

Small distances can keep bacteria at bay for days

Bram A. D. van Bunnik^{a,b,c,1,2}, Amos Ssematimba^{a,b,1}, Thomas J. Hagenaars^b, Gonnie Nodelijk^b, Manon R. Haverkate^d, Marc J. M. Bonten^{d,e}, Mary K. Hayden^f, Robert A. Weinstein^{f,g}, Martin C. J. Bootsma^{d,h}, and Mart C. M. De Jong^{a,2}

^aDepartment of Quantitative Veterinary Epidemiology, Wageningen University, 6700 AH, Wageningen, The Netherlands; ^bDepartment of Epidemiology, Crisis Organization and Diagnostics, Central Veterinary Institute of Wageningen UR, 8200 AB, Lelystad, The Netherlands; ^cCentre for Immunity, Infection and Evolution, University of Edinburgh, Edinburgh EH9 3JT, United Kingdom; ^dJulius Center for Health Sciences and Primary Care, University Medical Center Utrecht, 3508 GA, Utrecht, The Netherlands; ^eDepartment of Medical Microbiology, University Medical Center Utrecht, 3508 GA, Utrecht, The Netherlands; ^fSection of Infectious Diseases and Department of Pathology, Rush University Medical Center, Chicago, IL 60612; ^gDivision of Infectious Diseases, John Stroger Hospital of Cook County, Chicago, IL 60612; and ^hFaculty of Science, Department of Mathematics, Utrecht University, 3584 CD, Utrecht, The Netherlands

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Transmission of pathogens between spatially separated hosts, i.e., indirect transmission, is a commonly encountered phenomenon important for epidemic pathogen spread. The routes of indirect transmission often remain untraced, making it difficult to develop control strategies. Here we used a tailor-made design to study indirect transmission experimentally, using two different zoonotic bacteria in broilers. Previous experiments using a single bacterial species yielded a delay in the onset of transmission, which we hypothesized to result from the interplay between diffusive motion of infectious material and decay of infectivity in the environment. Indeed, a mathematical model of diffusive pathogen transfer predicts a delay in transmission that depends both on the distance between hosts and on the magnitude of the pathogen decay rate. Our experiments, carried out with two bacterial species with very different decay rates in the environment, confirm the difference in transmission delay predicted by the model. These results imply that for control of an infectious agent, the time between the distant exposure and the infection event is important. To illustrate how this can work we analyzed data observed on the spread of vancomycin-resistant *Enterococcus* in an intensive care unit. Indeed, a delayed vancomycin-resistant *Enterococcus* transmission component was identified in these data, and this component disappeared in a study period in which the environment was thoroughly cleaned. Therefore, we suggest that the impact of control strategies against indirect transmission can be assessed using our model by estimating the control measures' effects on the diffusion coefficient and the pathogen decay rate.

diffusion model | transmission experiment | *Campylobacter jejuni* | *Escherichia coli*

Indirect transmission, i.e., transmission without direct contact between hosts, is a ubiquitous mechanism of disease spread in epidemics as has been demonstrated in plants (e.g., refs. 1–3), in livestock (e.g., refs. 4–8), and in humans (e.g., refs. 9–12). Indirect transmission is important because, although control measures can prevent direct contacts, it is unclear how indirect contacts can best be avoided. For example, indirect transmission in health care facilities is believed to be the underlying mechanisms for a number of hospital infections, and as such has been implicated for example in the spread of methicillin-resistant bacterium *Staphylococcus aureus* associated with hospitals. Transmission via (the hands of) health care workers or contaminated surfaces are thought to be important routes for these infections (9–11). Similarly, in the experimental study of the airborne transmission of *Bordetella pertussis* (12), it was found that there can be pathogen transmission without physical contact and that distance between separately housed animals plays an important role in determining whether naïve animals can actually get infected and the time it will take for infection to happen. Although highly important, knowledge of the possible routes of transmission alone is often insufficient to understand the mechanisms and dynamics of the disease transmission. A better understanding of the mechanisms that underlie indirect transmission

is needed to improve effectiveness of biosecurity measures to control disease spread.

Here we obtain mechanistic insight by studying indirect transmission in controlled experiments and by using mathematical modeling to understand the experimentally observed transmission patterns. In previous experiments where a single bacterium species, *Campylobacter jejuni* (*C. jejuni*), was used, a delay in the onset of the first transmission events was observed when there is a (small) distance between colonized animals and recipients; however, when birds are in direct contact this delay is not observed, showing that the early pathogen excretion is sufficient to cause infection (13, 14). These observations have led to the hypothesis that the observed delay is the result of a combination of diffusive movement of pathogen in the environment and decay of this pathogen while traveling from colonized animals to recipient animals. To test this hypothesis, tailor-made experiments were carried out, in which we concurrently inoculated broilers with two different pathogens with very different decay rates in the environment, namely *C. jejuni* and *Escherichia coli* (*E. coli*), and then studied the indirect transmission of these pathogens to spatially separated susceptible recipients.

In the mathematical model, we assume that pathogen-containing particles are randomly displaced through the environment according

Significance

The failure to identify avoidable contacts makes indirect transmission the prime cause of difficulties in controlling epidemic spread (e.g., bacteria in hospitals, livestock infections). Our results from tailored indirect transmission experiments show that there is a clear delay in onset of transmission when there is a small distance between sender and recipient, and that this delay differs considerably between bacterium species. We showed that the observed patterns can, as we understand it, only be explained by taking into account the slow (random) transport of infectious material from sender to recipient and mortality of the bacterium during that transport. The implication of this is that hygiene measures can influence indirect transmission as shown for an antibiotic-resistance bacterium in a hospital.

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¹B.A.D.v.B. and A.S. contributed equally to this work.

²To whom correspondence may be addressed. E-mail: bram.vanbunnik@ed.ac.uk or mart.dejong@wur.nl.

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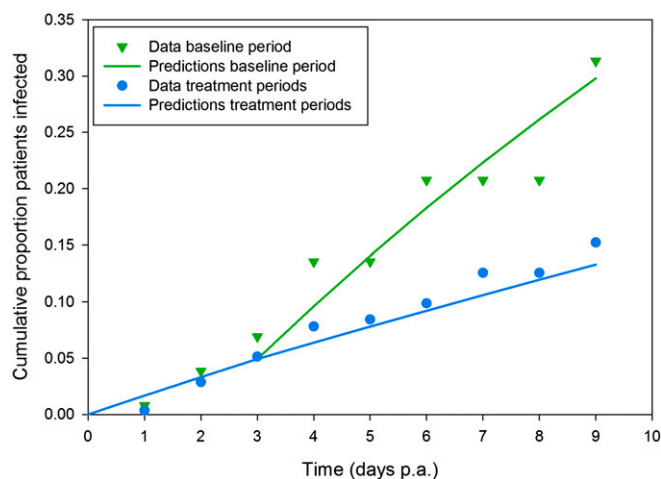


Fig. 2. Cumulative proportion of patients infected per day after intensive care admission for the baseline period and all periods combined. Day 0 is the day of admission. Baseline refers to the period with no intervention, treatment periods refers to the treatment periods in which intensified cleaning was carried out (see *Materials and Methods* for further details). Note that only from day 4 onward, the slope of the baseline period is different from the treatment periods.

As a result, the accumulation of pathogens in the environment is much slower for *C. jejuni* compared with *E. coli*. At a given location and for a steady emission of pathogens from colonized/infected sources, the model predicts a saturation level of pathogen accumulation. That level is determined by the time needed to reach a location and the decay occurring during that time. This level is predicted to be lower for *C. jejuni* compared with *E. coli*. In addition, the model predicts that there is a limit to the distance that pathogens can reach in substantial amounts. Formulated more precisely, for every pathogen quantity level there is a maximum distance at which that quantity level can be realized (Fig. S2). This distance limit is determined by the decay rate and the diffusion coefficient D .

The data (Fig. 1) clearly show that there is a delay between the excretion by the senders and the infection of the recipients. We argue that the delay occurs during the travel/transfer of infectious material from sender to recipient, as separate observations imply the absence of a delay on either the sender or the recipient side: As was shown in direct transmission experiments using *C. jejuni* (13), the sender is excreting the bacteria from very soon after inoculation onward and these bacteria are immediately able to infect direct-contact recipients. Furthermore, it was observed in other previous experiments (14) that the recipients showed excretion always within one or two days after inoculation.

Therefore, the delay has to occur during the travel/transfer of infectious material from sender to recipient. Modeling the travel as a random walk, i.e., diffusion in 2D space (and including the decay of the pathogen while traveling), has the advantage that it explains both the delay and the difference in delay between the two bacterium species. Modeling it only as a buildup in the environment without a spatial component (16) would not explain the difference in delay. Although in our current model the diffusion is precisely following a 2D diffusion equation, this might not necessarily be the case; the movement of the infectious material in space does not have to be a random walk as we assumed. However, we believe that the random walk is a sensible model to start with as it needs no additional information.

Furthermore, the model predicts that infections with microorganisms having low decay rates can occur at distant locations (long) after the source of infectious material has been removed. This would have important consequences in, for example,

hospital intensive care units where this would imply that removing (or quarantining) a patient colonized with a certain pathogen might not prevent subsequent transmission if that pathogen survives well in the environment. As an example of this, we investigated data from a study of VRE in an ICU (15). In the original study, intensified environmental cleaning was associated with reduced acquisition of VRE. Given that newly admitted patients enter the ICU in a clean (and sterilized) bed, we assume that the surfaces immediately surrounding such a patient are initially not contaminated with VRE. However, without sufficient cleaning, the farther inanimate environment of a patient may still be contaminated with VRE from patients previously occupying the unit. Indeed our analysis of the ICU data shows the presence of a delayed transmission component, with a delay of 4 d. During intensified cleaning, the contamination level of the environment would be reduced whenever cleaning removes VRE from surfaces more rapidly than contamination occurs through diffusion. In those situations, we expect indirect transmission to be absent and indeed we observe that during the intensified cleaning period, thus indicating that colonization is most probably due to surface contamination near the patient (Fig. S1). This emphasizes the importance of evacuation, cleaning, and disinfection measures that are often taken to avoid such transmission. Furthermore, a delay of 4 d implies that—in this ICU—regular cleaning of the environment (at least once a week) is sufficient to counteract diffusive delayed transmission of VRE. As noted above, indirect transmission is often caused by multiple difficult-to-quantify mechanisms. Our diffusion model provides a means to understand and quantify the expected transmission risks and the impact of control measures. Our results indicate that 2D diffusion modeling is a promising approach to describing indirect transmission in a parsimonious manner; with only a few parameters and, moreover, parameters that could feasibly be estimated. The approach was successful in explaining key features of the indirect transmission of the two bacteria studied here and can also provide an explanation of a delayed component that we identified in the transmission of VRE in an ICU.

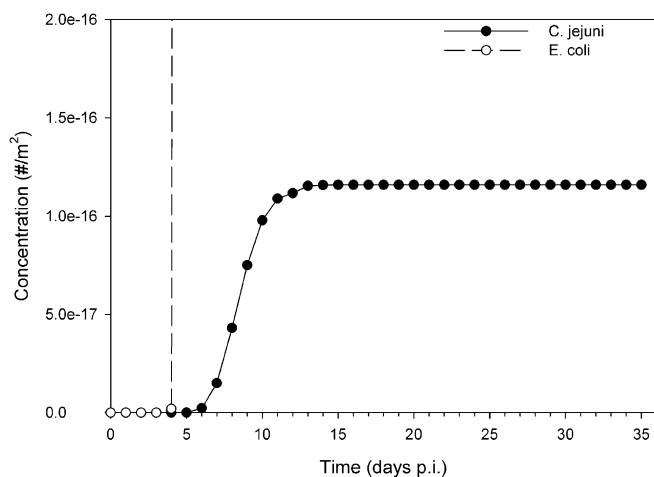


Fig. 3. Prediction of the amount of infectious material per unit area in a recipient cage as a function of time. Note that the curve for *E. coli* quickly rises beyond the scale of this graph because, for *E. coli*, a decay rate value of 0.0 d^{-1} was used. Open circles depict the amount of viable *E. coli*. Closed circles depict the amount of viable *C. jejuni*. For the construction of the figure, the center cage (Fig. 4) was taken as the area source and a cage alongside the center cage as the recipient source. Parameter values used: $D = 0.0025 \text{ m}^2/\text{d}$, $\alpha_{\text{campy}} = 2.25 \text{ d}^{-1}$, $\alpha_{\text{coli}} = 0 \text{ d}^{-1}$.

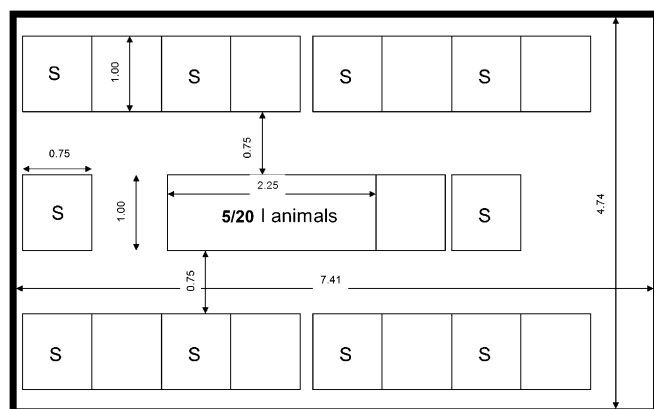


Fig. 4. Schematic overview of the housing of the experimental groups of 5 or 20 inoculated animals in a center cage and 10 susceptible recipient animals in individual surrounding cages. Alongside the arrows, distances are given in meters.

Materials and Methods

Analyses of the Experimental Data. The experimental setup consisted of, in each replicate, inoculated infectious broilers in a center cage surrounded by 10 recipient broilers placed individually in cages at a distance of ~ 75 cm both from the center cage and from each other (Fig. 4). All broilers in the center cage were inoculated with either *C. jejuni* or both *C. jejuni* and a labeled *E. coli* (see Table S1 for inoculation scheme). Both being commensal organisms to broilers, we expect no important interference between the two species, and comparison of the data for *C. jejuni* only replicates and those with both *C. jejuni* and *E. coli* show no signs of interference in terms of colonization times (Student *t* test: $P = 0.27$ for the group with 5 inoculated animals and $P = 0.31$ for the group with 20 inoculated animals). The occurrence of indirect transmission events was monitored by a daily collection of cloaca swab samples from all recipient broilers. The experiment ended 35 d p.i. (see Supporting Information for full description of experiment). In mathematical models, direct pathogen transmission is usually assumed to occur instantaneously when susceptible and infectious individuals are at the same location at the same time (17–19). Modeling indirect transmission necessitates inclusion of the transport of infectious material in the environment between hosts, thereby allowing for time delays between pathogen shedding by an infectious host and subsequent exposure of a recipient host (20, 21). To quantify the indirect infection pressure experienced by a susceptible recipient at a specific location at a specific time, the full history of how many infectious individuals were present at particular locations up until the time of interest needs to be taken into account. Here we developed a model in which the transport process was assumed to be diffusion of particles, i.e., infectious material was assumed to move with small random steps (22, 23). One appealing consequence of this simplification is that we do not have to parameterize unobserved individual displacements of infectious material through the environment. Instead, we fit a single parameter (the diffusion coefficient) to the observed pattern, averaging over all transport routes. We assume that the diffusion of both *C. jejuni* and *E. coli* through the environment is governed by one and the same diffusion coefficient. This is motivated by the fact that both *C. jejuni* and *E. coli* are transmitted fecal–orally, thus, both pathogens are

most probably transported on the same material. Moreover, in this case the two bacteria were excreted by the same animals. Cages with infectious broilers are modeled as an area source of pathogen-containing particles from which diffusion at rate D to the recipient cages occurs. For an area source emitting with strength Q_0 during a time interval $[0, \tau]$, the concentration of viable infectious material at a given location (x, y) at time t is obtained by integrating the point-source solution of the diffusion equation over both space and time taking into account the decay rate (α):

$$S_{cont}(x, y, t) = \int_0^\tau \int_{y_1}^{y_2} \int_{x_1}^{x_2} \frac{Q_0}{4\pi D(t-t')} \exp\left[-\alpha(t-t') - \frac{(x-x')^2 + (y-y')^2}{4D(t-t')}\right] dx' dy' dt'$$

The force of infection (FOI) experienced by a recipient animal is assumed to be proportional to the average concentration across its cage floor area. However, this is true for as long as the concentration is (much) smaller than an “exposure capacity” K (24). For larger concentrations, the FOI is assumed to be bounded by a maximum equal to βK (with β being the transmission parameter, see below and Supporting Information), which is determined, for instance, by limitations in access to and/or uptake of infectious material by recipient animals. This formulation ensures that, even in the limit of negligible pathogen decay, the infection rate will remain within biologically plausible bounds. See Supporting Information for the resulting equation. The model parameters and their dimensions are listed in Table 3. The parameters that need to be estimated from experimental observations are the diffusion coefficient D , the transmission parameter β_{campy} for *C. jejuni*, β_{coli} for *E. coli*, the exposure capacity K and the decay rates of the pathogens α_{campy} and α_{coli} . The two decay rates are estimated independently from the transmission experiments in separate survival experiments (see Supporting Information for full description of experiments), carried out under the same conditions as the transmission experiments. Estimated decay rates were 2.25 d^{-1} for *C. jejuni* and we used 0 for *E. coli*, as we observed 100% survival during more than 100 d (see Table S2). The remaining parameters were estimated using a maximum likelihood estimation approach (see Supporting Information for the derivation of the likelihood equation).

Analysis of the ICU Data. The data of Hayden et al. (15), on the spread of VRE in an ICU, were reanalyzed in this study to evaluate if the observed pattern of transmission provides evidence for a delayed/diffusive transmission component. A detailed description of the setup of this study can be found in the original paper.

In brief, the original study was intended to assess the performance of three different intervention schemes on the spread of VRE. It comprised of four study periods, each with different (sets of) interventions: a baseline period (baseline, period 1); a period with intensified environmental cleaning (treatment 1, period 2); a “washout” period without any specific intervention (treatment 2, period 3); and a period with multimodal hand hygiene (treatment 3, period 4). During the study period, rectal swab samples were taken daily from patients starting on the day of admission throughout the admission period. Cultures for VRE were performed of those swabs.

Improved environmental cleaning (treatment 1, period 2) involved explaining to housekeepers the importance of environmental cleaning and increased monitoring of housekeeper performance in addition to the actual environmental cleaning. It also involved daily cleaning of ventilator control panels as well as sensitizing nurses and other ICU staff about the problem of VRE and the interventions.

There were a total of 21 ICU beds available for admission of patients throughout the study period. In total, 748 admissions to the ICU were studied

Table 3. Dimension and description of parameters used in the model

Parameter	Dimension	Description
S_{cont}	$\#/m^2$	Concentration of pathogen on the time and location of interest
t'	d	Time of release of the particles
t, T	d	Time of interest
(x', y')	(m, m)	Location in the source cage
(x, y)	(m, m)	Location in the recipient cage
$x_{1r}, x_{2r}, y_{1r}, y_{2r}$	m	Coordinates of the source cage corners
$x_{1ar}, x_{2ar}, y_{1ar}, y_{2ar}$	m	Coordinates of the recipient cage corners
D	m^2/d	Diffusion coefficient
α	d^{-1}	Decay rate of the pathogen
K	$\#/m^2$	Exposure capacity
β	d^{-1}	Transmission parameter

and the average duration of stay was not significantly different for the four periods. Using this data, the daily infection rate per person after being admitted to the ICU was calculated as a function of days postadmission. Differences between rates of colonization for two window periods were analyzed using a Fisher's exact test with the level of significance set at $P < 0.05$.

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