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Pathogen survival in the external environment and the evolution of virulence

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ABSTRACT

Recent studies have provided evolutionary explanations for much of the variation in mortality among human infectious diseases. One gap in this knowledge concerns respiratory tract pathogens transmitted from person to person by direct contact or through environmental contamination. The sit-and-wait hypothesis predicts that virulence should be positively correlated with durability in the external environment because high durability reduces the dependence of transmission on host mobility. Reviewing the epidemiological and medical literature, we confirm this prediction for respiratory tract pathogens of humans. Our results clearly distinguish a high-virulence high-survival group of variola (smallpox) virus, *Mycobacterium tuberculosis*, *Corynebacterium diphtheriae*, *Bordetella pertussis*, *Streptococcus pneumoniae*, and influenza virus (where all pathogens have a mean percent mortality $\geqslant 0.01\,\%$ and mean survival time >10 days) from a low-virulence low-survival group containing ten other pathogens. The correlation between virulence and durability explains three to four times of magnitude of difference in mean percent mortality and mean survival time, using both across-species and phylogenetically controlled analyses. Our findings bear on several areas of active research and public health policy: (1) many pathogens used in the biological control of insects are potential sit-and-wait pathogens as they combine three attributes that are advantageous for pest control: high virulence, long durability after application, and host specificity; (2) emerging pathogens such as the 'hospital superbug' methicillin-resistant *Staphylococcus aureus* (MRSA) and potential bioweapons pathogens such as smallpox virus and anthrax that are particularly dangerous can be discerned by quantifying their durability; (3) hospital settings and the AIDS pandemic may provide footholds for emerging sit-and-wait pathogens; and (4) studies on food-borne and insect pathogens point to future research considering the potential evolutionary trade-offs and genetic linkages between virulence and durability.

Key words: virulence, durability, mortality, transmission, sit-and-wait pathogens, evolution, infectious disease.

CONTENTS

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I. INTRODUCTION

Life-threatening human diseases such as smallpox, tuberculosis and malaria have been present throughout recorded history (Manchester, 1984; Des Prez & Goodwin, 1985; Benenson, 1989; Klayman, 1989; Neff, 1990). The persistence of high virulence over millennia runs counter to the traditional view that all pathogens should eventually evolve to a state of commensalism with their host (Dubos, 1965; Hoeprich, 1989). This inconsistency is especially apparent when one considers the rapid rate at which pathogens can evolve. Antibiotic resistance, for example, can evolve dramatically within months (Gezon, Schaberg & Klein, 1973; Mhalu, Mmari & Ijumba, 1979; Rowe & Threlfall, 1984; Saunders, 1984). Likewise, virulence of myxoma virus in Australian rabbit populations declined within a decade (Fenner & Ratcliffe, 1965).

During the last 15 years, the traditional view that well-adapted pathogens are mild pathogens has been rejected on theoretical and empirical grounds (Levin & Pimentel, 1981; Anderson & May, 1982; May & Anderson, 1983; Ewald, 1983, 1988, 1991*a*, *b*, 1994, 1995, 1996*a*, 1998; Levin, 1983; Bull, 1994; Lenski & May, 1994; Frank, 1996). The view displacing it proposes that the evolved levels of virulence reflect tradeoffs between counterbalancing selective pressures. Competition among pathogens within individual hosts should generally favour those genotypes that reproduce most rapidly, leading to increased virulence. Requirements for transmission from infected to susceptible hosts provide counterbalancing selection, often favouring relatively benign genotypes. Much of the research during the last 15 years has attempted to determine how different aspects of transmission have influenced the strength of this counterbalancing selection, and, hence, the virulence to which host-pathogen relationships evolve.

When transmission from immobilized infectious hosts to susceptible hosts can readily occur, high levels of virulence should evolve. For such pathogens the fitness costs should tend to rise relatively slowly as pathogen reproduction increases. The level of pathogen reproduction at which fitness costs increase faster than fitness benefits therefore should be greater for such pathogens, which should consequently evolve to relatively high levels of virulence (Ewald, 1983). Statistical analyses of data in the literature on human infectious diseases show that the potential for transmission from immobilized hosts explains much of the existing variation in virulence. Pathogens transmissible from immobilized hosts by arthropod vectors are significantly more lethal than are pathogens that are transmitted directly from person to person (Ewald, 1983). Similarly, much of the variation in virulence among gastrointestinal bacterial pathogens can be explained by the presence of cultural vectors, which are collections of environmental, biological, and cultural characteristics that transmit pathogens from immobilized infectious hosts (Ewald, 1988, 1991*b*).

Although vector-borne transmission explains much of the variation in virulence, substantial variation remains unexplained. Nonvector-borne pathogens are generally benign, but some, such as the smallpox virus and tuberculosis bacterium, are often lethal. Some of this variation in virulence among nonvector-borne pathogens might be explained by the prevalence of a 'sit-and-wait' strategy (Ewald, 1987, 1994). Like the vector-borne strategy, the sit-and-wait strategy requires mobility of another organism to permit transmission from an immobilized, infected host to a susceptible host; but instead of relying on the mobility of a vector for transmission, the 'sit-and-wait' pathogen makes use of the mobility of susceptibles. Pathogens that can survive outside of the host until a new susceptible host contacts them can still be transmitted from immobilized hosts (the differential mobility of infected hosts is crucial to this hypothesis, see Discussion). Because the costs of host immobilization are relatively low for durable pathogens, sit-and-wait transmission should favour evolution towards high levels of virulence (Ewald, 1987, 1994). This hypothesis predicts that the duration of pathogen survival outside of the host will be positively correlated with virulence. This study reports the first test of this prediction.

II. METHODS

To test for the predicted correlation between virulence and survival in the external environment, we quantified these two variables for respiratory pathogens of humans. We began our literature search using general medical and microbiological texts, and then moved to the original literature using cited references and computer databases to obtain data that were absent from or obliquely described in the texts.

We restricted the analysis to pathogens of the human respiratory tract because we wanted to eliminate variation due to host species or site of infection. We consider a pathogen to be a human pathogen if it is acquired exclusively or almost exclusively from other humans. On this basis we

excluded, for example, the rodent virus that causes Lassa fever. Influenza virus, however, was included because phylogenetic reconstructions indicate that once influenza enters humans it cycles almost exclusively among humans (Gorman *et al.*, 1991).

We define respiratory tract pathogens as those pathogens that are transmitted by nonrespiratory routes rarely relative to respiratory routes. On this basis we excluded pathogens for which infections by nonrespiratory routes were known to occur frequently (e.g. adenovirus, coronavirus, coxsackievirus, echovirus, enterovirus, herpesvirus, poliovirus, reovirus, *Staphylococcus aureus*, *Streptococcus pyogenes*). Other pathogens were excluded because of insufficient information about survival in the external environment (e.g. *Bordetella parapertussis*, *Haemophilus parainfluenzae*, and *Chlamydia pneumoniae*) or modes of transmission (*Mycobacterium leprae*).

III. RESULTS AND DISCUSSION

(1) Ranking of mortality as an indicator of virulence

(*a*) *Procedures for quantifying mortality*

To quantify virulence, we calculated mortality per infection (*M/I*) for each pathogen (which is also sometimes referred to as case mortality, see Day, 2002*a*). Day (2002*a*) suggested that this measure (or lethal dose which is impossible to determine in comparative studies) is best suited as a single measure of parasite virulence. We define infection broadly to include all people within which the pathogen has multiplied sufficiently to be detectable serologically or by isolation (this definition therefore includes those people who according to a narrower definition of infection may have been considered 'colonized' rather than 'infected'). Because *M/I* is rarely presented in the literature, we usually calculated it by multiplying mortality per case (*M/C*) by cases per infection (*C/I*). To reduce effects of treatment on *M/I*, estimates were obtained from data gathered prior to the initiation of effective antibiotic treatment against the pathogen in question (usually a bacterium). To reduce effects of secondary infections by different pathogens on *M/I*, estimates were obtained for cases with antibiotic treatment against the secondary pathogens whenever such treatment was ineffective against the primary pathogen (usually a virus). If a vaccine is based on the virulence-determining components it should favour evolutionary reductions in virulence (Ewald, 1994, 1996*b*). To quantify *M/I* for pathogens controlled by such vaccines, we therefore used data gathered just prior to the introduction of these vaccines.

To reduce geographic and cultural influences on *M/I*, data from the United States were compared whenever possible. For those pathogens that were successfully controlled with antibiotics we chose the latest time periods before introduction of these antibiotics under the assumption that these later statistics would be the most accurate. If the direct effects of a pathogen on mortality were quantifiable only after antibiotics successfully controlled secondary infections (see above), we used the earliest time periods after introduction of such antibiotics and accurate reporting.

Particularly vulnerable individuals, such as newborns, the aged, the immuno-compromised, and the poorly nourished, should be prevalent among the reported deaths. We consider these individuals to be useful indicators of the inherent virulence of pathogens, like the canary in the mine, but we did not want to over-represent them in sampled populations. We therefore sought data sets that reflected the composition of the community-at-large, and omitted data sets collected exclusively from particularly vulnerable populations, such as populations of hospitals or nursing homes. To reduce effects of host resistance, we excluded data sets from especially vulnerable groups such as neonates and immuno-compromised individuals. We also excluded data from studies that examined populations living under crowded conditions, such as populations of schools and military installations. Because of the effects of crowding and/or cultural vectors, transmission in these institutions may change the pathogen's virulence substantially (Bisno, 1990; Ewald, 1994).

(*b*) *Mortality values for different pathogens*

Variola (smallpox) virus. Variola viruses have been divided into two groups, designated major and minor. Mortality is positively related to level of viremia during the first two days of disease (Kempe, 1979), but not to co-occurring bacterial infections (Downie, 1965). The *M/C* of variola major ranges from 0.05 to 0.4, averaging about 0.2; that of variola minor ranges from 0.001 to 0.02, averaging about 0.01 (Fenner *et al.*, 1988). Because *C/I* ranges between 0.95 and 1.0 for both groups (Benenson, 1990; Nathanson, 1990), *M/I* is about 0.2 for variola major and 0.01 for variola minor, or **0.1** for variola in general.

Mycobacterium tuberculosis. Antibiotics effective against *M. tuberculosis* were introduced during the latter half of the 1940s (Des Prez & Heim, 1990; Mitchison, 1992). Prior to this time about half of the active cases of tuberculosis resulted in death (Des Prez & Heim, 1990). *C/I* averages about 0.1 (NIAID, 1989). *M/I* was thus about **0.05**.

Corynebacterium diphtheriae. In the U.S. from the early 1920s to the mid 1970s, *M/C* remained relatively constant, averaging about 0.1 (Goldstein & Hoeprich, 1977). The dramatic decreases in *C/I* during this period have been attributed to vaccination, which was introduced during the mid 1920s (Goldstein & Hoeprich, 1977). The vaccine used a modified version of the diphtheria toxin, which is the key determinant of lethality. By preventing the transmission of toxigenic strains of *C. diphtheriae* to vaccinated individuals, widespread vaccination undoubtedly provided a selective pressure against the toxigenic strains, increasing the relative frequency of non-toxigenic strains (Schuman & Doull, 1940; Stebbins, 1940; Pappenheimer & Gill, 1972, 1973; Ewald, 1994), thereby decreasing *C/I*. These considerations indicate that *C/I* should be estimated from data obtained prior to the widespread use of vaccination. At the onset of vaccination *C/I* was about 0.02 (Frost, 1928). *M/I* was therefore **0.002** prior to the evolutionary effects of vaccination.

Bordetella pertussis. The mortality per recorded cases of whooping cough decreased steadily in the U.S. from about 0.05 in the 1920s to 0.004 in the 1970s (Brooks & Buchanan, 1970). This reduction was not a consequence of effective

antibiotic treatment against *B. pertussis* because no effective antibiotic treatment had been put into use during this time. Some of the reduction may be attributable to a preponderance of unreported non-lethal cases, which may have inflated *M/C* by five- to tenfold early in the 20th century (Luttinger, 1916). Although the accuracy of reporting improved substantially during the first half of the century, *M/C* was probably overestimated at least until the mid-century (Gordon & Hood, 1951). Most of the mortality that occurred during the first half of the century was attributable to secondary infections, which became effectively controlled by antibiotics during the period (Morse, 1977). We therefore consider the most recent figure from the 1970s mentioned above to be the best indicator of *M/C* attributable to *B. pertussis*. Estimates of *C/I* among the very young average 0.78 (Gordon & Hood, 1951; Wilfert, 1988*a*; Giammanco *et al.*, 1991), but about two-thirds of the infections occur after the age of three (Giammanco *et al.*, 1991). *M/I* is therefore about **0.001** (i.e. $0.004 \times 0.78/3$).

Streptococcus pneumoniae. Each year in the U.S. *S. pneumoniae* causes about 150 000–570 000 cases of pneumonia, 2600–6200 cases of meningitis, and 16 000–55 000 cases of bacteremia. Prior to effective antibiotic treatment, the deaths per case for these three diseases were 0.2–0.3, 0.3, and 0.2, respectively (Heffron, 1939; Finland, 1982; Roberts, 1985). Using the midpoints for the preceding ranges, these figures yield about 90 000 deaths from pneumococcal pneumonia, 1320 deaths from meningitis and 7100 from bacteremia, for a total of 98 420 deaths due to *S. pneumoniae* each year.

Carriage frequencies are about 38% for children 0–5 years old, 29% for children 6–12 years old, 9% for children 13–17 years old, 19% for adults with children and 6% for adults without children (Hendley *et al.*, 1975). Combining these carriage frequencies with population estimates from this period (i.e. 1970) (US Bureau of the Census, 1975) yields a total of about 40 million people in the U.S. carrying *S. pneumoniae* at any given time. Given that the average length of carriage is about 7.5 weeks (mean of estimates given by Heffron, 1939; Dowling, Sheehe & Feldman, 1971; Hendley *et al.*, 1975), the annual number of infections is about 277 million. Thus, *M/I* is approximately 98 420 deaths/277 million infections = **0.00036**.

Influenza virus. Mortality data prior to the 1957 pandemic should tend to overestimate actual *M/C* attributable to the influenza virus because deaths due to the virus were not distinguished from deaths due to secondary bacterial infections (Stuart Harris, 1961). Moreover, unusual epidemiological circumstances of the 1918 pandemic may have enhanced virulence to unnaturally high levels (Ewald, 1991*a*). During the 1957 pandemic, about 59 300 deaths occurred in the U.S. (Dauer & Serfling, 1961), about 25% of which can be attributed to viral pneumonia (Stuart Harris, 1961). These 14 825 virally caused deaths occurred among 70 million cases (Francis & Maassab, 1965); thus, *M/C* is about 0.0002. Estimates for the *C/I* of influenza vary, but average about 0.5 (Mulder, 1960; Francis & Maassab, 1965; WHO, 1969; Davis *et al.*, 1973; Jackson & Muldoon, 1975), yielding a value of **0.0001** for *M/I*.

Neisseria meningitidis. Prior to effective antibiotic treatment, *M/C* of meningococcal disease was about 0.5 (Feldman, 1972). Laboratory surveillance yielded a national incidence in the U.S. of 3100 cases during 1986 (Pinner *et al.*, 1991); earlier surveys suggest that this incidence differs little from the incidences during the 1960s and 1970s (Feldman, 1972). About 7.5% of the U.S. population carries *N. meningitidis* at any given time; the median duration of carriage is about 9.6 months (Greenfield, Sheehe & Feldman, 1971). Assuming that 9.4% (i.e. $7.5\% \times 12$ months/9.6 months) of the 242 million people in the US (US Bureau of the Census, 1991) were infected with *N. meningitidis* during 1986, a total of 22.7 million infections occurred. Thus, *C/I* was 0.000137 (i.e. 3100 cases/22.7 million infections). *M/I* is therefore about **0.00007**.

Rubeola (measles) virus. In the U.S. and other industrialized countries, the mortality per case of measles decreased about 20 fold during the 20th century (Marcy & Kibrick, 1977*a*). As with whooping cough, most of the mortality historically associated with measles was due to secondary bacterial infections, the negative effects of which have been lessened by antibiotics and improved nutrition (Marcy & Kibrick, 1977*a*; Krugman, 1983). After this transition, *M/C* was approximately 0.0001 (Barkin, 1975*a*). Approximately 63% of these deaths are caused by respiratory complications (Barkin, 1975*b*), and about half of these deaths from respiratory complications are still caused by secondary bacterial infection (Marcy & Kibrick, 1977*a*). Thus, 68.5% of all deaths can be attributed to the viral infection alone (= infections without complications plus half of the infections with complications = 37% + 63%/2). *C/I* for measles is between 0.95 and 1.0 (Nathanson, 1990). *M/I* is therefore approximately **0.00007** (i.e. $0.0001 \times 0.685 \times 0.975$).

Mumps virus. Virtually all mumps deaths result from encephalitis, which has a mortality of 1.4% (Wolinsky & Server, 1985; Benenson, 1990) and occurs in about 0.38% of all mumps cases (mean of percentages given by Benenson, 1990 and Wilfert, 1988*b*), yielding an *M/C* of 0.00005 while Frenkel and Bellanti (1981) estimated *M/C* to be 0.0001. Multiplying the average of these two estimates with the *C/I* of mumps, which averages 0.7 (Marcy & Kibrick, 1977*b*; Horstmann, 1979; Benenson, 1990), results in **0.00005** for *M/I*.

Parainfluenza virus. Parainfluenza infections can be lethal as a consequence of laryngotracheobronchitis (LTB), which is caused by virus types 1, 2 and 3, and bronchiolitis, which is caused almost exclusively by type 3 virus (Hendley, 1990). The overall *M/I* in children below six years of age is 0.00036 (Table 1). The infection rate for these children is 1.5 times the infection rate of adults (Chanock & McIntosh, 1985). Assuming a life expectancy of 74 years (Southwick, 1985), 88% of parainfluenza infections occur in people older than six years of age and cause virtually no death (Brady, Evans & Cuartas, 1990). Thus, overall *M/I* is about **0.00004**.

Mycoplasma pneumoniae. Without antibiotic treatment, *M/C* of *Mycoplasma pneumonia* is 0.0–0.1% (Liu, 1977). Clinically apparent pneumonia develops in about 6.3% of people infected with *M. pneumoniae* (Foy *et al.*, 1979). Using the midpoint for *M/C* results in **0.00003** for *M/I*.

Respiratory syncytial virus (RSV). RSV is responsible for approximately 2000 deaths annually in the U.S. (Weiss, 1989), virtually all of them in very young children

Table 1. Mortality per infection from parainfluenza virus in children below six years of age

Type i	Disease	Frequency per case*	Deaths per case M_i/C_i	Cases per infection C_i/I_i	Deaths per infection M_i/I_i	Prevalence p_i	Prevalence-weighted deaths per infection p_iM_i/I_i
1	LTB	0.09†‡	0.00072	0.504†	0.00036	0.27§	0.00010
2	LTB	0.03†	0.00024	0.67†	0.00016	0.21§	0.00003
3	LTB	0.07‡	0.00056	0.78†	0.00067	0.34§	0.00023
	B	0.02‡	0.00030				
4	NA	0	0	NA	NA	0.18§	0.00000
							$M/I=\Sigma p_iM_i/I_i$ $=0.00036$

Abbreviations: LTB = laryngotracheobronchitis, B = bronchiolitis, NA = not applicable because mortality is virtually zero (Hendley, 1990).
* Frequency per case refers to the frequency of the life-threatening disease specified in the second column per symptomatic infection of the parainfluenza type specified in the first column. The frequency per case was multiplied by 0.8 % for LTB [mean of percentage deaths of LTB cases from eight studies cited in Adair *et al.* (1971)] and 1.5 % for bronchiolitis [percentage deaths of bronchiolitis cases as cited in Liu (1983)] to obtain M_i/C_i (fourth column).
† Chanock & Parrott (1963).
‡ Wright (1984).
§ Hendley (1990).

(Kravetz *et al.*, 1961; Chanock & Parrott, 1965; Liu, 1983; Benenson, 1990). The overall annual infection rate is 0.216 per person (Cooney, Fox & Hall, 1975). Assuming a 74-year life-span (Southwick, 1985), the 4 million infants born in a given year (Hoffman, 1990) should experience about 64 million infections during their lifetimes. Because nearly all deaths occur very early in life, the 2000 deaths caused annually by RSV correspond to approximately 2000 deaths expected during the lifetimes of each annual cohort. *M/I* is therefore approximately **0.00003** (i.e. 2000 deaths/64 million infections).

Varicella-zoster (chickenpox) virus. In the U.S. about 100 deaths occur among 2.82 million varicella cases each year (Preblud, 1981), yielding 0.000035 for *M/C*. Because *C/I* ranges between 0.95 and 1.0 (Gelb, 1985), *M/I* is about **0.00003**.

Rubella virus. Virtually all of the mortality due to rubella virus results from encephalitis which is associated with a mortality of 20–50 % (Marcy & Kibrick, 1977*c*; Kilbourne, 1979; Gershon, 1990). Encephalitis occurs in approximately 0.02 % of rubella cases (Gershon, 1990). Using the average mortality, *M/C* for rubella is about 0.00007. *C/I* averages about 0.4 (Rawls, 1972), yielding **0.00003** for *M/I*.

Haemophilus influenzae. Of all clinical cases due to *H. influenzae*, meningitis accounts for 55 %, pneumonia for 12 %, epiglottitis for 10 %, and bacteremia for 3 % (mean of percentages given by Dajani, Asmar & Thirumoorthi, 1979; Granoff & Basden, 1980; Murphy *et al.*, 1987); the remaining 20 % of clinical cases (e.g. arthritis, cellulitis, etc.) are non-fatal. Prior to effective antibiotic treatment, about 96 % of meningitis cases were fatal (Alexander, Ellis & Leidy, 1942). Mortality associated with the other categories was high but not well quantified (Alexander *et al.*, 1942; Turk & May, 1967). Assuming 50 % mortality for all severe cases other than meningitis yields 0.65 for *M/C*.

Approximately 10 000 cases of meningitis caused by *H. influenzae* type b infection were reported each year in the U.S. prior to the use of *H. influenzae* vaccines in the 1980s (Glode *et al.*, 1980; Moxon, 1990), but active surveillance indicates that such reporting identified only about 60 % of cases (Adams *et al.*, 1993). Therefore, about 16 700 cases of meningitis occurred annually prior to vaccination. On the basis of the percentages given above, a total of approximately 23 560 cases of meningitis, pneumonia, epiglottitis, and bacteremia occurred each year prior to effective vaccination. At any moment in time, about 158.46 million people in the U.S. carry one of the *H. influenzae* strains. This number is derived by multiplying the U.S. population of 228 million (1980 census, see US Bureau of the Census, 1991) by the average prevalence of all the *H. influenzae* strains in the population [taking the midpoint of prevalences (called carriage rates in Table 2 of Moxon, 1990) for each strain, average prevalence is 0.695]. Since the mean carriage duration of *H. influenzae* is 0.2 years (Murphy *et al.*, 1987), a total of 158.46 million/0.2 = 792.30 million infections occur annually in the U.S. Thus *C/I* is 23 560 cases/792.30 million infections = 0.0000297. Assuming that *M/C* = 0.65 (see preceding paragraph), *M/I* is **0.00002**.

Rhinovirus. No fatalities due to rhinovirus infection have been reported (Benenson, 1990). We therefore consider *M/I* to be **0**.

(2) Ranking of survival in the external environment

(*a*) *Procedures for ranking durability*

Studies of pathogen survival in the external environment have been carried out under a wide variety of environmental conditions. Temperature, substrate, and source of infectious material may have marked effects on pathogen survival. We therefore categorized survival times according to these variables to facilitate comparisons between pairs of pathogens under similar environmental conditions. We used these pair-wise comparisons to generate a ranking of survival durations among the pathogens described in the preceding section. However, we did not include relative humidity in our

Table 2. The maximum durability of respiratory pathogens under different environmental conditions. References for durability of mumps, rubella and varicella-zoster virus are given in the text

Substrate*†	Immediate source	Light†	Temperature‡†	Durability§	References
Variola virus					
exudate	patient	dark	R (15–30 °C)	$13 y	Wolff & Croon (1968)
exudate	patient	dark	R	97–417 d‖	Downie & Dumbell (1947)
exudate	patient	indirect	R (20–24 °C)	$530 d	MacCallum & McDonald (1957)
exudate	patient	indirect	R	$196 d	Downie & Dumbell (1947)
exudate	patient	dark?	30 °C	60–185 d	MacCallum & McDonald (1957)
glass	vesicle fluid	dark	R	84 d	Downie & Dumbell (1947)
glass	vesicle fluid	indirect	R	35 d	Downie & Dumbell (1947)
Corynebacterium diphteriae					
exudate	patient	indirect	R	63–150 d	Mitscherlich & Marth (1984)
glass	suspension	indirect	R	<1–175 d	Laurell *et al.* (1949); Ouchterlony (1949)
dust	floor	indirect	R	<61–112 d‖	Crosbie & Wright (1941)
dust	floor	dark	R	7–102 d	Engley (1955)
dust	suspension	indirect	R	104–175 d	Laurell *et al.* (1949); Ouchterlony (1949)
sand	suspension	indirect	R	18–189 d	Laurell *et al.* (1949); Ouchterlony (1949)
soil	suspension	indirect	R	10–208 d	Laurell *et al.* (1949); Ouchterlony (1949)
fabric	suspension	indirect	R	2–175 d	Laurell *et al.* (1949); Ouchterlony (1949)
fabric	suspension	indirect	R	8–140 d	Mitscherlich & Marth (1984)
paper	suspension	indirect	R	91–175 d	Laurell *et al.* (1949); Ouchterlony (1949)
wood	patient	dark	R	180 d‖	Mitscherlich & Marth (1984)
aerosol	suspension	dark	R (21–27 °C)	2.2 d¶	Wells & Stone (1934)
Mycobacterium tuberculosis					
glass	sputum	dark	R	90–142 d‖	Smith (1942)
glass	sputum	dark	R	42–309 d	Soparkar (1917); Abe (1949)
glass	sputum	indirect	R	6–124 d	Soparkar (1917); Twichell (1905)
glass	suspension	dark	R	41 d	Mitscherlich & Marth (1984)
glass	suspension	indirect	R	1.3 d	Mitscherlich & Marth (1984)
dust	fabric & floor	dark?	R	10–120 d	Mitscherlich & Marth (1984)
sand	sputum	indirect	R	30–123 d	Mitscherlich & Marth (1984)
fabric	sputum	indirect	R	10–75 d	Mitscherlich & Marth (1984)
paper	sputum/ suspension	dark	15–30 °C	14–135 d	Smith (1942); Mitscherlich & Marth (1984)
paper	sputum	indirect	R	1–31 d‖	Kenwood & Dove (1915); Smith (1942)
wood	sputum	indirect	R	70 d	Mitscherlich & Marth (1984); Twichell (1905)
Streptococcus pneumoniae					
glass?	sputum	dark?	R	6 d	Williams & Kauffman (1978)
glass	suspension	dark	27 °C	1–11 d	Mitscherlich & Marth (1984)
glass	suspension	indirect	27 °C	<1–10 d	Mitscherlich & Marth (1984)
dust	sputum	dark?	15–20 °C	20 d	Mitscherlich & Marth (1984)
dust	pus	dark?	15–20 °C	12 d	Mitscherlich & Marth (1984)
soil	sputum	dark?	15–20 °C	100–140 d‖	Mitscherlich & Marth (1984)
soil	pus	dark?	15–20 °C	16 d	Mitscherlich & Marth (1984)
fabric	suspension	dark	27 °C	2–15 d	Mitscherlich & Marth (1984)
fabric	suspension	indirect	27 °C	1–10 d	Mitscherlich & Marth (1984)
aerosol	suspension	dark	R (21–27 °C)	1.5 d¶	Wells & Stone (1934)
aerosol	suspension	indirect	33 °C	0.4–0.7 d¶	Dunklin & Puck (1948)
aerosol	suspension	indirect	22 °C	0.03–0.7 d¶	Dunklin & Puck (1948)
aerosol	suspension	indirect	14 °C	0.02–0.4 d¶	Dunklin & Puck (1948)
Influenza virus					
glass	mucin suspension	dark	R	0.13–45 d‖	Parker *et al.* (1944)
glass	suspension	dark	R	<0.08–45 d‖	Parker *et al.* (1944); Edward (1941)
glass	suspension	dark?	20 °C	0.12–0.25 d¶	Buckland & Tyrrell (1962)
glass	suspension	dark	22 °C	28 d	Edward (1941)
glass	suspension	dark	37 °C	3 d	Edward (1941)
glass	droplet nuclei	dark	R	0.21 d	Edward (1941)
dust	suspension	dark	R	14 d	Edward (1941)
talc	suspension	dark	R	4 d	Parker *et al.* (1944)
fabric	suspension	dark	R	7–14 d	Edward (1941)

Table 2 (*cont.*)

Substrate*†	Immediate source	Light†	Temperature‡†	Durability§	References
fabric	droplet nuclei	dark	R	21 d	Edward (1941)
fabric	suspension	dark	22 °C	3–<7 d	Edward (1941)
fabric	suspension	dark	37 °C	<1 d	Edward (1941)
fabric	suspension	indirect	R	1 d	Edward (1941)
skin	suspension	indirect	R	0.03 d‖	Parker & MacNeal (1944)
aerosol	suspension	dark	32 °C	0.15–2.1 d¶	Harper (1961, 1963)
aerosol	suspension	dark	R	0.02 d	Wells & Brown (1936)
aerosol	suspension	dark?	R	0.03–0.51 d¶	DeJong & Winkler (1964); Schaffer *et al.* 1976)
aerosol	suspension	dark	21–24 °C	0.54–8.3 d¶	Harper (1961, 1963)
aerosol	suspension	dark	10 °C	2.1–5.6 d¶	Harper (1963)
aerosol	suspension	dark	7–8 °C	2.4–24 d¶	Harper (1961)
Bordetella pertussis					
glass	suspension	indirect?	R	<0.04h–5 d	Ocklitz & Milleck (1967)
plastic	suspension	indirect?	R	3–5 d	Ocklitz & Milleck (1967)
rubber	suspension	indirect?	R	<0.04–0.2 d	Ocklitz & Milleck (1967)
fabric	suspension	dark	17–20 °C	6 d	Ocklitz & Milleck (1967)
fabric	suspension	indirect?	R	<0.04–4 d	Ocklitz & Milleck (1967)
paper	suspension	dark	17–20 °C	2 d	Ocklitz & Milleck (1967)
paper	suspension	indirect?	R	0.2–1 d	Ocklitz & Milleck (1967)
skin	suspension	indirect?	R	<.04–0.25 d	Ocklitz & Milleck (1967)
aerosol	suspension	dark	17–20 °C	0.83 d	Ocklitz & Milleck (1967)
Rubeola virus					
aerosol	suspension	dark?	R	0.05–0.8¶	DeJong & Winkler (1964)
Neisseria meningitidis					
glass	suspension	dark	R	<1 d	Flügge (1906)
glass	suspension	indirect	R	<0.42 d	Flügge (1906)
fabric	patient	indirect	7–11 °C	0.17–0.29 d	Downie & Aberd (1940)
fabric	patient	indirect	17–18 °C	0.29 d	Downie & Aberd (1940)
fabric	suspension	dark	20–25 °C	0.13 d	Mitscherlich & Marth (1984)
fabric	suspension	indirect	R	0.29–1.25 d	Mitscherlich & Marth (1984); Flügge (1906)
Parainfluenza virus					
glass	suspension	dark?	20 °C	0.5–0.7 d¶	Buckland & Tyrrell (1962)
glass	suspension	dark?	R	4.3–6.7 d¶	Parkinson *et al.* (1983)
plastic	suspension	indirect	R	0.02 d	Brady *et al.* (1990)
steel	suspension	indirect	R	0.08 d	Brady *et al.* (1990)
fabric	suspension	indirect	R	0.25 d‖	Brady *et al.* (1990)
paper	suspension	indirect	R	0.08 d	Brady *et al.* (1990)
skin	mucin suspension	indirect	R	0.04 d	Ansari *et al.* (1991)
skin	suspension	indirect	R	0.04 d‖	Brady *et al.* (1990)
aerosol	suspension	dark?	24 °C	0.12–1.7 d¶	Miller & Artenstein (1967)
Mycoplasma pneumoniae					
aerosol	suspension	dark	38 °C	0.11–0.44 d¶	Wright *et al.* (1969)
aerosol	suspension	dark	27 °C	0.17–2.1 d¶	Wright *et al.* (1968)
aerosol	suspension	dark	15 °C	2.8–4.2 d¶	Wright *et al.* (1969)
aerosol	suspension	dark	10 °C	1.7–3.3 d¶	Wright *et al.* (1969)
Respiratory syncytial virus					
plastic	suspension	dark?	R	1–3 d	Kingston (1968)
plastic	suspension	dark?	R	2.1–2.8 d¶	Kingston (1968)
plastic	nasal secretion	indirect	22–25 °C	0.25–0.29 d	Hall *et al.* (1980)
rubber	nasal secretion	indirect	22–25 °C	0.04–0.17 d	Hall *et al.* (1980)
fabric	nasal secretion	indirect	22–25 °C	0.02–0.08 d	Hall *et al.* (1980)
paper	nasal secretion	indirect	22–25 °C	0.02 d	Hall *et al.* (1980)
skin	nasal secretion	indirect	22–25 °C	0.01–0.02 d	Hall *et al.* (1980)
aerosol	suspension	dark?	20.5 °C	0.08–0.3 d¶	Rechsteiner & Winkler (1969)
Rhinovirus					
plastic	mucus	indirect	23 °C	0.13–0.2 d‖	Hendley *et al.* (1973)

Table 2 (*cont.*)

Substrate*†	Immediate source	Light†	Temperature‡†	Durability§	References
plastic	mucus suspension	indirect	23 °C	0.06–1 d	Hendley *et al.* (1973)
plastic	suspension	indirect	23 °C	0.13–2.5 d‖	Reed (1975); Hendley *et al.* (1973)
steel	suspension	indirect	23 °C	0.04–3 d	Reed (1975); Hendley *et al.* (1973)
glass	suspension	dark?	20 °C	0.5–2.1 d¶	Buckland & Tyrrell (1962)
fabric	suspension	indirect	23 °C	0.04–1 d	Reed (1975); Hendley *et al.* (1973)
paper	suspension	indirect	23 °C	0.04 d	Reed (1975); Hendley *et al.* (1973)
wood	suspension	indirect	23 °C	0.04–0.13 d	Hendley *et al.* (1973)
skin	suspension	indirect	23 °C	0.04–0.13 d‖	Reed (1975); Hendley *et al.* (1973)
aerosol	suspension	dark?	19–21 °C	0.02–7.6 d¶	Karim *et al.* (1985)
Haemophilus influenzae					
glass	suspension	indirect	R	0.29 d	Mitscherlich & Marth (1984)
sputum	patient	?	R?	2 d	Burrows (1968)
fabric	suspension	?	R?	<1 d	Burrows (1968)
fabric	patient	indirect?	R?	0.25 d	Mitscherlich & Marth (1984)
wood	suspension	indirect	R	0.08 d	Mitscherlich & Marth (1984)
aerosol	suspension	dark	R (21–27 °C)	0.03 d¶	Wells & Stone (1934)

* 'Exudate' refers to the false membrane that forms on the mucous surface during diphtheria and the crusts that form during smallpox. 'Fabric' includes textiles and their raw materials, such as cotton and linen.
† A question mark indicates that the particular information was not stated explicitly; if descriptions of materials and methods yielded a best guess, that best guess precedes the question mark.
‡ R = room temperature.
§ The values refer to the maximum durations over which viable pathogens were found for individual replicates.
‖ Studies that were ended before maximum durability was ascertained. y = years, d = days.
¶ Time for 99.99 % reduction of survival as calculated from the exponential decay equation.

comparisons, because the effect of humidity on individual pathogens is highly variable. High humidity increases survival for some pathogens, decreases survival for others and has no discernible effect on still others. We therefore compared maximum survival durations regardless of humidity within the range of survival durations.

We report survival on all substrates that are considered to be of epidemiological relevance. When researchers disagreed about the precise role of different substrates and routes of transmission, and conclusive proof was lacking, we included all routes and substrates suggested by these researchers. For example, decades of experimental study of rhinovirus transmission have not yet permitted a consensus of the importance of aerosol transmission relative to direct contact with contaminated skin and fomites (Gwaltney & Hendley, 1978; Gwaltney, Moskalski & Hendley, 1978; Dick *et al.*, 1987; Tyrrell, 1992). Similarly, *M. tuberculosis*, *S. pneumoniae* and influenza virus are often categorized as being transmitted primarily by aerosol droplets or droplet nuclei (the residues of dried aerosol droplets; Betts & Douglas, 1990; Mufson, 1990; Starke, Jacobs & Jereb, 1992). However, observations and experimentation, especially during the first half of the 20th century, led many experts to incriminate dust and disintegrating sputum as major sources of infection (Otis, 1909; Knopf, 1910; Lange, 1926; Augustine, 1929; Loosli *et al.*, 1943; Duguid & Wallace, 1948). The relative importances of these various routes of air-borne transmission are still uncertain (Grange, 1984). The experiments completed during the intervening years have supported the importance of droplet nuclei, for at least some respiratory pathogens (Riley, 1982; Nardell, 1990), but do not address the degree to which air-borne particles of comparable size may be generated from other forms of environmental contamination. Another source of uncertainty stems from the time scale and environmental conditions of the studies. The importance of dust-borne infections, for example, could be vastly underestimated if the pathogen under study survived on dust far longer than the duration of the study, if studies were conducted under relatively dust-free conditions, or if the smallest dust fragments were not represented in the study. Even if transmission occurred solely by droplet nuclei, we presume that survival on surfaces is relevant for pathogens whose aerosol survival has not been measured, because survival on surfaces is probably positively correlated with air-borne survival.

We did not include durations of survival in aqueous media because survival in solution is of little if any epidemiological importance to respiratory tract pathogens that are regularly transmitted between humans. Similarly, we did not use survival in sun to establish rankings because pathogens typically die rapidly when exposed to direct sunlight and therefore have little opportunity for sit-and-wait transmission. Variations in virulence due to variations in sit-and-wait transmission therefore would be more dependent on the much greater variation in survival that occurs in the presence of little or no direct sunlight (e.g. inside houses).

(*b*) *Durability values for different pathogens*

The documented survival durations of variola virus are often lower limits of maximum survival (Table 2) because

experimenters exhausted their supply of virus for assaying viability or terminated their experiments before the viruses became no nviable. Even so, durations of survival are generally greater for variola virus than for *C. diphtheriae* (Table 2). When these pathogens were in dried exudate exposed to indirect light, the lower bound of variola survival was greater than the upper bound of *C. diphtheriae* survival (196 *versus* 150 days; Table 2). On the basis of these differences, variola virus was ranked above *C. diphtheriae* with regard to survival in the external environment.

C. diphtheriae survived longer than *M. tuberculosis* on glass (suspension, indirect light), paper and wood (Table 2). Survival on dust was comparable for the two pathogens (Table 2). Therefore *C. diphtheriae* was ranked above *M. tuberculosis*.

M. tuberculosis survived longer on glass in the dark than *S. pneumoniae* (Table 2). All other possible comparisons between these two pathogens differ in at least one environmental variable, but in similar environments *M. tuberculosis* generally survived longer than *S. pneumoniae*. After application to glass from suspension, *M. tuberculosis* survived longer than influenza virus in the dark and for comparable durations in indirect light. *M. tuberculosis* also survived longer in sputum and on fabric (Table 2). *M. tuberculosis* survived longer than *B. pertussis*, *N. meningitidis*, parainfluenza virus (on glass and fabric for each), respiratory syncytial virus (on fabric) and rhinovirus (on glass and fabric) (Table 2). *M. tuberculosis* was therefore ranked above all of these pathogens in terms of durability in the external environment.

Aside from variola virus, *C. diphtheriae*, and *M. tuberculosis*, only two pathogens regularly survive for longer than a week in external environments: *S. pneumoniae* and influenza virus (Table 2). The data do not permit differentiation between these two pathogens; their survival in air was not distinguishable, nor was there any consistent difference across all tested conditions. These two pathogens were therefore assigned a tied rank, below variola virus, *C. diphtheriae* and *M. tuberculosis* and above all other pathogens. Accordingly, in a direct comparison using a single apparatus and a fixed protocol, aerosol survival times of *S. pneumoniae* and *C. diphtheriae* were consistently longer than that of *H. influenzae* (Wells and Stone, 1934) (Table 2). The other available measurements on *H. influenzae* conform to the ranking of *H. influenzae* below *S. pneumoniae* (Table 2).

B. pertussis was the only other pathogen that regularly survived for a day or more on substrates on which the pathogens were tested (i.e. glass, plastic, fabric paper), but its survival never reached the durations found for variola virus, *C. diphtheriae*, *M. tuberculosis*, *S. pneumoniae*, or influenza virus. Its aerosol survival was within or above the ranges for all of the remaining pathogens. *B. pertussis* was therefore ranked below influenza virus and *S. pneumoniae*.

Few quantitative data exist for rubeola virus. The research laboratory that measured the rubeola strain presented in Table 2 compared their result to measurements of an influenza strain that were made in the same laboratory a few years previously. They found no statistically reliable difference below 40 % humidity and slightly higher survival by rubeola virus above 40 % (DeJong & Winkler, 1964). But the data from all strains of influenza virus presented in Table 2 suggest that the survival of influenza virus used in those experimental conditions was unrepresentatively short. Lozovskaia (1959) found that survival of influenza virus at approximately 15 °C was consistently greater than that of rubeola when freeze-dried preparations were kept in the dark or exposed to indirect light. Qualitative descriptions suggest that rubeola virus, unlike influenza virus, *S. pneumoniae*, and *B. pertussis*, is typically inactivated rapidly when dried on surfaces (Black, 1984). We therefore believe that, on balance, the limited evidence available favours a ranking of influenza virus and *B. pertussis* above rubeola virus.

N. meningitidis, *M. pneumoniae*, *H. influenzae*, rubeola virus, mumps virus, parainfluenza virus, respiratory syncytial virus, varicella-zoster virus, rubella virus, and rhinovirus cannot be distinguished from each other. All tended to survive in the external environment for a matter of hours and occasionally more than a day or two (Table 2). We assigned these pathogens the same rank on the basis of these similarities and the following pair-wise comparisons.

On skin, survival durations were longer for rhinovirus than respiratory syncytial virus. On fabric they were similar, but on plastic, respiratory syncytial virus survived longer (Table 2). When released as aerosols the range of survival times for RSV was within the range of survival times for rhinovirus. Similarly, comparisons under corresponding environmental conditions revealed no consistent differences between *N. meningitidis* and rhinovirus (on fabric and glass), and parainfluenza (glass, skin, steel) (Table 2).

Although the data from rubella virus are scanty, the durability of this virus is comparable to that of the others in this group. Its half-life is about an hour at 37 °C (Marcy & Kibrick, 1977*c*). Rawls (1972) states that 'under normal laboratory conditions 10–50 % of the infectivity is lost per hour at 37 °C'. We also did not find precise measurements of survival of varicella-zoster and mumps viruses, but descriptions indicate that their infectivity is generally lost within a few hours (Cruickshank, Duguid & Swain, 1965; Davis *et al.*, 1973; Douglas, 1973; Schmidt, 1974; Marcy & Kibrick, 1977*d*; Richman, 1982). We therefore assigned varicella-zoster and mumps viruses the same rank as these other labile pathogens.

(3) The overall trend and alternative hypotheses

The results of our test are summarized in Table 3 (these results were previously summarized in Walther, 1993; Ewald, 1994, 1995, 1996*a*, 1998; Gandon, 1998 and Ewald & de Leo, 2001). The mortality attributable to respiratory pathogens is positively correlated with pathogen durability in the external environment (Spearman rank test corrected for ties, $N=16$, $r_s=0.86$, $P=0.0008$). To assess whether this association is present at higher taxonomic levels we grouped the pathogens according to genera and according to family. Grouping pathogens according to genera combines mumps and parainfluenza viruses within the genus *Paramyxovirus*, but still yields a statistically significant association ($N=15$, $r_s=0.88$, $P=0.001$). Grouping pathogens according to families merges parainfluenza, mumps, rubeola, and respiratory syncytial viruses within the family Paramyxoviridae, and also yields a statistically significant association ($N=13$, $r_s=0.90$, $P=0.002$).

Table 3. Mortality (measured as mean per cent mortality per infection $= 100 \times M/I$) and mean survival time in the external environment (in days) for 16 human respiratory pathogens. For ranking of each pathogen, see text

	Mortality (M/I)		Survival in external environment	
Pathogen	(%)	(rank)	(days)	(rank)
Variola virus (DNA)	10	1	885.1	1
Mycobacterium tuberculosis	5	2	244.3	3
Corynebacterium diphtheriae	0.2	3	369.8	2
Bordetella pertussis	0.1	4	11.6	6
Streptococcus pneumoniae	0.036	5	28.6	4.5
Influenza virus (RNA)	0.010	6	13.7	4.5
Neisseria meningitidis	0.007	7.5	1.9	11.5
Rubeola virus (RNA)	0.007	7.5	4.4	11.5
Mumps virus (RNA)	0.005	9	0.9	11.5
Parainfluenza virus (RNA)	0.004	10	4.6	11.5
Mycoplasma pneumoniae	0.003	12.5	1.9	11.5
Respiratory syncytial virus (RNA)	0.003	12.5	1.1	11.5
Varicella-zoster virus (DNA)	0.003	12.5	0.9	11.5
Rubella virus (RNA)	0.003	12.5	0.9	11.5
Haemophilus influenzae	0.002	15	1.3	11.5
Rhinovirus (RNA)	0.000	16	2.3	11.5

This correlation between durability and mortality is not a consequence of some bias associated with lumping of bacteria and viruses in a single test. If separate tests are run on these two groups, each is statistically significant (for bacteria: $N=7$, $r_s=0.89$, $P=0.03$; for viruses: $N=9$, $r_s=0.74$, $P=0.04$). The overall statistical significance resulting from combining statistically (Sokal & Rohlf, 1981) the bacteria test with the virus test was comparable to that conducted on the mixed data set (d.f. $=4$, $X^2=13.45$, $P<0.01$).

The preceding across-species and across-taxons comparisons show that the associations between durability and mortality are robust with regard to taxonomic groupings, but the analyses do not directly test whether variation in mortality is independent of phylogenetic relatedness. Since species are not statistically independent data points because of their shared evolutionary history (Harvey & Pagel, 1991), comparative methods need to be used to correct for any influence of phylogenetic relatedness on the statistical results. To correct for phylogenetic relatedness, we used the method of generating phylogenetically independent contrasts within an established phylogenetic tree. Such a tree represents the relationships between the investigated species and can thus be used to control for phylogenetic relatedness. We calculated phylogenetically independent contrasts using comparative methods developed by Felsenstein (1985), Harvey & Pagel (1991) and Pagel (1992). These methods correct for any influence of phylogenetic relatedness by considering only the evolved difference between related taxa. Specifically, evolutionary change is modelled by a process of Brownian motion along the branches of a phylogenetic tree depicting the relationships of the different taxa. Any species character is assumed to evolve independently along each branch, so that branch lengths can be assumed to represent the expected variance of evolutionary change (Pagel, 1992). By subtracting character values between related sister taxa and weighing them according to the expected evolutionary change, phylogenetically independent contrasts are generated which can be correlated to test for covariation between changes in different character values. We used the CAIC program (Purvis & Rambaut, 1995) using Pagel's (1992) method to generate 15 independent contrasts within a phylogenetic tree based on recent literature on pathogen phylogenies (Melnick, 1984; Ito *et al.*, 1987; Kawano *et al.*, 1990; Balows *et al.*, 1992). Branch lengths were set from the topology (Grafen, 1989). Per cent mortality (Table 3) and durability (measured in days, see below) were transformed using $\log_{10}(1000x+1)$ prior to the CAIC analysis. The resulting contrasts were analysed with a Model I regression forced through the origin (Grafen, 1989; Garland, Harvey & Ives, 1992).

Durability in days was determined using comparisons of survival data (Table 2) under the same conditions of substrate (glass, fabric), source of isolate, and light exposure. The pathogens in our study survive, on the average, 6.4 times longer in dark than in indirect light conditions (mean of nine examples in Table 2) and 10.2 times longer on substrates than in aerosols (mean of seven examples in Table 2). When comparisons involved survival data under different light or substrate conditions, values from indirect light and from aerosols were multiplied by these correction factors. The mean of all the resultant values was used as the average durability for each pathogen species. Plotting the resulting mean values for each species shows the overall positive correlation described above (Fig. 1). We then subjected these mean values to the comparative method described above. Again, we found a significant correlation between pathogen-induced mortality and durability ($N=15$ independent contrasts, $F_{1,14}=33.91$, $P<0.0001$). Residuals were plotted and found to be normally distributed; none of the residuals had a Cook's D greater than $4/n$ and a leverage

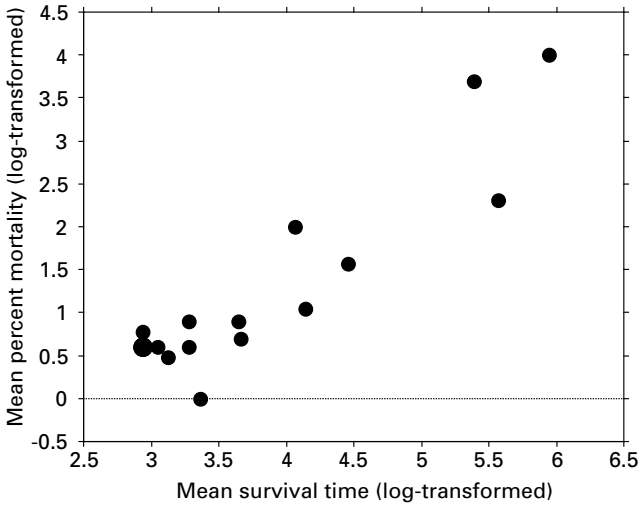

Fig. 1. Plot of mean survival time *versus* mean per cent mortality for 16 human respiratory pathogens (data from Table 3; both axes were $\log_{10}(1000x+1)$ transformed). The larger circle denotes two overlying data points.

greater than $3k/n$ (for discussion of outliers, see Myers, 1990).

Our calculations of mortality may have overestimated the death rate among the mildest group of pathogens because data were insufficient to assess the extent to which death was due to secondary infections. Correction of any overestimates, however, would generate little if any change in the correlation coefficient because these pathogens share the lowest durability rank (Table 3).

Indeed, our results clearly distinguish a high-virulence high-survival group of variola virus, *M. tuberculosis*, *C. diphtheriae*, *B. pertussis*, *S. pneumoniae*, and influenza virus (where all pathogens have a mean per cent mortality $\geqslant 0.01$ % and mean survival time >10 days) and a low-virulence low-survival group containing the ten remaining pathogens, in accordance with the sit-and-wait hypothesis. Even if our estimates of mean per cent mortality and mean survival time may be subject to some uncertainty due to the various methods and sources from which they were collected, such uncertainty cannot explain the three to four times of magnitude of difference in mean per cent mortality and mean survival time which distinguishes the typical sit-and-wait pathogens from the other pathogens. Still, because of the limited number of studies and difficulties inherent in comparing values from studies with different protocols and environmental conditions, we were forced to incorporate a large number of ties in our statistical tests. The cross-specific test could be improved by prospectively evaluating the durability of the pathogens in Table 3, particularly those that were assigned tied ranks. The variability in durability documented by past studies also draws attention to the need to measure the durability of numerous strains for each pathogen species using identical protocols thus yielding much stronger comparative evidence.

Whenever comparative studies are conducted, cause and effect remain uncertain. One can imagine correlates of durability that might be directly responsible for the variation in mortality and hence for the association between durability and mortality. Perhaps the most feasible alternative hypothesis is the degree of within-host genetic heterogeneity of parasites. Theory predicts that increased genetic variability within hosts should favour evolution of increased virulence (Lewontin, 1970; Levin & Pimentel, 1981; Bremermann & Pickering, 1983; Ewald, 1983; Bull, 1994; Nowak & May, 1994; Frank, 1996). One might argue that genetic variation within hosts is higher when pathogens are more durable because pathogens that remain viable in the external environment may experience more mixing of different genotypes, yielding more heterogenous inocula. By this argument, genetic heterogeneity within hosts rather than a reduced reliance on host mobility might cause the variation in mortality rates shown in Table 3. Genetic heterogeneity can arise from many sources, however, such as increased rates of mutations and recombinations and epidemic spread, which might increase the number of sources of infection per infected individual. However, the less mutation-prone DNA viruses in Table 3 do not tend to be less lethal than the RNA viruses. In fact, the smallpox virus is the most deadly virus and one of the least variable. The potential for epidemic spread also shows little if any association with lethality. The smallpox virus, *M. tuberculosis*, *S. pneumoniae* and *H. influenzae* have relatively low potential for epidemic spread; measles, influenza, and rubella viruses have a high potential; and the rest are characterized by both epidemic and endemic spread (see Mandell, 1990 and also values for the intrinsic reproductive rate of infection, R_o, given by Anderson & May, 1982). Respiratory syncytial virus, *S. pneumoniae* and *H. influenzae* are characterized by vulnerability to re-infection and therefore ought to be particularly prone to heterogeneity (Mandell, 1990). Overall, these data suggest that, although this heterogeneity hypothesis needs more direct analysis, the variation in mortality presented in Table 3 does not appear to be associated with either the degree of mutation-induced variation or epidemic-driven heterogeneity.

Another alternative hypothesis is that the more virulent pathogens are associated with a higher initial inoculum size (Day, 2002*b*). This hypothesis is difficult to assess without lethal dose experiments (Day, 2002*a*), but given the choice between equal inocula of variola and rhinovirus, we doubt many people would think for too long which one to choose. Another hypothesis suggests that higher virulence evolves in pathogens that are transmitted over longer distances (Boots & Sasaki, 1999). Again, we cannot assess the importance of this hypothesis with the data at hand, but one should realize that the Boots & Sasaki (1999) hypothesis is really just the reverse of the sit-and-wait hypothesis. They suggest that pathogens that can reach more distant populations should evolve towards higher virulence, while the sit-and-wait hypothesis suggests that those pathogens that can survive for long enough until hosts that used to be 'distant' contact them should also evolve towards higher virulence. In both cases, the more susceptible 'distant' individuals can be infected, the higher the evolved virulence.

Some mathematical modelling studies have also investigated the sit-and-wait hypothesis. Bonhoeffer, Lenski & Ebert (1996) developed a model based on host and parasite

birth and death rates. They concluded that the optimal virulence at equilibrium does not depend on durability, but only on the host's natural death rate and the relationship between the pathogen's virulence and the resulting discharge rate of pathogens from the host. Their model does, however, predict a correlation between durability and virulence if the host-parasite system is in disequilibrium, e.g. during an epidemic. Gandon (1998) extended their model to include the effects of competing pathogen strains and dispersal (see also Frank, 1996). For most model parameter settings, this model predicts a positive correlation between durability and virulence.

However, neither model incorporates the role of host mobility on different routes of transmission. As originally formulated, the sit-and-wait hypothesis is based on the distinction between direct transmission through close contact between infected and susceptible individuals and indirect transmission involving the movement of susceptibles to a place in the external environment where the pathogen lies 'waiting' (see Introduction). Critical to this distinction is the negative effect of host illness on the frequency of contacting other susceptibles due to decreased mobility of the infected individual or avoidance of infected individuals by susceptible individuals (e.g. Hart, 1997). This negative effect of illness on host contact results in a hump-shaped transmission coefficient for direct transmission. The models by Bonhoeffer *et al.* (1996) and Gandon (1998) do not consider this negative effect of host illness, but rather assume a linear relationship between virulence and their coefficients of transmission. To incorporate this effect, Ewald & de Leo (2001) used a hump-shaped relationship between virulence and the coefficient of transmission for directly transmitted pathogens in their model. Their conclusion was that pathogens less dependent on host mobility for transmission should evolve towards higher levels of virulence and should also have a higher potential for epidemic spread. This general conclusion should hold for various transmission modes, e.g. waterborne, attendant-borne or sit-and-wait transmission. Given that the 'hump-shaped' model best explains the variation found in this study and previous studies (Ewald, 1983, 1988, 1991*b*, 1994, 1995), the 'hump-shaped' model has better empirical support than the previous models.

(4) General relevance of sit-and-wait transmission

(*a*) *Sit-and-wait transmission and the traditional view*

Our test of the sit-and-wait hypothesis offers a resolution for the inconsistency between the traditional view that all pathogens will evolve towards benignness and the ancient and lethal history of smallpox and tuberculosis. According to the sit-and-wait hypothesis, these pathogens have remained severe throughout history because host immobilization is less detrimental to their transmission than to the transmission of less durable pathogens. Pathogen variants that reproduce to high levels in people, and thereby inadvertently cause severe disease, achieve a competitive advantage. By relying on the mobility of susceptible hosts for transmission they may accrue the fitness advantages from extensive use of host resources yet incur relatively low costs from immobilization of hosts.

By quantifying mortality per infection we avoid potential inaccuracies associated with intuitive impressions about the virulence of the different pathogens (see also Day, 2002*a*). Considering virulence to be synonymous with pathogen-induced mortality, Bonhoeffer *et al.* (1996), for example, questioned the general validity of the sit-and-wait hypothesis by suggesting some pathogens that seemed inconsistent with it. When discussing human pathogens they cited the measles virus, which they considered to be highly virulent but not particularly durable. Our quantification shows that the measles virus does in fact accord with the sit-and-wait hypothesis: its lethality and durability are both intermediate.

(*b*) *Pathogens of non-human hosts*

Although this study has tested the sit-and-wait hypothesis using human respiratory pathogens, the same arguments should apply to many other directly transmitted pathogens (Myers & Rothman, 1995). Highly lethal, directly transmitted pathogens of insects tend to be extremely long-lived in the external environment. One of the most lethal pathogens of the honey bee, for example, is *Bacillus larvae* which causes American foulbrood disease (Shimanuki, 1990). Viability of spores in the external environment is at least 40–50 years (Borchert, 1966; H. Shimanuki, personal communication *contra* Bonhoeffer *et al.*, 1996). *B. larvae* therefore has a large window of time within which a suitable nest site such as a tree hole can be re-colonized after the pathogenic destruction of the original colony. The nuclear polyhedrosis viruses that infect many different insect species (Payne, 1988) are often sit-and-wait pathogens (Ewald, 1987); for example, the nuclear polyhedrosis virus that infects *Trichoplusia ni* remains viable in soil for at least six years (Tinsley & Entwistle, 1974; Hostetter & Bell, 1985). It is not surprising that these sit-and-wait pathogens are among the most commonly used biological control agents (Payne, 1988) as they combine three attributes that are advantageous for pest control: high virulence, long durability after application, and host specificity (Anderson & May, 1981; Ewald, 1987). The sit-and-wait hypothesis therefore should be useful in guiding research on and determining appropriate policies for controlling insect pests in agriculture, as well as managing beneficial insects.

The sit-and-wait hypothesis also provides an explanation for the high virulence of pathogens like *Bacillus anthracis*, the viruses causing foot-and-mouth disease, and the prion agents of scrapie and bovine spongiform encephalopathy. Each of these pathogens cause severe disease in their hosts and can survive outside the host for long periods of time, often for many years.

(*c*) *Emerging pathogens, hospitals, and AIDS*

The AIDS pandemic has drawn attention to the need to guard against emerging pathogens (Lederberg, 1988; Kilbourne, 1991; Morse, 1991; Krause, 1992; Ewald, 1996*a*, 1998; Stephens *et al.*, 1998). Fulfilling this need is complicated by several factors: the vast number of organisms that can occasionally cause severe disease in humans, most of which cause very restricted outbreaks (e.g. *Legionella*

pneumophila, Lassa Fever virus, Marburg virus), the limited availability of economic resources, and the restricted intervals of time within which decisive interventions may be feasible. Given these complications, efficiency could be improved by distinguishing those severe pathogens that are predisposed to cause small ephemeral outbreaks from those that have potential for causing severe disease indefinitely if they can achieve a foothold.

The sit-and-wait hypothesis has a bearing on this problem because it identifies pathogens that have the potential for causing severe disease stably over time (Ewald, 1996*a*, 1998). By quantifying durations of durability in the external environment, researchers should be able to focus more quickly on particularly dangerous pathogens. Directly transmitted pathogens pose a relatively great long-term threat if they can remain viable for a long period of time outside of the host. If durable pathogens are severe during their emergence they may remain severe over time. If durable pathogens are not severe during their emergence, they may evolve toward increased severity. This argument draws attention to the need to quantify promptly the durability of emerging pathogens in the external environment. Durable pathogens warrant particularly intensive research activity.

We believe that these considerations may be especially relevant to hospital environments. Some of the most dangerous hospital pathogens are also capable of surviving for relatively long periods of time. *Pseudomonas aeruginosa*, for example, can survive for six months in dust (Mitscherlich & Marth, 1984), longer than the maximum durability documented for *M. tuberculosis* or *C. diphtheriae* (Table 2). *P. aeruginosa* is commonly spread on invasive equipment and is lethal in about half of those whose bloodstream it infects, even with antibiotic therapy (Gallagher & Watanakunakorn, 1989).

Because hospital-acquired pathogens are typically transmitted *via* fomites, on the external surfaces of attendants (e.g. on hands or clothes of nurses), or in air currents, durable pathogens are especially suited for transmission within hospitals. The more durable a pathogen is, the more chances it will have of being picked up by an attendant, of being wafted into the air (e.g. on dust particles) or of staying on materials until susceptible patients arrive. *M. tuberculosis*, for example, is particularly prone to air-borne spread in hospitals (Dooley *et al.*, 1992*b*). *Streptococcus pyogenes* and *Staphylococcus aureus* can survive for months on fabric or dust particles (Mitscherlich & Marth, 1984); they can be transmitted by attendants, fomites and probably by air-borne dust, and are a common cause of lethal, hospital-acquired infections (Cruickshank & Godber, 1939; Cruickshank & Muir, 1940; Cruickshank, 1941; Hare, 1941; Thomas, 1941; Wolinsky *et al.*, 1960; Mortimer *et al.*, 1962; Crossley, Landesman & Zaske, 1979; Florman & Holzman, 1980; Thompson, Cabezudo & Wenzel, 1982; Pavillard *et al.*, 1982; Borg, 2003).

Because the resistance of people in hospitals is often compromised by therapy, invasive procedures, stress, or states of disease, microbes that would not be able to infect healthy humans may be able to infect compromised patients, which then serve as a stepping stone for the evolution of increased potential for infecting healthy humans (Wallace, 1989). If the use of antibiotics always provided effective control, these issues would be of limited significance. Existing antibiotics, however, are often of little value against emerging hospital acquired pathogens such as *Clostridium dificile* and *Pseudomonas aeruginosa* (Gallagher & Watanakunakorn, 1989; Greenough & Bennett, 1990; Hamon-Poupinel *et al.*, 1991; Fujita *et al.*, 1992; Neu, 1992). Moreover, the rapid development of antibiotic resistance in hospitals (Gezon, Schaberg & Klein, 1973) raises the possibility that virulent strains may cause substantial mortality before appropriate antibiotics can be found. The 'hospital superbug' methicillin-resistant *S. aureus* (MRSA) offers one worrisome illustration (Pavillard *et al.*, 1982; Borg, 2003; Enright, 2003; Melzer *et al.*, 2003), but *M. tuberculosis* seems to be even more dangerous. The development of antibiotic resistant *M. tuberculosis* appears to be a major factor increasing its spread and lethality in institutional environments (Iseman, 1992).

The AIDS pandemic has also heightened the relevance of the sit-and-wait hypothesis by increasing the importance of tuberculosis. After declining for decades, the incidence of tuberculosis began to rise again during the mid-1980s largely as a result of HIV infection. The global death toll from tuberculosis now stands at about 3 million per year and is rising, particularly in areas hard-hit by AIDS (Sudre, Ten Dam & Kochi, 1992). Because AIDS patients are frequent visitors to medical care institutions, the effects of HIV, antibiotic resistance, and hospital environments are jointly enhancing the resurgence of lethal tuberculosis (Iseman, 1992; Dooley *et al.*, 1992*a*; Fischl *et al.*, 1992*a*, *b*; Pearson *et al.*, 1992).

The AIDS pandemic may also be opening the door for new sit-and-wait pathogens of humans. Like many hospitalized patients, AIDS patients are particularly vulnerable to opportunistic pathogens. The sit-and-wait hypothesis may help researchers to identify particularly dangerous opportunistic pathogens. Bacteria within the *Mycobacterium avium/M. intracellulare* complex, for example, often infect AIDS patients (Gradon, Timpone & Schnittman, 1992) and can survive for years outside of the host (Mitscherlich & Marth, 1984). They are also naturally resistant to anti-tuberculosis drugs (Wolinsky, 1992). Should they evolve an enhanced ability to complete infectious cycles among uncompromised hosts we may be confronting a particularly dangerous human pathogen. The current incidence of *M. avium* pulmonary disease in the U.S. is a testament to this danger: excluding AIDS patients, *M. avium* now causes about one case of pulmonary disease for every eight cases caused by *M. tuberculosis* (Wolinsky, 1992).

(*d*) *Variation in durability and the emergence of virulent food-borne pathogens*

While we hypothesized that high virulence can be costly to pathogens in terms of decreased host mobility, we have so far not discussed possible costs of high durability. After all, if high durability entailed no cost, why would not all pathogens evolve towards high durability and high virulence? Recent research on food-borne pathogens suggests that not only is durability costly to pathogens, but that durability and virulence may in some cases be genetically linked. To survive environmental stresses such as heat, cold, acid shock, starvation, oxidation or anaerobiosis, food-borne pathogens

produce a variety of protective proteins, some of which offer protection to more than one environmental stress (Archer, 1996; Sheridan & McDowell, 1998). Genes regulating the production of these protective proteins are found in various food-borne pathogens. Moreover, experiments with food-borne pathogens have shown that variation exists among different sub-populations in their ability to survive environmental stresses, and that the more durable pathogens spread in the overall population if environmental stresses persist (Sheridan & McDowell, 1998). Besides such phenotypic stress responses, higher durability is often achieved by increased mutation rates, and such stress-induced hypermutation may be an adaptation to survive in hostile environments (Archer, 1996; Sheridan & McDowell, 1998). The same regulatory systems that are used for protective responses apparently also trigger virulence factors inside the host (Archer, 1996; Sheridan & McDowell, 1998). Mutant bacteria whose protective response to environmental stresses was negatively affected by mutation were also found to be less virulent, suggesting a genetic linkage between durability and virulence in a wide range of pathogens (Archer, 1996; Sheridan & McDowell, 1998).

These studies on food-borne pathogens suggest that (1) mounting regulatory responses to environmental stresses is costly in terms of additional production of protective proteins, (2) increased durability can be achieved by both phenotypic and genotypic adaptation (i.e. production of protective proteins and increased mutation rate), (3) mutation creates genetic variation in durability, and (4) high durability may be genetically linked to high virulence. Durability in food-borne pathogens may even be enhanced by modern food preservation methods as new food treatments may actually 'stress harden' pathogens by applying several sublethal stresses intended to slow or inhibit bacterial growth instead of outright killing the bacteria (Archer, 1996; Sheridan & McDowell, 1998). By allowing food-borne pathogens to mount an adaptive phenotypic and/or genotypic stress response, such food treatments may thus increase both durability and virulence and may lead to the emergence of new highly virulent pathogen strains which can cause disease even at low dosages (Archer, 1996). Moreover, such durable food-borne pathogens probably have a higher chance of successful transmission to a new host. However, whether food-borne pathogens actually reap the same adaptive benefits from high durability as sit-and-wait pathogens depends on the intricacies of their transmission cycles. Therefore, researchers should consider the details of transmission of food-borne pathogens before making predictions about the effects of durability on virulence levels (Ewald, 1991*a*, *b*, 1994, 1995, 1996*a*, 1998; Ewald & de Leo, 2001).

Similar studies investigating the genetic mechanisms of durability and virulence in sit-and-wait pathogens should be highly rewarding. For example, durability and virulence are two highly variable and heritable characters in nuclear polyhedrosis viruses (Witt & Stairs, 1975; Brassel & Benz, 1979; Witt & Hink, 1979; Shapiro *et al.*, 1984; Shapiro, Lynn & Dougherty, 1992) which tempted us to conduct experimental tests on the possible evolutionary trade-off between durability and virulence in such a virus, but the results were unfortunately inconclusive (Walther, 1993). While durability may change due to phenotypic and genotypic adaptation, durability may also vary depending on the substrate or environment in which the pathogen has to survive (Table 2). Just as survival of food-borne bacteria is enhanced by the carbohydrate content of the food product (Sheridan & McDowell, 1998) and the survival of nuclear polyhedrosis virus by the presence of host tissues (David, 1969; Shapiro, 1984), smallpox virus survives longer in skin crusts than in vesicle fluids (Table 2). Therefore, some sit-and-wait pathogens such as *C. diphtheriae* and the smallpox and nuclear polyhedrosis viruses may enhance their durability by encasing themselves inside host tissues for protection. Whether food-borne pathogens can use food resources in a similar way to increase their durability remains to be investigated.

(*e*) *Biological warfare*

The recent terror attacks using anthrax *B. anthracis* and the widespread fear of a smallpox bioweapon attack underline the potential danger posed by sit-and-wait pathogens (Medical aspects of chemical and biological warfare, http://www.nbc-med.org/SiteContent/HomePage/WhatsNew/MedAspects/contents.html). Other potential bioweapon pathogens can unfortunately be identified using the sit-and-wait hypothesis. Science sometimes brings to light dangerous possibilities, and the sit-and-wait hypothesis is such a case. One should hope that biological warfare using sit-and-wait pathogens will only be waged against pest animals (e.g. nuclear polyhedrosis virus against insect pests), but such hope may be in vain. However, health and emergency authorities may at least identify potentially hazardous pathogens prior to bioweapons attacks and thus be more prepared. The Biological and Toxin Weapons Convention (http://www.bradford.ac.uk/acad/sbtwc/) whose aim is to 'establish and maintain the security and oversight of pathogenic microorganisms and toxins' (Pearson and Dando, 2003) could also be strengthened if it gave special attention to potential sit-and-wait pathogens.

IV. CONCLUSIONS

(1) The sit-and-wait hypothesis predicts that virulence should be positively correlated with durability in the external environment because high durability reduces the dependence of pathogen transmission on host mobility. Since sit-and-wait pathogens can still be transmitted from a highly immobilised host by simply sitting around and waiting until a new host picks them up, they should evolve towards higher levels of host exploitation (i.e. virulence).

(2) Reviewing the epidemiological and medical literature, we confirm this prediction for respiratory tract pathogens of humans. We find significant correlations between durability and mean per cent mortality (as a measure of virulence), no matter whether we use across-species or phylogenetically controlled analyses. Our results distinguish a high-virulence high-survival group of variola (smallpox) virus, *Mycobacterium tuberculosis*, *Corynebacterium diphtheriae*, *Bordetella pertussis*, *Streptococcus pneumoniae*, and influenza virus (where all pathogens

have a mean per cent mortality $\geqslant 0.01\,\%$ and mean survival time >10 days) from a low-virulence low-survival group containing ten other respiratory pathogens.

(3) As with all correlative studies, cause and effect remain uncertain, and alternative hypotheses may also explain the observed correlation. Alternative explanations involve within-host genetic heterogeneity of parasites, higher initial inoculum size, and long-distance transmission, and should be considered in future comparative or experimental studies. Previous mathematical modelling studies investigating the sit-and-wait hypothesis did not incorporate the role of host mobility on transmission rate. A new model using a hump-shaped relationship between virulence and the coefficient of transmission shows that pathogens less dependent on host mobility for transmission should evolve towards higher levels of virulence, in accordance with the empirical results of this study and previous comparative studies.

(4) Assuming the validity of the sit-and-wait hypothesis, the high virulence of non-human pathogens may be explained. Many pathogens used in biological control of insects are potential sit-and-wait pathogens as they combine three attributes that are advantageous for pest control: high virulence, long durability after application, and host specificity.

(5) Our findings bear on public health policy because they implicate characteristics of emerging pathogens that are particularly dangerous, especially in the context of hospital settings and the AIDS pandemic. Emerging sit-and-wait pathogens should thrive in unhygenic hospital settings, especially when immuno-compromised patients are involved, and may more easily gain a foothold in such conditions. Therefore, sit-and-wait pathogens have the potential for causing severe disease for long time periods. By quantifying durability in the external environment, researchers should be able to focus more quickly on potentially dangerous pathogens.

(6) Studies on food-borne pathogens suggest that higher durability entails costs in terms of additional production of protective proteins, that increased durability can be achieved by both phenotypic and genotypic adaptation, and that high durability may be genetically linked to high virulence. New food treatment methods may allow food-borne pathogens to mount an adaptive stress response and thus lead to the emergence of new highly virulent pathogen strains. Whether similar genetic mechanisms control durability and virulence in sit-and-wait pathogens needs to be investigated.

(7) Recent terror attacks using anthrax and the widespread fear of a smallpox attack underline the potential danger posed by sit-and-wait pathogens. Health and emergency authorities may identify potentially hazardous pathogens prior to attacks using the sit-and-wait hypothesis.

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