

# Small distances can keep bacteria at bay for days

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Edited by Burton H. Singer, University of Florida, Gainesville, FL, and approved January 10, 2014 (received for review May 29, 2013)

**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.

Author contributions: B.A.D.v.B., T.J.H., G.N., M.J.M.B., and M.C.M.D.J. designed research; B.A.D.v.B. performed research; B.A.D.v.B., A.S., T.J.H., M.R.H., M.C.J.B., and M.C.M.D.J. analyzed data; T.J.H., G.N., M.J.M.B., M.K.H., R.A.W., M.C.J.B., and M.C.M.D.J. contributed analytic tools; M.K.H. and R.A.W. contributed dataset; and B.A.D.v.B., A.S., T.J.H., and M.C.M.D.J. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

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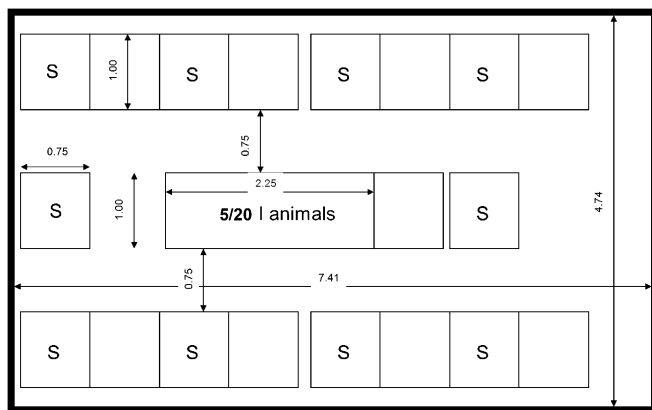
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This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1310043111/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1310043111/-DCSupplemental).









**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 ( $\alpha$ ):

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

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  $\beta K$  (with  $\beta$  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  $\beta_{campy}$  for *C. jejuni*,  $\beta_{coli}$  for *E. coli*, the exposure capacity  $K$  and the decay rates of the pathogens  $\alpha_{campy}$  and  $\alpha_{coli}$ . 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  $2.25 \text{ d}^{-1}$  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
$x_1, x_2, y_1, y_2$	m	Coordinates of the source cage corners
$x_{ar}, x_{br}, y_{ar}, y_b$	m	Coordinates of the recipient cage corners
$D$	$m^2/d$	Diffusion coefficient
$\alpha$	$d^{-1}$	Decay rate of the pathogen
$K$	$\#/m^2$	Exposure capacity
$\beta$	$d^{-1}$	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  $P < 0.05$ .

**ACKNOWLEDGMENTS.** We thank the animal caretakers for their assistance with the experiments; F. Putirulan and N. Bolder for their assistance in the laboratory; and M. Woolhouse and J. van Leeuwen for their insightful comments on earlier versions of the manuscript. This research was funded by the Ministry of Economic Affairs of The Netherlands (project code BO-08-010-010), and by the Economic Structure Enhancing Fund (FES) in The Netherlands: FES Program on Avian Influenza.

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