote the time spent in state (s,i), and $\delta = 0$ if the first route is followed and $\delta = 1$ if the second route is followed. Then, for the time to absorption Z we have say:

$$Z = T_{1,1} + \delta(T_{0,2} + T_{0,1}). \qquad (6)$$

Calculation of the mean and variance of Z using the above formulations is straightforward:

$$E(Z) = \frac{4+3R_0}{2(R_0+2)} \qquad (7)$$

and

$$\mathrm{Var}(Z) = \frac{R_0(4+5R_0)}{4(R_0+2)^2}. \qquad (8)$$

The mean time to absorption of a highly infectious pathogen, $R_0 \to \infty$, is 1·5 infectious periods. Based on

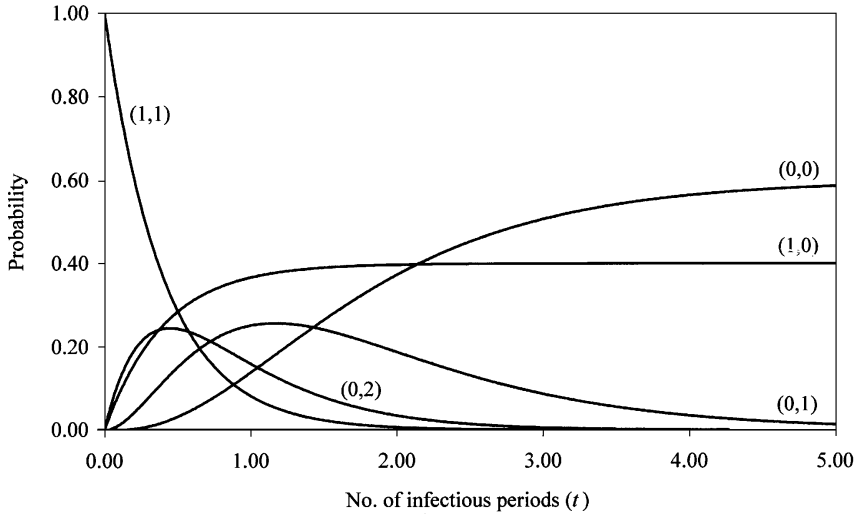

Fig. 2. The state probabilities of the five states in a one-to-one trial as a function of time. In this particular example $R_0 = 3$.

(7) and (8) 95% of the one-to-one trials will have reached an absorbing state within 3·69 infectious periods. In the case of a pathogen that is hardly infectious ($R_0 \to 0$), the mean time to absorption is 1 infectious period.

PRACTICAL CONSIDERATIONS

It is often difficult to distinguish between recovered and infectious individuals in transmission experiments. Consider, for instance, the bacterial pathogen *A. pleuropneumoniae* in pigs where under experimental conditions the excretion pattern varies widely between individuals [8]. It may occur that individuals cease to excrete the bacteria but that excretion is resumed after a few days. Therefore, it cannot be concluded that a pig has stopped being infectious when there has been no excretion of the bacteria for a few days. Susceptible individuals are easier to identify, because they are consistently negative in bacteriological culturing and serology during the experimental period.

To quantify R_0 it is desirable to use a probability distribution over the number of susceptible individuals (s) instead of the number of infectious and susceptible animals (s,i). The probability of the number of susceptible individuals in a one-to-one trial is easily obtained by adding all state probabilities with equal numbers of susceptibles, i.e., $s = 1$ or $s = 0$. Consequently, the number of infectious individuals becomes irrelevant in the quantification of R_0. The probability of having no susceptible individual at time t for the TS algorithm is the sum of the state probabilities $p_{0,2}(t)$, $p_{0,1}(t)$ and $p_{0,0}(t)$:

$$p_{s=0}(t) = \sum_i p_{0,i}(t) = \frac{R_0}{2+R_0} - \frac{R_0}{2+R_0} \exp\left(-\frac{2+R_0}{2} t \right). \quad (9)$$

The probability of having one susceptible individual left at time t is equal to the sum of state probabilities $p_{1,1}(t)$, and $p_{1,0}(t)$. The state probabilities of having one respectively no susceptible individual according to the FS algorithm equal the state probabilities of the two absorbing states (5), i.e., $p_{1,0}$ respectively $p_{0,0}$.

When planning a one-to-one experiment, it is possible to calculate the minimal experimental period depending on the expected R_0. Since the original number of state probabilities is reduced from five in (4) to the two state probabilities in (9) where only the number of susceptibles is considered, the minimal experimental period can be determined by use of the mean sojourn time and its variance in starting state (1,1), i.e., $2/(R_0+2)$ and $(2/(R_0+2))^2$, respectively. This because, the observable final size situation is reached immediately after state (1,1) has been left.

ONE-TO-ONE EXPERIMENTS

In a single one-to-one trial the outcome of the infection process is a binary variable, since an infection will occur or not. Hence, the total number of infection events k from n mutually independent replications of an one-to-one trial is binomially distributed with index n, and parameter $p_{s=0}(t)$ (9):

$$Q(K = k;t) = \binom{n}{k} \cdot p_{s=0}(t)\, k \cdot (1 - p_{s=0}(t))^{n-k}. \quad (10)$$

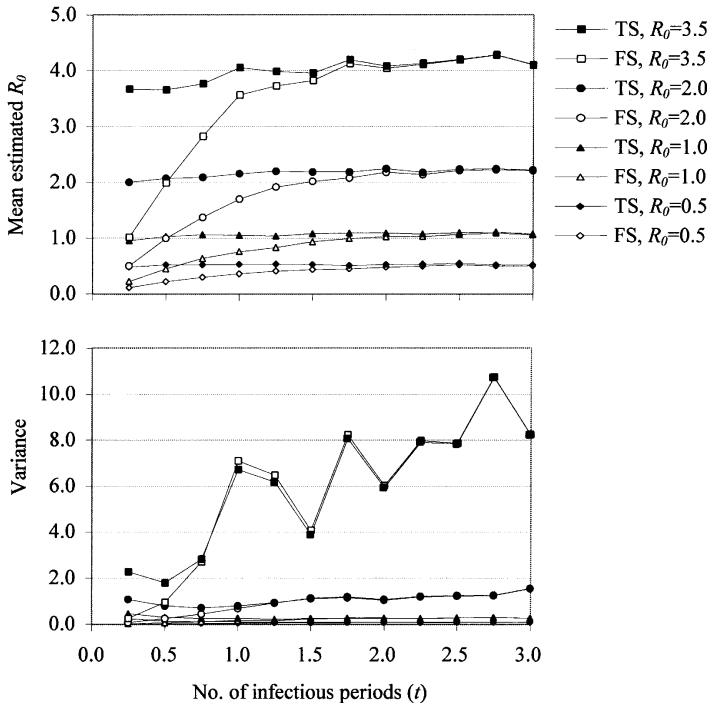

Fig. 3. The mean estimated R_0 (top panel) and the variance (bottom panel) as a function of the number of infectious periods (t) calculated from 1000 simulations for given combinations of R_0 and t.

The binomial parameter for the FS algorithm is $p_{0,0} = \lim_{t \to \infty} p_{0,0}(t)$. Since the FS algorithm is a limiting case of the TS algorithm, it is interesting to investigate the effect of early stopping, i.e., using the FS algorithm instead of the TS algorithm. The error made when using the FS algorithm instead of the TS algorithm, where the latter should have been used, can be quantified by comparing infinite t (FS algorithm) to finite or even small t (TS) algorithm. In the subsections to follow this will be done for different aspects.

Estimation of R_0

The maximum likelihood estimator (MLE) of parameter $p_{s=0}(t)$ is simply the observed proportion of successes, i.e., k/n. Hence, the MLE of R_0 is obtained as the solution of:

$$\frac{\hat{R}_0}{2+\hat{R}_0} - \frac{\hat{R}_0}{2+\hat{R}_0} \exp\left(-\frac{2+\hat{R}_0}{2} t \right) = \frac{k}{n}. \qquad (11)$$

One-sided and two-sided statistical tests about R_0 can be performed on the basis of the probability distribution given in (10). A two-sided 95% confidence interval (CI) for R_0 can be constructed as usual. In case of extreme outcomes, i.e., $k = n$ or $k = 0$, a one-sided interval would be more appropriate.

To quantify the behaviour of the two estimators of R_0 considered here, we simulated 1000 experiments of 20 one-to-one trials for a given pair of R_0 and t, by drawing 1000 random numbers from the binomial distribution (10). From each of the simulated experiment, R_0 was estimated with both the TS and the FS algorithm to give $R_{0\text{-TS}}$ and $R_{0\text{-FS}}$. This procedure resulted in two arrays of 1000 estimated R_0s. From these arrays we calculated the mean estimated $R_{0\text{-TS}}$ and $R_{0\text{-FS}}$, and the variance. This whole procedure was performed for several combinations of R_0 and t, and the results are given in Figure 3.

The top panel of Figure 3 shows that the $R_{0\text{-FS}}$ underestimates R_0 for small values of t, while it overestimates R_0 for high values of t. The $R_{0\text{-FS}}$ approaches $R_{0\text{-TS}}$ when t increases. So if the experimental period is relatively short and the FS algorithm is used instead of the TS algorithm, then it means that R_0 is underestimated. Note $R_{0\text{-TS}}$ overestimates R_0 for all t and this overestimation increases with the real R_0.

R_0 and the threshold value 1

An important purpose of transmission experiments is to assess whether a particular intervention can be used

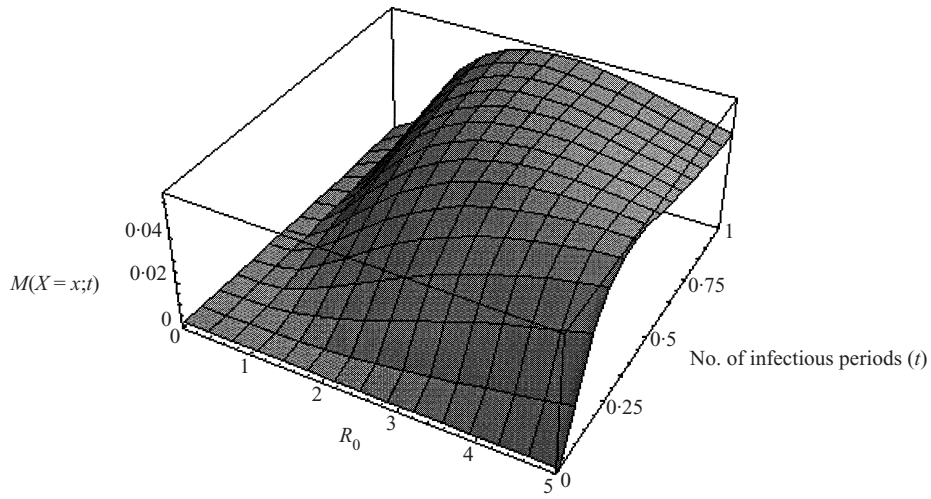

Fig. 4. The cumulative probability $M(X = x;t)$ (14) to observe at least x contact infections, plotted as a function of R_0 and t.

to eradicate an infectious agent. To achieve eradication R_0 should be brought below 1 so that the infectious agent cannot persist, and only small outbreaks can occur. To test whether an intervention brings R_0 below 1 the hypothesis $H0: R_0 \geqslant 1$ against $H1: R_0 < 1$ should be considered. Application of the usual test for a binomial parameter, which in this case is $p_{s=0}(t)$ (9), and the observation that $p_{s=0}(t)$ is a monotone, increasing function of R_0, means that rejection of $H0$ sustains $H1$, i.e., R_0 is assumed to be smaller than 1. To this end, the probability that k or less infections have occurred is calculated under the null hypothesis, $R_0 = 1$, and should be smaller than 0·05 to reject $H0$:

$$\Pr(K \leqslant k;t \mid R_0 = 1) = \sum_{i=0}^{k} Q(K = i;t \mid R_0 = 1) \leqslant 0.05. \tag{12}$$

Probability $Pr(K \leqslant k;t \mid R_0 = 1)$ is a decreasing function of t. Thus, if the FS algorithm ($t \to \infty$) is used for testing the above-mentioned hypothesis it is possible to reject $H0: R_0 \geqslant 1$ with a greater probability than the indicated error rate. On the other hand, if the FS algorithm does not rejects $H0$ the TS algorithm will not reject it either. In other words the FS algorithm is too liberal when testing $H0: R_0 \geqslant 1$ against $H1: R_0 < 1$.

Another hypothesis that may be of interest is $H0: R_0 \leqslant 1$ against $H1: R_0 > 1$. Rejecting $H0$ suggests that R_0 is greater than 1. If this is so, it is unsure whether the infectious agent can be eradicated from the population and major outbreaks can occur. In this situation eradication will take place only by chance and minor outbreaks are possible depending on the size of R_0. Like (15), to reject $H0$ the probability that k or more infections are observed should be lower than 0·05. This probability $Pr(K \geqslant k;t \mid R_0 = 1)$ is an increasing function of t, so using the FS algorithm to test $H0: R_0 \leqslant 1$ may lead to the wrong acceptance of $H0$. However, if $H0$ is rejected with the FS algorithm, it will surely be rejected with the TS algorithm. So, the FS algorithm is conservative when testing $H0: R_0 \leqslant 1$.

The effect of an intervention of R_0

One application of transmission experiments is to assess the effect of an intervention on the transmission of an infectious agent. Here we compare the level of transmission in two populations, e.g., one vaccinated and the other unvaccinated. Although, in the simple case of a one-to-one experiment with equal stopping times in the control and treatment groups elementary tests like Fisher's one for testing equality of binomial proportions could be applied, we propose a more generally applicable method, which also can be applied to experiments with larger numbers of animals per trial or with different stopping times. Methods to test the difference in transmission between two groups are available [19]. The hypothesis to be tested is that there is no difference in transmission between the treatment group and the control group, $H0: R_{0\text{-control}} = R_{0\text{-treatment}}$ versus $H1: R_{0\text{-control}} \neq R_{0\text{-treatment}}$. Rejection of $H0$ in favour of its alternative makes it plausible that the transmission in the treatment group

differs from the control group. A natural test statistic is the difference in the number of contact infections between the two groups:

$$X = |K_{\text{control}} - K_{\text{treatment}}|.$$

To test $H0: R_{0\text{-control}} = R_{0\text{-treatment}}$ the probability that the observed difference in contact infections x or more has to be calculated under the assumption that the R_0 is equal in both groups. The probability to obtain a difference of x contact infections is twice the sum of all possible products of $Q(K = i;t)$ and $Q(K = i+x;t)$ for $x \neq 0$:

$$D(X = x;t) = 2 \cdot \sum_{i=0}^{n-x} Q(K = i;t) \cdot Q(K = i+x;t). \quad (13)$$

$H0$ is rejected if the probability of a difference of x, say $M(X = x;t)$, is smaller than 0·05, i.e., if

$$M(X = x;t) = \sum_{j=x}^{n} D(X = j;t) \leqslant 0{\cdot}05. \quad (14)$$

Note that we assume that the number of one-to-one trials is equal in both groups. It is also possible to calculate the above mentioned probabilities in situations where the numbers differ between treatment groups. However, for simplicity we only present results for the case where the numbers are equal.

Since the parameter $p_{s=0}(t)$ in $Q(K = k;t)$ depends on both parameters of interest R_0 and t, $M(X = x;t)$ depends also on R_0 and t. A conservative way to reject $H0$ is to require that the maximum of $M(X = x;t)$ is smaller than 0·05 for any arbitrary R_0 and t for the TS algorithm, and for any arbitrary R_0 with $t \to \infty$ for the FS algorithm. In Figure 4 the surface of $M(X = x;t)$ is plotted against R_0 and t in the situation where the observed difference is 4 in 20 one-to-one trials per treatment group. The whole surface of $M(X = x;t)$ is below 0·05 and its maximum is on the same height for any value of t. This is due to the fact that $M(X = x;t)$ depends on R_0 and t only through $p_{s=0}(t)$. Hence, for this test it is sufficient to use $p_{0,0}$ according to the FS algorithm.

In addition, one would also like to know how many trials one should conduct in order to find a significant difference between $R_{0\text{-control}}$ and $R_{0\text{-treatment}}$. Thus we have to calculate the power of the test, i.e., the probability to find a significant difference given that there is a difference. The power is determined by adding all probabilities $D(X = x;t)$ for all x for which the difference, given $R_{0\text{-control}}$, $R_{0\text{-treatment}}$ and n, is significant.

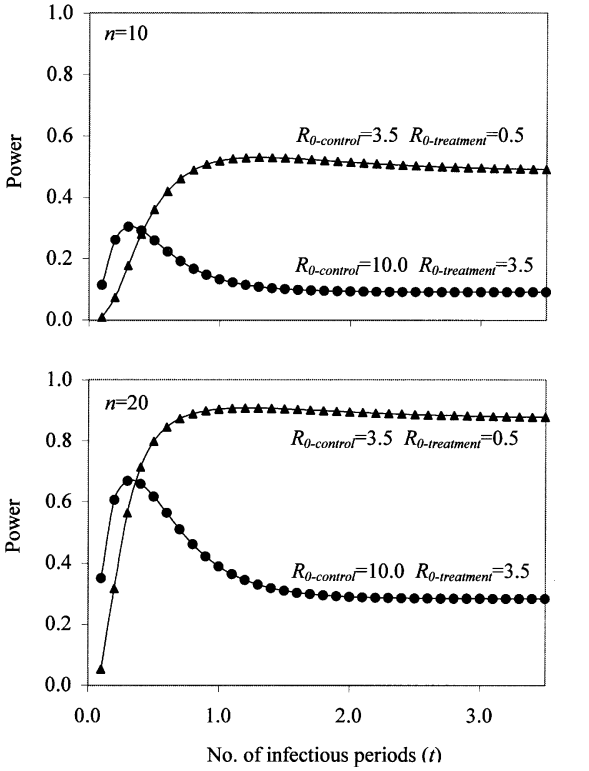

Fig. 5. The power of the test $H0: R_{0\text{-control}} = R_{0\text{-treatment}}$ versus various alternative hypotheses. In the top panel the number of replicates is $n = 10$, while $n = 20$ in the bottom panel. Two scenarios are considered: $R_{0\text{-control}} = 3{\cdot}5$ vs. $R_{0\text{-treatment}} = 0{\cdot}5$, and $R_{0\text{-control}} = 10{\cdot}0$ vs. $R_{0\text{-treatment}} = 3{\cdot}5$. The error rate is set at 0·05.

Figure 5 shows an example of a power calculation. The top panel shows the results if the number of replicates is $n = 10$, while $n = 20$ in the bottom panel. Two scenarios are considered: $R_{0\text{-control}} = 3{\cdot}5$ versus $R_{0\text{-treatment}} = 0{\cdot}5$, and $R_{0\text{-control}} = 10{\cdot}0$ versus $R_{0\text{-treatment}} = 3{\cdot}5$. The error rate is set at 0·05. It appears that high power can only be obtained if R_0 exceeds 1 in one treatment group and is less than 1 in the other. If R_0 exceeds 1 in both treatment groups, highest power is obtained for small t. In this particular example reasonable power (say $> 0{\cdot}80$) is obtained if $R_{0\text{-control}} > 1$ and $R_{0\text{-treatment}} < 1$ and if the number of trials is large (here $n = 20$).

ILLUSTRATION

The results presented above are illustrated by application to the one-to-one experiment of Nodelijk et al. [6], who investigated the effect of vaccination on the transmission of porcine reproductive and respiratory syndrome virus (PRRSV). Two sets of ten replicate one-to-one trials were carried out. In one set

Table 1. *Results of the one-to-one experiment carried out to test the effect of vaccination on the transmission of PRRSV*

	Virus isolated from contact pig in	
Days post Inoculation	Control group (n = 10)	Vaccine group (n = 9)
3	0	0
7	3	1
10	9	1
14	10	6
17	10	9
56	10	9

of trials all pigs were vaccinated, while in the other they were left unvaccinated in the other experiment. At day 1, one pig from each couple was inoculated intranasally with PRRSV, while the other pig was placed in a separate pen. At day 2, the contact pigs were placed back to their original pens. To determine the onset and duration of viremia, sera were collected from all the pigs at day 1, and thereafter every third or fourth day. This continued until the end of the experiment, 56 days post inoculation. A PRRSV infection was confirmed by virus detection in the sera.

Table 1 shows the results of this experiment for the contact pigs. One inoculated pig from a vaccinated couple remained uninfected and was excluded from the analysis. All unvaccinated contact pigs were infected at day 14, while all vaccinated contact pigs were infected at day 17. Thus, in all one-to-one trials the final size of the outbreak had been reached in both treatment groups before the end of the experiment, making this experiment an ideal test case for an illustration.

Now, let us assume that the experiment was not stopped at day 56, but at day 7, 10 or 14, i.e., before the final size was reached in all trials. The question is, would there be a difference in the conclusions drawn if the TS algorithm had been used instead of the FS algorithm?

Table 2 gives the estimated reproduction ratios in the unvaccinated ($R_{0\text{-c}}$) and the vaccinated group ($R_{0\text{-v}}$) together with the 95%-CIs and the *p*-values under the different *H0* hypotheses for the different scenarios. For the estimates with the TS algorithm we assumed that the duration of the infectious period was 56 days [20].

Let us first assume that the experiment was stopped at day 7. On this day only 3 out of 10 unvaccinated, and 1 out of 9 vaccinated contact animals were infected. Table 2 shows to what extent the FS algorithm underestimates $R_{0\text{-c}}$ and $R_{0\text{-v}}$. The FS algorithm does not reject the hypothesis $H0: R_{0\text{-c}} < 1$ while the TS algorithm does. All other conclusions drawn with the FS algorithm are the same as with the TS algorithm.

Next, let us assume that the experiment was stopped at day 10. At this day, 9 out of 10 unvaccinated, and only 1 out of 9 vaccinated contact animals were infected. As before, the FS algorithm underestimates the $R_{0\text{-c}}$ and $R_{0\text{-v}}$, but all other conclusions are the same for both algorithms.

Third, assuming that the experiment was stopped at day 14, by which time all unvaccinated couples, and 6 out of 9 vaccinated couples were infected, the final size was reached in all unvaccinated couples but not in all vaccinated couples. According to both algorithms $R_{0\text{-c}}$ tends to infinity. The $R_{0\text{-v}}$ remains underestimated with FS algorithm. All other conclusions are the same for both algorithms.

Overall, this example suggests that the FS algorithm is a good algorithm to test the different *H0* hypothesis, except for $H0: R_{0\text{-c}} < 1$ at day 7. However, the estimated reproduction ratios, $R_{0\text{-c}}$ and $R_{0\text{-v}}$, will be underestimated when the FS algorithm is used instead of the TS algorithm.

DISCUSSION

In this paper we compared two algorithms to quantify the transmission of an infectious agent from one-to-one experiments. The first algorithm, the transient state (TS) algorithm, takes the time course of the experimental epidemic into account. The second algorithm, the final size (FS) algorithm, does not take time into account, and assumes that the final size of the epidemic process has been reached before the experiment was stopped.

The stochastic *SIR*-model on which both algorithms are based was originally proposed by Bartlett [9], and formal solutions are attributable to Billard [17] and Kryscio [21]. Bailey [2] derived a likelihood function for parameter estimation, although it was not applied to real-world data. Inspired by observational data human diseases, Becker [22] described methods for the analysis of a single epidemic in a large community. Kroese and De Jong [19] considered methods to analyse transmission experiments that have been applied to experimental data [3, 4, 23, 24].

Table 2. *The estimated $R_{0\text{-c}}$ and $R_{0\text{-v}}$ 95%-CI, and the p-values under the different H0 hypotheses for four different scenarios, assuming that the experiment was stopped at days 7, 10, 14 and 17 post inoculation*

	Day 7		Day 10		Day 14		Day 17	
	FS	TS	FS	TS	FS	TS	FS	TS
$R_{0\text{-c}}$	0·86	6·12	18·0	43·41	∞	∞	∞	∞
95%-CI	0·14–3·75	1·18–18·53	2·49–∞	14·08–∞	4·48–∞	13·93–∞	4·48–∞	11·40–∞
$H0: R_{0\text{-c}} > 1$	0·56	1·00	1·00	1·00	1·00	1·00	1·00	1·00
$H0: R_{0\text{-c}} < 1$	0·70	0·00	0·00	0·00	0·00	0·00	0·00	0·00
$R_{0\text{-v}}$	0·25	3·42	0·25	2·01	4·00	12·95	∞	∞
95%-CI	0·01–1·86	0·08–19·29	0·01–1·86	0·05–11·4	0·85–24·72	4·04–36·13	3·95–∞	10·49–∞
$H0: R_{0\text{-v}} > 1$	0·14	0·96	0·14	0·91	0·99	1·00	1·00	1·00
$H0: R_{0\text{-v}} < 1$	0·97	0·27	0·97	0·41	0·04	0·00	0·00	0·00
$H0: R_{0\text{-c}} = R_{0\text{-}v}$	0·34	0·34	0·00	0·00	0·09	0·09	1·00	1·00
$R_0 \mid H0$	5·58	39·58	2·29	13·25	2·75	10·06	∞	∞

Their methods, however, are restricted to the final size of the experimental epidemics.

Thus far, quantification of the reproduction number from transmission experiments has relied almost exclusively on the FS algorithm. This is not surprising since the FS algorithm is easy to understand and readily implemented on a personal computer, while the computational burden of the TS algorithm quickly becomes insurmountable as the size of the population increases. On the other hand, the applicability of the FS algorithm is not always warranted, as the epidemic process may not have ended in one or more of the trials when the experiment is stopped. The assumption that the epidemic processes have ended before the end of the experiment may be justified for viral infections with relatively fast transmission dynamics. However, the transmission dynamics of many bacterial infections are much slower, more variable, and less easy to keep track of.

Well-known examples of slow and highly variable infections include *Salmonella enteritidis* in chickens, *Mycobacterium paratuberculosis* in cattle, and *Actinobacillus pleuropneumoniae* in pigs. For instance, Velthuis et al. [8] studied the transmission of *Actinobacillus pleuropneumoniae* among pigs by means of a transmission experiment. The excretion pattern of the bacterium in tonsillar swabs and nasal swabs was highly variable. In all trials the bacterium could still be isolated from some pigs on the last day of the experimental period.

Hence, we are faced with the problem that while it is desirable to base the analysis of bacterial transmission experiments on the TS algorithm, it is not always feasible in practice. For one-to-one trials, however, this problem does not arise since a full analytical comparison of the TS algorithm is within reach. In this paper we have presented different aspects of the statistical inference based on the FS and TS algorithms on data from one-to-one trials.

First, in case of one-to-one trials it is still possible to estimate beforehand the time until absorption or until an infection-event has occurred. In particular, the mean time to absorption and its variance are expressed in terms of R_0 by equations (7) and (8).

Second, the results show that the FS algorithm underestimates R_0 when the final size has not yet been reached. If the experimental period is short compared to the infectious period, the degree of underestimation is high. If, on the other hand, the experimental period is relatively long, R_0 will only slightly be underestimated. Furthermore, both algorithms lead to overestimated $R_0 s$. This bias is a consequence of the fact that R_0 is a convex function of the proportion of successful infection-events. Although this proportion is an unbiased estimate of the success probability, Jensen's inequality leads to a biased overestimation of R_0, which increases with time and R_0 [25].

Third, we conclude that use of the FS algorithm is liberal in testing the null hypothesis $H0: R_0 \geqslant 1$ against the alternative hypothesis $H1: R_0 < 1$. In other words, it is possible that the null hypothesis would be rejected with the FS algorithm, whereas it would not be rejected with the TS algorithm. The implication is that conclusions based on the FS algorithm may overestimate the possibility of eradication. On the other hand, use of the FS algorithm yields conservative $H0: R_0 \leqslant 1$ in testing against its alternative $H1: R_0 > 1$. The implication is that the FS algorithm can safely be used for testing $H0: R_0 \leqslant 1$ even if the final size has not been reached in all trials.

Finally, there is no difference in p-value between both algorithms when testing: $H0: R_{0\text{-control}} = R_{0\text{-treatment}}$ against $H1: R_{0\text{-control}}\ R_{0\text{-treatment}}$. This is due to the fact that $M(X = x;t)$ depends on R_0 and t only through $p_{s=0}(t)$. Thus, in principle at least, the FS and TS algorithm are equally good when testing for the effect of an intervention. Note, however, that the power of the test does depend on both R_0 and t. In fact, the largest power is achieved if $R_{0\text{-control}}$ is greater than 1 while $R_{0\text{-treatment}}$ is smaller than 1. Moreover, the power of the test is largest at intermediate t. We conclude that both algorithms can safely be used to test for differences, but that the power of the test is affected by both R_0 and t.

To the best of our knowledge, there are two studies that use one-to-one experiments [6, 7]. Nodelijk et al. [6] carried out 20 one-to-one trials, 10 with vaccinated pigs and 10 with unvaccinated pigs. The aim of the study was to test whether vaccination reduces the transmission of porcine reproductive and respiratory syndrome virus (PRRSV) among pigs. All the susceptible contact pigs were infected at the end of all the trials. In the control as well as in the vaccine groups both algorithms lead to the conclusion that R_0 exceeds 1. The authors concluded, with use of the FS algorithm, that there was no proof that vaccination reduced the transmission of PRRSV. However, it could still be that vaccination reduces the reproduction ratio, albeit not below 1. The analysis presented in this paper shows that there is indeed evidence that vaccination has a significant effect on the transmission on day 10 ($p = 0{\cdot}00$) and a marginally significant effect at day 14 ($p = 0{\cdot}09$).

Mars et al. [7] carried out a one-to-one experiment with 32 trials to test if cows infected with a gE-negative bovine herpes virus 1 vaccine strain could re-excrete the strain and transmit it to contact-exposed cows. The number of trials was chosen such that the null hypothesis $H0: R_0 \geqslant 1$ should be rejected in favour of $H1: R_0 < 1$ when no contact infections would be observed. The experiment lasted 5 weeks, and no contact infections were observed. As a consequence Mars et al. conclude that R_0 of the vaccine strain is below 1. In fact, using the FS algorithm R_0 was estimated at 0·0 with a 95% confidence interval of (0·0; 0·91).

Finally, in this paper we have presented a first step towards the statistical inference of transmission experiments. Of course, much remains to be done. For instance, one could think of extension to experiments involving more animals per trial, to infectious processes with non-exponentially distributed infectious periods, or so that differentiation in individual levels of susceptibility and infectivity is allowed. To what extent the results of the present paper still hold in a more general setting, is at present an open question.

ACKNOWLEDGEMENTS

The authors wish to thank NOADD, a co-operation between ID-Lelystad, Wageningen University and the Utrecht University for their financial support.

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