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How to find natural reservoir hosts from endemic prevalence in a multi-host population: A case study of influenza in waterfowl

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ABSTRACT

The transmission dynamics of infectious diseases critically depend on reservoir hosts, which can sustain the pathogen (or maintain the transmission) in the population even in the absence of other hosts. Although a theoretical foundation of the transmission dynamics in a multi-host population has been established, no quantitative methods exist for the identification of natural reservoir hosts. For a host to maintain the transmission alone, the host-specific reproduction number (U), interpreted as the average number of secondary transmissions caused by a single primary case in the host(s) of interest in the absence of all other hosts, must be greater than unity. If the host-excluded reproduction number (Q), representing the average number of secondary transmissions per single primary case in other hosts in the absence of the host(s) of interest, is below unity, transmission cannot be maintained in the multi-host population in the absence of the focal host(s).

The present study proposes a simple method for the identification of reservoir host(s) from observed endemic prevalence data across a range of host species. As an example, we analyze an aggregated surveillance dataset of influenza A virus in wild birds among which dabbling ducks exhibit higher prevalence compared to other bird species. Since the heterogeneous contact patterns between different host species are not directly observable, we test four different contact structures to account for the uncertainty. Meeting the requirements of $U>1$ and $Q<1$ for all four different contact structures, mallards and other dabbling ducks most likely constitute the reservoir community which plays a predominant role in maintaining the transmission of influenza A virus in the water bird population. We further discuss epidemiological issues which are concerned with the interpretation of influenza prevalence data, identifying key features to be fully clarified in the future.

Introduction

Reservoir hosts of an infectious disease serve as a source of infection and sustain the pathogen in a population (Dorland, 1994). Although ideal disease control efforts, intended to eliminate an infection, should be aimed at controlling infections in the reservoir hosts, the identification of these reservoir hosts and the clarification of their role have yet to be fully understood in many instances (Haydon et al., 2002). The difficulty in identifying reservoirs is largely due to poor understanding of the epidemiology of multi-host pathogens (Woolhouse et al., 2001). To date, only a limited number of observed data have been analyzed to elucidate the transmission dynamics in a multi-host population with a particular emphasis on the reservoir host (Begon et al., 1999; Craft et al., 2008; Hudson et al., 1995; Lembo et al., 2008; Rhodes et al., 1998).

The epidemiological conditions for a particular host to act as the reservoir, proposed by Cleaveland and Dye (1995), in conjunction with theoretical foundation of the transmission dynamics in a multi-host population (Roberts, 2007) provide a sound conceptual framework for investigating reservoir hosts. However, quantitative methods to fill the gap between theory and observation have yet to be developed. Although we cannot extensively identify all maintenance hosts (i.e. hosts that can sustain pathogen alone) in nature, due mainly to limitations in the observed data in wildlife ecology, this type of quantitative application may help justify both disease control measures as well as host-specific targeted surveillance.

The present study proposes a simple method for the identification of a specific host or group of hosts as a reservoir using observed prevalence data of an endemic disease. In light of different definitions of the reservoir host in literature (Ashford, 1997, 2003; Cleaveland and Dye, 1995; Haydon et al., 2002; Swinton et al., 1998), we define a reservoir community as a minimum set of hosts who can sustain the pathogen alone, and thereby all other hosts other than the reservoir

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community cannot maintain transmission by themselves. As an example, we consider the epidemiological dynamics of influenza A virus in migratory waterbirds, which have been suggested as potential reservoir hosts of influenza A virus. Although these wild birds mainly harbor low pathogenic avian influenza (LPAI) virus, full clarification of the dynamics is directly relevant to epidemiological understanding of the emergence of highly pathogenic avian influenza (HPAI). From various studies of ecological surveillance of influenza A virus in waterbirds it is known that wild birds infected with LPAI virus transport and excrete the virus, and thus may infect poultry (Easterday et al., 1968; Homme et al., 1970; Fouchier et al., 2007; Webster et al., 1992). The switch from a LPAI phenotype to a HPAI is achieved by the introduction of basic amino acid residues into the HA0 cleavage site (Fouchier et al., 2005). A previous study summarized the surveillance results of global prevalence patterns in waterbird populations (Olsen et al., 2006). In this and other studies (e.g. see Munster et al. (2005) and Wallensten et al. (2007) for northern Europe), higher prevalence has been observed in dabbling ducks (genus *Anas*), especially in mallards (*Anas platyrhynchos*), compared to other species. It was thus postulated that mallards may be a reservoir host of influenza A virus (Munster et al., 2005; Songserm et al., 2006; Sturm-Ramirez et al., 2005). However, high prevalence in a specific host alone is not sufficient to deem that host as a natural reservoir. A species with a low prevalence, yet, an extremely long infectious period that interacts with other species frequently enough to cause inter-specific transmissions can also satisfy the requirements of a maintenance host. Therefore, the assessment of whether a particular host is a reservoir needs to be made on the basis of theoretical approaches with a firm understanding of the underlying transmission dynamics.

By our definition, a transmission cannot be maintained in the multi-host population without the reservoir community. If there are one or more maintenance hosts in the population, the reservoir community must include these maintenance hosts. Moreover, the reservoir community can also include non-maintenance hosts, i.e., the host species which cannot sustain the pathogen on their own. To identify specific hosts as constituting the reservoir community in a multi-host population we have to regard three different conditions. First, the infected host of interest must be able to cause secondary transmissions among susceptible individuals (i.e. the potential for secondary transmission). Second, the infection should persist in the reservoir community in the absence of other host species (which we refer to as *necessary condition*; previously described by Cleaveland and Dye (1995) as a condition of the reservoir host for a single host population). If the average number of secondary transmissions per single primary case in the reservoir community is greater than 1, the transmission will be maintained in that host species. Third, if the transmission cycle cannot be maintained in the absence of the reservoir community (which we refer to as *sufficient condition*), this indicates that the specific host (or a combination of hosts which constitute the reservoir community) plays a critical role in maintaining the transmission in the population as a whole.

In the case of influenza in wild birds, secondary transmission, the first condition, can be proven experimentally and has already been examined among ducks (Yamamoto et al., 2007). The second and third conditions can be assessed by epidemiological modeling, quantifying the threshold quantities for a particular infection in the multi-host population. Although earlier reservoir definitions have emphasized that the pathogen of interest should be non-pathogenic to the reservoir host (which may permit a long infectious period, and thereby increase the number of secondary transmissions), theoretically the non-pathogenicity condition is not essential (Haydon et al., 2002; Latorre-Margalef et al., 2009). Thus, we here focus on the transmission dynamics and persistence of infection in the multi-host population.

Here, we aim to develop a simple quantitative method to estimate threshold quantities for transmission and persistence of viral infections in a multi-host population. This method is exemplarily applied to the identification of the reservoir host(s) for influenza A virus in a multi-host waterbird population. Through our application, we test if a specific host or a combination of multiple hosts can satisfy the above-mentioned conditions to be a reservoir community of influenza.

Methods

Host types and influenza prevalence data

In the present study, we use "type" to denote a group of hosts of our interest. The type does not necessarily correspond to taxonomic or phylogenetic groups. We use the prevalence data of influenza A virus for different waterbird species (Fig. 1; Olsen et al., 2006). These published data summarize the worldwide distribution up to 2005 based on a total of 71859 samples across 47 species of waterbirds. Based on ecological and prevalence characteristics we selected only the waterfowl and wader species from this data set and grouped them into five types. We distinguish dabbling ducks, diving ducks, geese and swans, and waders. Furthermore, as dabbling ducks differ in prevalence and abundance, we further subdivide dabbling ducks into two types: (I) mallards and (II) all other dabbling ducks but mallards. Other species with greatly deviating ecology (e.g. with respect to habitat preference) or because of extremely low prevalence, e.g., rails and cormorants, were ignored. Gulls were also removed, because infections with only a few specific subtypes of influenza virus have been reported in this group of birds (Munster et al., 2007). We used the prevalence data, because other epidemiological information (e.g. incidence of infection) is usually not collected.

The prevalence in dabbling ducks (including mallards, wigeon, teals and gadwall) has been reported to be as high as 10.1%, while that of diving ducks (e.g. pochards and tufted ducks) was 1.6% (Olsen et al., 2006; Fouchier et al., 2007). Among the five types, Fig. 1 ranks the prevalence in descending order; we label the types accordingly (i.e. the type with highest prevalence (i.e. mallards) is labeled as 1). Observing the highest prevalence in mallards, and the second highest prevalence in other dabbling ducks, they have been suggested to be the reservoir hosts, or, at least, playing a key role for maintenance. Nevertheless, high prevalence alone does not suffice to constitute the reservoir community. Below, we will present a model that permits identification of the reservoir community from endemic prevalence data.

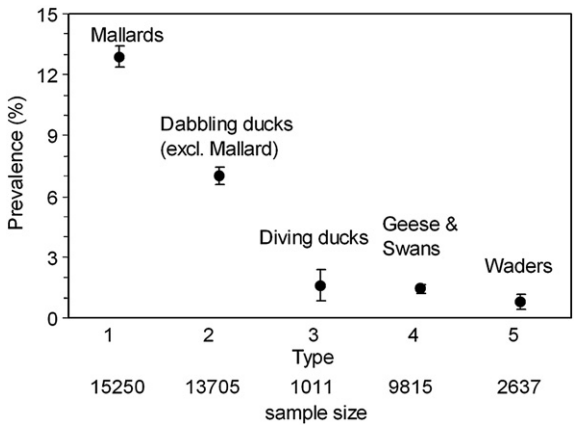

Fig. 1. Observed prevalence of influenza A virus infection by different types of hosts. Each dot represents the expected value of prevalence for each host type. The whiskers indicate the lower and upper 95% confidence intervals. According to rank of the observed prevalence levels, we label the types from 1 to 5 in descending order. See Olsen et al. (2006) for original data.

Model

We consider the population dynamics of influenza A virus in a multi-host wild bird population with n different host types (i.e. $n=5$ in our example). For each type k, we divide the population into susceptible (s_k) and infectious (i_k) individuals. Both s_k and i_k are expressed as proportion and thus we assume $s_k(t)+i_k(t)=1$, for any time t. Due to the absence of adequate data, we ignore periodic fluctuations both in the prevalence and the host population dynamics, despite realizing that models for infectious diseases in natural populations may yield additional insights into the transmission dynamics when incorporating temporally fluctuating dynamics in prevalence and host demography. The stationary assumption for host demography is in accordance with the assumption that birth and death rates are identical, μ_k (per unit time), and constant in time. Let the force of infection (i.e. the rate at which susceptible individuals experience infection) and the recovery rate of type k be λ_k and γ_k, respectively. The transmission dynamics of influenza A virus in a waterbird population are described by an SIS (susceptible-infected-susceptible) model:

$$\begin{aligned}\frac{ds_k}{dt} &= \mu_k + \gamma_k i_k - (\lambda_k + \mu_k)s_k \\ \frac{di_k}{dt} &= \lambda_k s_k - (\gamma_k + \mu_k)i_k\end{aligned} \tag{1}$$

We adopt an SIS-type model rather than an SIR (susceptible-infected-recovered)-type approach, because influenza infection in ducks is known not to elicit subtype specific immunity against further infections, and thus, the host experiences frequent re-infections (Kida et al., 1980). Although influenza epidemiology may be better described by incorporating protective immunity both in humans (Pease, 1987) and perhaps also in wild birds (Latorre-Margalef et al., 2009), we tentatively assume the SIS model to be valid for the purpose of demonstration of our method. We further assume the infection process to be frequency-dependent (de Jong et al., 1995; McCallum et al., 2001), again due to the absence of more specific data or evidence of the contrary. Population size estimates could be extracted from the literature (Rose and Scott, 1997), potentially permitting an estimate of density-dependent transmission; however, we used the aggregated prevalence data which involved considerable variations in the species-specific and location-specific sampling of influenza A virus within the same type. The force of infection λ_k can be written as

$$\lambda_k = \sum_{l=1}^{n} \beta_{kl} i_l \tag{2}$$

where β_{ij} is the transmission rate from type j to i. Ignoring periodic fluctuations in the prevalence indicates that we implicitly regard the prevalence data in Fig. 1 as reflecting an endemic steady state of system (1) and thus the wild birds as one globally interacting community. We assume homogeneous mixing within the same type due to the absence of adequate data for further systematic explorations of potential heterogeneities. The steady state solutions of Eq. (1) are given by

$$\begin{aligned}s_k^* &= \frac{\mu_k + \gamma_k}{\lambda_{k0} + \mu_k + \gamma_k} \\ i_k^* &= \frac{\lambda_{k0}}{\lambda_{k0} + \mu_k + \gamma_k}\end{aligned} \tag{3}$$

where i_k^* represents the prevalence level of type k in endemic equilibrium and λ_{k0} is the force of infection of type k in the endemic steady state. If M_k out of N_k individuals of type k were infected in the observed data, we assume

$$M_k \sim \text{Binomial}\left(N_k, i_k^*\right) \tag{4}$$

That is, the loglikelihood kernel l for the prevalence survey data is given by

$$l(\lambda_{10}, \lambda_{20}, \ldots, \lambda_{n0}) = \sum_{k=1}^{n} \left\{ M_k \ln \frac{\lambda_{k0}}{\lambda_{k0} + \mu_k + \gamma_k} + (N_k - M_k) \ln \frac{\mu_k + \gamma_k}{\lambda_{k0} + \mu_k + \gamma_k} \right\}, \tag{5}$$

from which we can estimate λ_{k0} for all types k. Since there are n unknown λ_{k0} with n inputs of prevalence, the average life-expectancy at birth $1/\mu_k$ and mean infectious period $1/\gamma_k$ for all types are extracted from literature and both are assumed known (Table 1). The pre-fledging mortality is known to be extremely high; however, individuals do not aggregate at this stage, and as such their presence (or absence) is expected to have little impact on the transmission dynamics. Thus, we utilize published post-fledging mortality as the known estimate of $1/\mu_k$. Although the mean infectious periods are typically derived from mean virus shedding periods in experimental infection trials, strictly speaking, the virus shedding periods do not directly offer the infectious period. Since the detailed distribution of infectious period has been suggested to greatly influence the estimates of threshold quantities in real-time (Lloyd, 2001; Wearing et al., 2005; Roberts and Heesterbeek, 2007; Wallinga and Lipsitch, 2007), we examine the sensitivity of threshold quantities to different infectious periods assuming plausible ranges (see Reservoir definition).

Threshold quantities

To capture the multi-species interactions and quantify the resulting threshold quantities, we consider the $n \times n$ matrix $\mathbf{B}$ with elements β_{ij}. As we will discuss in the following, parameter

Table 1
Parameter estimates for the average life-expectancy at birth and mean infectious period of influenza A virus infection in waterbird populations.

Type (k)		$1/(365\times\mu_k)$, average life-expectancy at birth (years)	Range	$1/\gamma_k$, mean infectious period (days)	Range	References
1	Mallards	1.6	(1.2–2.0)	4.0	(1.0–7.0)	Cramp and Simmons (1977), Homme and Easterday (1970), Webster et al. (1978), Isoda et al. (2006), Keawcharoen et al. (2008)
2	Dabbling ducks (excl. mallards)	2.0	(1.5–2.5)	6.5	(4.0–10.0)	Cramp and Simmons (1977), Kida et al. (1980)
3	Diving ducks	2.0	(1.5–2.5)	4.0	(2.0–7.0)	Cramp and Simmons (1977), Blums et al. (1996), Keawcharoen et al. (2008)
4	Geese and swans	7.0	(3.0–20.0)	5.0	(2.0–8.0)	Balmer and Peach (1997), Homme and Easterday (1970), Otsuki et al. (1982), Brown et al. (2008), Schekkerman and Slaterus (2008)
5	Waders	7.0	(3.0–20.0)	5.0	(3.0–14.0)	Hilden (1978), Otsuki et al. (1982), Krauss et al. (2004)

Although the mortality before fledging is known as extremely high, the individuals do not aggregate and have little impact on the transmission dynamics. Following the fledging, juveniles start aggregation and their mortality is greatly improved. Note that the mean estimate of mortality ignores deaths before fledging.

identifiability can be arranged by restricting the matrix **B** to no more than n degrees of freedom (e.g. specifying a structure for **B** involving only n distinct parameter values). That is, there is a unique matrix $\mathbf{D}(\lambda)$ which satisfies

$$\lambda = \mathbf{D}(\lambda)\beta \tag{6}$$

where β is the n-vector of distinct parameters $(b_1, b_2, \ldots, b_n)^{\mathrm{T}}$ (Anderson and May, 1985; Farrington et al., 2001). The Eq. (6) can be derived by further examining Eqs. (2) and (3); i.e.

$$\lambda_{k0} = \sum_{l=1}^{n} \frac{\beta_{kl}\lambda_{l0}}{\lambda_{l0} + \mu_l + \gamma_l} \tag{7}$$

Solving Eq. (7) for β, the parameters β are described as a function of λ and are estimated as $\hat{\beta} = \mathbf{D}(\hat{\lambda})^{-1}\hat{\lambda}$. We assume $\mathbf{D}(\lambda)^{-1}\lambda > 0$, so that matrix **B** has a regular configuration for the data (i.e., the estimation is constrained by this condition).

In general, the contact frequencies between different host types are not directly observable and quantifiable, introducing uncertainties to the model. We therefore investigate four different contact structures, employing the so-called WAIFW (who acquires infection from whom) matrix (Fig. 2). The first assumption, $\mathbf{B}_1$, adopts a separable mixing assumption, where β_{ij} can be decomposed as $a_i a_j$ (Dietz and Schenzle, 1985; Greenhalgh and Dietz, 1994), while three others, $\mathbf{B}_2$–$\mathbf{B}_4$, assume different qualitative patterns of contact. The biological interpretation of separable mixing $\mathbf{B}_1$ is that irrespective of its own type, an individual can acquire infection from any given infectious individual (i.e., the transmission rate from host j to i is determined by host j; Diekmann and Heesterbeek (2000)). All other matrices, $\mathbf{B}_2$, $\mathbf{B}_3$ and $\mathbf{B}_4$, assume that the transmission rates between type 1 (mallards) and the other types are smaller than the rate among type 1 individuals, because the prevalence in mallards is by far greater than the prevalence in the other types, while the infectious periods are not markedly different. $\mathbf{B}_2$ assumes that the transmission is high in type 1 and decreases according to the ascending label number of the other types (similar to the assumption of age-related heterogeneity in human disease transmission (Anderson and May, 1985)). $\mathbf{B}_4$ assumes the extreme case where different host types rarely interact and especially types 1–4 primarily experience infection within their own type. $\mathbf{B}_3$ is an intermediate between $\mathbf{B}_2$ and $\mathbf{B}_4$. It should be noted that these qualitative patterns are arbitrarily defined. Restricting the matrices with 5 parameters alone does not generally represent fully realistic contact patterns.

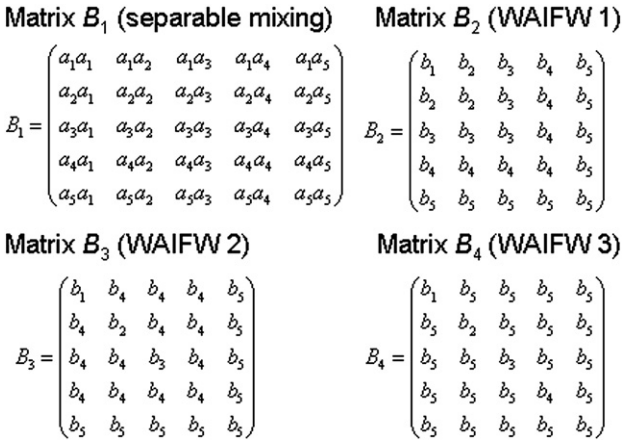

Fig. 2. Contact matrix structures. We assume four different contact structures for the transmission of influenza A virus in waterbird populations. Matrix $\mathbf{B}_1$ assumes that the contact between types i and j is separable. Matrix $\mathbf{B}_2$ assumes higher transmission rates among those with higher prevalence levels. Matrix $\mathbf{B}_4$ is an extreme assumption where types 1–5 experience the majority of contacts within the same type alone and seldom experience contacts with different types. Matrix $\mathbf{B}_3$ is an intermediate assumption between $\mathbf{B}_2$ and $\mathbf{B}_4$. In all cases, there are in total 5 parameters to be estimated.

To discuss the different threshold quantities in the multi-host population, we quantify the $n \times n$ next-generation matrix, **K**. Let **C** denote the $n \times n$ diagonal matrix of death/removal rates. Following Diekmann and Heesterbeek (2000), we consider the following vector $\boldsymbol{x}(\tau)$

$$\frac{\mathrm{d}\boldsymbol{x}(\tau)}{\mathrm{d}\tau} = -\mathbf{C}\boldsymbol{x}(\tau) \tag{8}$$

which describes the probability to be in the infectious state at infection-age τ. Since the matrix **B** is regarded as the vector of infectivity of the various hosts, the next-generation matrix **K** is given by

$$\mathbf{K} = -\mathbf{B}\left(-\mathbf{C}^{-1}\right) = \mathbf{B}\mathbf{C}^{-1} \tag{9}$$

where $\mathbf{C}^{-1}$ is the $n \times n$ diagonal matrix, $\mathrm{diag}(1/(\mu_1+\gamma_1), 1/(\mu_2+\gamma_2), \ldots, 1/(\mu_n+\gamma_n))$, which describes the average duration of infectiousness among infected individuals in each host type. Thus, each element k_{ij} of **K** is given by $\beta_{ij}/(\mu_j+\gamma_j)$ (Roberts, 2007). The basic reproduction number, R_0, is given by the dominant eigenvalue of **K**:

$$R_0 = \rho(\mathbf{K}) \tag{10}$$

In the present study, the estimate of R_0 is expected to be greater than 1 because we assume the system (1) has reached an endemic equilibrium, i.e., that an epidemic took off in the past. In addition to R_0, we examine two threshold quantities (Roberts and Heesterbeek, 2003): the host-specific (U) and the host-excluded (Q) reproduction numbers, which are defined as

$$U = \rho(\mathbf{P}\mathbf{K}) \tag{11}$$

and

$$Q = \rho((\mathbf{I} - \mathbf{P})\mathbf{K}) \tag{12}$$

where **I** and **P** are identity and projection matrices, respectively. The elements of the projection matrix are $P_{ii} = 1$ if $i \in \sigma$ and 0 otherwise (where σ represents the type(s) that are tested as potential reservoir host(s)). In practical terms, U measures the average number of secondary transmissions produced by a single primary case in the focal host(s) in the absence of other hosts (e.g. the reproduction number of influenza for dabbling ducks (i.e. types 1 and 2) in the absence of all other types). Q is the average number of secondary transmissions produced by a single primary case in other hosts in the absence of the focal host(s) (e.g. the reproduction number of influenza among all types other than dabbling ducks (i.e. types 3–5)).

Reservoir definition

In the multi-host population with n different host types, the maintenance host and reservoir community are defined as follows:

(A) For a host i to be a maintenance host, the host has to satisfy

$$U_i = \rho(\mathbf{P}_i\mathbf{K}) > 1 \tag{13}$$

The maintenance host should be considered as the minimum set of hosts to satisfy Eq. (13).

(B) The reservoir community consists of m different types, where $1 \le m \le n$, which is a minimum set of hosts satisfying both

$$U_m = \rho(\mathbf{P}_m\mathbf{K}) > 1 \tag{14}$$

and

$$Q_m = \rho((\mathbf{I} - \mathbf{P}_m)\mathbf{K}) < 1 \tag{15}$$

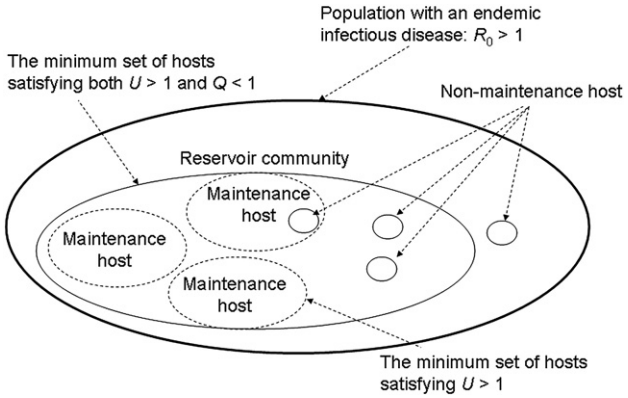

Fig. 3. Definition of the reservoir host. Given an endemic infection, R_0 of the population is greater than unity. In some instances, a maintenance host, defined as the minimum set of hosts satisfying $U>1$, can be found in the population. The reservoir community is defined as the minimum set of hosts satisfying both $U>1$ and $Q<1$, which must include all the maintenance host(s) and perhaps also the non-maintenance host(s). In the absence of the reservoir community, the remaining hosts cannot sustain the pathogen. If a single maintenance host is identical to the reservoir community, the host is referred to as the unique reservoir host.

By "minimum" m, the reservoir community is regarded as the minimum essential group of host(s) to maintain the transmission in the population as a whole.

(C) If there is only a single maintenance host and if the reservoir community consists of the maintenance host alone, the host is referred to as the unique reservoir species.

Fig. 3 shows the relationships between the different hosts. There can be multiple maintenance hosts in the single multi-host population. The definition of the maintenance host is irrelevant to Q. However, in the absence of the reservoir community, the transmission cannot be maintained. It should be noted that the reservoir community can include not only maintenance hosts, but also non-maintenance hosts. If maintenance hosts exist, the reservoir community must include all of the maintenance hosts in satisfying Eq. (15).

It is also possible that we cannot find any single type of maintenance host in the population. As an example, consider a mosquito-born disease with 3 or more hosts; e.g., the Japanese encephalitis virus is transmitted between *Culex* spp. and swine (the latter of which is referred to as an amplifying host), and human, horses and cattle are believed not to cause any secondary transmissions and thus referred to as the dead-end hosts (Vaughn and Hoke, 1992). Neither *Culex* spp. nor swine can maintain the transmission alone ($U<1$ for both *Culex* spp. alone and swine alone) and hence both are not the maintenance host. Nevertheless, if $U>1$ for the combination of *Culex* spp. and swine, the reservoir community is that combination.

Accordingly, the *necessary condition* for the reservoir community is that the infection persists in the presence of the reservoir community alone and the *sufficient condition* is that the transmission cycle cannot be maintained in the absence of the reservoir community, and are given by $U>1$ and $Q<1$, respectively. It should be noted that U based on type k is equivalent to Q based on all types other than type k (and vice versa); i.e., let $\overline{\mathbf{P}}$ be $\mathbf{I}$–$\mathbf{P}$, U_k (U for type k) is given by $\rho(\mathbf{PK}) = \rho((\mathbf{I}-\overline{\mathbf{P}})\mathbf{K}) = Q_{\text{non\_k}}$ (i.e., Q for all types other than type k).

We estimate R_0, U and Q from prevalence data of the five different host types. Since we assume four different WAIFW matrices, there are four different sets of these estimates. U and Q are estimated for all five types, and moreover, we also estimate U and Q for all possible combinations of two types to identify the reservoir community. The maximum likelihood estimates of R_0, U and Q are obtained directly from the maximum likelihood estimates of parameters for $\mathbf{B}$. The 95% percentile confidence intervals are obtained by employing a bootstrapping method (Efron and Tibshirani, 1993). Given the observed data $x_1, x_2, \ldots, x_n$, an estimate of some population parameter θ is calculated by taking a sample (with replacement) of size $m \leq n$ from the original set, $x_1, x_2, \ldots, x_n$. Based on simple random sampling (i.e. sampling which is independent of the types of host), an empirical distribution of θ is created by repeating the sampling procedure, which yields an estimate of θ. The 95% percentile confidence interval estimate of θ describes the 2.5th and 97.5th percentile points of the empirical distribution function of θ. The method is deemed particularly useful when it is difficult to estimate the sampling distribution of θ (e.g. in the case of R_0, U and Q).

Sensitivity analysis

We determine the sensitivity of U and Q to different infectious periods in different host types. Since the natural mortality rates μ_k would have only negligible impact on thresholds (compared to recovery rates γ_k), we here focus on varying recovery rates only. Furthermore, we examine the sensitivity of U and Q to different infectious periods for type 1 and types 1 and 2 only, because these are the only types that have the potential to constitute the reservoir community (see Results section). As the next-generation matrix is employed to quantify the threshold quantity, the sensitivity S_{ij} of R_0 to changes in the element of $\mathbf{K}$, $k_{ij}(\gamma_j)$, can be calculated as

$$S_{ij} = \frac{\partial R_0}{\partial k_{ij}} \frac{\partial k_{ij}}{\partial \gamma_j} \tag{16}$$

when focussing on γ_j. The fundaments and examples of sensitivity analysis in ecology can be found elsewhere (Caswell, 2001; see Hartemink et al., 2008 for use in epidemiology). Although the concept is useful for an analytical understanding of $\mathbf{K}$ where all other elements are constant and for numerical simulations, $\mathbf{K}$ in the present study is obtained partially by $\mathbf{B}$, which is estimated from the observed prevalence data, $p_1, p_2, \ldots, p_5$. The relative change in $k_{ij}(\gamma_j)$ would therefore influence all other elements of $\mathbf{K}$, and thus, we have to calculate maximum likelihood estimates of parameters for $\mathbf{B}$ in each relative change of a single parameter γ_j. That is, the expected values of U and Q are conditioned on p_k and, thus, we estimate

$$\frac{\partial \mathrm{E}(U \mid p_1, p_2, \ldots, p_5)}{\partial \gamma_j} \tag{17}$$

and

$$\frac{\partial \mathrm{E}(Q \mid p_1, p_2, \ldots, p_5)}{\partial \gamma_j} \tag{18}$$

as our sensitivity analysis. According to the plausible ranges of γ_k in Table 1, we examine four different relative changes in the mean

Table 2
Maximum likelihood estimates of the force of infection and the basic reproduction number for influenza A virus transmission in waterbird populations.

	Expected	95% confidence interval
Force of infection ($\times 10^{-2}$ (/year))		
λ_1 (Mallard)	3.72	(3.55, 3.90)
λ_2 (Dabbling ducks excluding mallards)	1.17	(1.09, 1.25)
λ_3 (Diving ducks)	0.40	(0.23, 0.63)
λ_4 (Gees and swans)	0.29	(0.25, 0.34)
λ_5 (Waders)	0.16	(0.10, 0.24)
R_0 (basic reproduction number)		
Matrix $\mathbf{B}_1$ (separable mixing)	1.13	(1.05, 1.23)
Matrix $\mathbf{B}_2$ (WAIFW 1)	1.14	(1.06, 1.22)
Matrix $\mathbf{B}_3$ (WAIFW 2)	1.14	(1.07, 1.23)
Matrix $\mathbf{B}_4$ (WAIFW 3)	1.14	(1.07, 1.29)

The 95% confidence intervals for the force of infection were derived from profile likelihood, while those for R_0 were obtained using bootstrap method.

Table 3
Maximum likelihood estimates of the host-specific reproduction number (U) for influenza transmission in waterbird populations.

Type(s)	Description	Matrix $\mathbf{B}_1$ (separable mixing)	Matrix $\mathbf{B}_2$ (WAIFW 1)	Matrix $\mathbf{B}_3$ (WAIFW 2)	Matrix $\mathbf{B}_4$ (WAIFW 3)
1	Mallards	0.96 (0.91, 1.02)	**1.01** (0.96, 1.07)	**1.11** (1.05, 1.16)	**1.13** (1.07, 1.18)
2	Dabbling ducks (excl. mallards)	0.15 (0.13, 0.17)	0.36 (0.34, 0.39)	0.89 (0.81, 0.97)	0.97 (0.89, 1.05)
3	Diving ducks	0.01 (0.00, 0.03)	0.07 (0.04, 0.11)	0.33 (0.17, 0.92)	0.64 (0.17, 1.23)
4	Geese and swans	0.01 (0.01, 0.01)	0.06 (0.05, 0.07)	0.06 (0.05, 0.07)	0.49 (0.19, 0.76)
5	Waders	0.00 (0.00, 0.00)	0.03 (0.02, 0.05)	0.03 (0.02, 0.05)	0.03 (0.02, 0.05)
1 and 2	All dabbling ducks	**1.11** (1.04, 1.19)	**1.12** (1.05, 1.18)	**1.12** (1.06, 1.19)	**1.13** (1.07, 1.20)
1 and 3	Mallards and diving ducks	0.97 (0.91, 1.04)	**1.02** (0.96, 1.08)	**1.11** (1.06, 1.18)	**1.13** (1.07, 1.26)
1 and 4	Mallards, geese and swans	0.97 (0.91, 1.03)	**1.01** (0.96, 1.07)	**1.11** (1.06, 1.17)	**1.13** (1.07, 1.19)
1 and 5	Mallards and waders	0.96 (0.91, 1.02)	**1.01** (0.96, 1.07)	**1.11** (1.05, 1.16)	**1.13** (1.07, 1.18)
2 and 3	Dabbling ducks (excl. mallards) and diving ducks	0.16 (0.14, 0.20)	0.39 (0.34, 0.45)	0.89 (0.82, 1.02)	0.97 (0.89, 1.25)
2 and 4	Dabbling ducks (excl. mallards), geese and swans	0.16 (0.14, 0.18)	0.38 (0.35, 0.41)	0.89 (0.82, 0.97)	0.97 (0.89, 1.06)
2 and 5	Dabbling ducks (excl. mallards) and waders	0.16 (0.14, 0.18)	0.37 (0.34, 0.40)	0.89 (0.81, 0.97)	0.97 (0.89, 1.06)
3 and 4	Diving ducks, geese and swans	0.02 (0.01, 0.04)	0.12 (0.09, 0.16)	0.34 (0.18, 0.92)	0.64 (0.20, 1.24)
3 and 5	Diving ducks and waders	0.01 (0.00, 0.03)	0.09 (0.05, 0.14)	0.33 (0.17, 0.92)	0.64 (0.17, 1.24)
4 and 5	Geese, swans and waders	0.01 (0.01, 0.02)	0.08 (0.06, 0.11)	0.08 (0.06, 0.11)	0.49 (0.19, 0.76)
1, 2 and 3	All ducks	**1.13** (1.05, 1.22)	**1.13** (1.06, 1.21)	**1.13** (1.07, 1.21)	**1.13** (1.07, 1.28)

The host-specific reproduction number, U, a measure of the capacity of host(s) of interest in maintaining the transmission alone, is given by $\rho(\mathbf{PK})$ where $\mathbf{K}$ and $\mathbf{P}$ are the next-generation matrix and projection matrix, respectively. Maximum likelihood estimates which satisfy $U>1$ are highlighted in bold. The parentheses show the 95% confidence interval using bootstrap method.

infectious period for types for $k=1$, 2 and 3–5 namely 0.50, 0.75, 1.25 and 1.50 times the baseline value.

Summary of assumptions

In summary, we make the following assumptions:

1. The observed prevalence data reflect influenza transmission in waterbirds in stationary state (i.e. achieving endemic steady state). We ignore periodic fluctuations in prevalence. Similarly, we ignore periodic fluctuations in the demographic dynamics of the host.
2. The infection does not elicit subtype specific immunity against further infections in waterbird populations. Infected hosts will become fully susceptible again upon recovery. Antigenic variation in influenza virus A is ignored.
3. We ignore variations in sampling frequencies with respect to time and place and regard the published data as representative of the worldwide prevalence data which permits ignoring migratory behavior.
4. Frequency-dependence is adopted to model the transmission within and between species. Due to the absence of more specific data, modes of transmission other than direct contact (e.g. transmission via surface water (Hinshaw et al., 1979; Markwell and Shortridge, 1982)) are ignored.
5. Due to limited data availability, heterogeneous contact patterns within the same host (e.g., space- or age-related) are ignored.
6. Interactions between wild birds and other hosts (e.g. poultry and swine) are far less frequent than those among wild birds, and are thus ignored.

If more detailed datasets become available, assumptions 3, 5 and 6 can be resolved by natural extensions of our concept (i.e. adding "types" to the model).

Results

Forces of infection and the basic reproduction number

Table 2 shows the maximum likelihood estimates of the force of infection for the five different types (which were estimated using Eq. (5)). Because we assumed that the infectious period would not greatly vary by types, the forces of infection almost directly reflect the prevalence levels in Fig. 1, which is certainly expected from Eq. (3). Estimates of R_0 are also shown for the four different assumptions of the WAIFW matrix. R_0 did not greatly vary for the different contact patterns. All four assumptions reflected the observed data equally well.

Table 4
Maximum likelihood estimates of the host-excluded reproduction number (Q) for influenza transmission in waterbird populations.

Type(s)	Description	Matrix $\mathbf{B}_1$ (separable mixing)	Matrix $\mathbf{B}_2$ (WAIFW 1)	Matrix $\mathbf{B}_3$ (WAIFW 2)	Matrix $\mathbf{B}_4$ (WAIFW 3)
1	Mallards	**0.17** (0.14, 0.22)	**0.41** (0.36, 0.49)	**0.90** (0.82, 1.04)	**0.98** (0.89, 1.26)
2	Dabbling ducks (excl. mallards)	**0.98** (0.92, 1.06)	1.02 (0.96, 1.09)	1.12 (1.06, 1.18)	1.13 (1.07, 1.27)
3	Diving ducks	1.12 (1.05, 1.20)	1.13 (1.06, 1.19)	1.13 (1.07, 1.20)	1.14 (1.07, 1.21)
4	Geese and swans	1.13 (1.05, 1.22)	1.13 (1.06, 1.21)	1.13 (1.07, 1.21)	1.14 (1.07, 1.28)
5	Waders	1.13 (1.05, 1.23)	1.13 (1.06, 1.21)	1.14 (1.07, 1.22)	1.14 (1.07, 1.29)
1 and 2	All dabbling ducks	**0.02** (0.01, 0.04)	**0.14** (0.10, 0.19)	**0.35** (0.19, 0.93)	**0.65** (0.21, 1.24)
1 and 3	Mallards and diving ducks	**0.16** (0.14, 0.19)	**0.38** (0.35, 0.42)	**0.90** (0.82, 0.98)	**0.98** (0.89, 1.07)
1 and 4	Mallards, geese and swans	**0.17** (0.14, 0.21)	**0.39** (0.35, 0.46)	**0.90** (0.82, 1.02)	**0.98** (0.89, 1.25)
1 and 5	Mallards and waders	**0.17** (0.14, 0.21)	**0.41** (0.36, 0.48)	**0.90** (0.82, 1.03)	**0.98** (0.89, 1.25)
2 and 3	Dabbling ducks (excl. mallards) and diving ducks	**0.97** (0.91, 1.03)	1.02 (0.96, 1.07)	1.11 (1.06, 1.17)	1.13 (1.07, 1.19)
2 and 4	Dabbling ducks (excl. mallards), geese and swans	**0.97** (0.91, 1.05)	1.02 (0.96, 1.08)	1.11 (1.06, 1.18)	1.13 (1.07, 1.26)
2 and 5	Dabbling ducks (excl. mallards) and waders	**0.98** (0.92, 1.05)	1.02 (0.96, 1.09)	1.11 (1.06, 1.18)	1.13 (1.07, 1.26)
3 and 4	Diving ducks, geese and swans	1.12 (1.04, 1.19)	1.12 (1.05, 1.19)	1.13 (1.07, 1.19)	1.13 (1.07, 1.20)
3 and 5	Diving ducks and waders	1.12 (1.05, 1.20)	1.12 (1.06, 1.19)	1.13 (1.07, 1.20)	1.13 (1.07, 1.21)
4 and 5	Geese, swans and waders	1.13 (1.05, 1.22)	1.13 (1.06, 1.21)	1.13 (1.07, 1.21)	1.13 (1.07, 1.28)
1, 2 and 3	All ducks	**0.01** (0.01, 0.02)	**0.08** (0.06, 0.11)	**0.08** (0.06, 0.11)	**0.49** (0.19, 0.76)

The host-excluded reproduction number, Q, a measure of the capacity of host(s) other than the types of interest in maintaining the transmission alone, is given by $\rho((\mathbf{I}-\mathbf{P})\mathbf{K})$ where $\mathbf{K}$, $\mathbf{I}$ and $\mathbf{P}$ are the next-generation matrix, identity matrix and projection matrix, respectively. Maximum likelihood estimates which satisfy $Q<1$ are highlighted in bold. The parentheses show the 95% confidence interval using bootstrap method.

The host-specific and the host-excluded reproduction numbers

Threshold quantities U and Q for all five types and for all two possible combinations are summarized in Tables 3 and 4. Except for mallards, expected values of U for all single types were smaller than unity, indicating that influenza transmission cannot persist without the presence of additional host type(s) in any type other than mallards. For mallards, U was greater than 1 in three out of the four contact structures ($\mathbf{B}_2$–$\mathbf{B}_4$). Only the separable mixing structure ($\mathbf{B}_1$) yielded $U=0.95$, suggesting that mallards alone will not be able to maintain the transmission cycle if the probability of infection given a contact in host i (a_i) and the contact rate which occurs with an infected host j (a_j) are independent (i.e. multiplicative). For the two combinations that include mallards, U was greater than 1 for three contact structures, $\mathbf{B}_2$–$\mathbf{B}_4$, but for separable mixing. Only a combination of types 1 and 2 (i.e. all dabbling ducks) satisfied $U>1$ for all four contact structures. U for other combinations of two types (not including type 1) was below unity.

The quantity Q was less than 1 for mallards alone and combinations of two hosts with type 1 (mallards and another host type) for all four contact structures indicating that influenza cannot persist in the absence of mallards. Q appeared to be greater than 1 for each type other than mallards except for an estimate for type 2 (dabbling ducks other than mallards) with the separable mixing assumption ($Q=0.98$). Thus, even in the absence of diving ducks, geese and swans, and waders, influenza transmission can be maintained in the

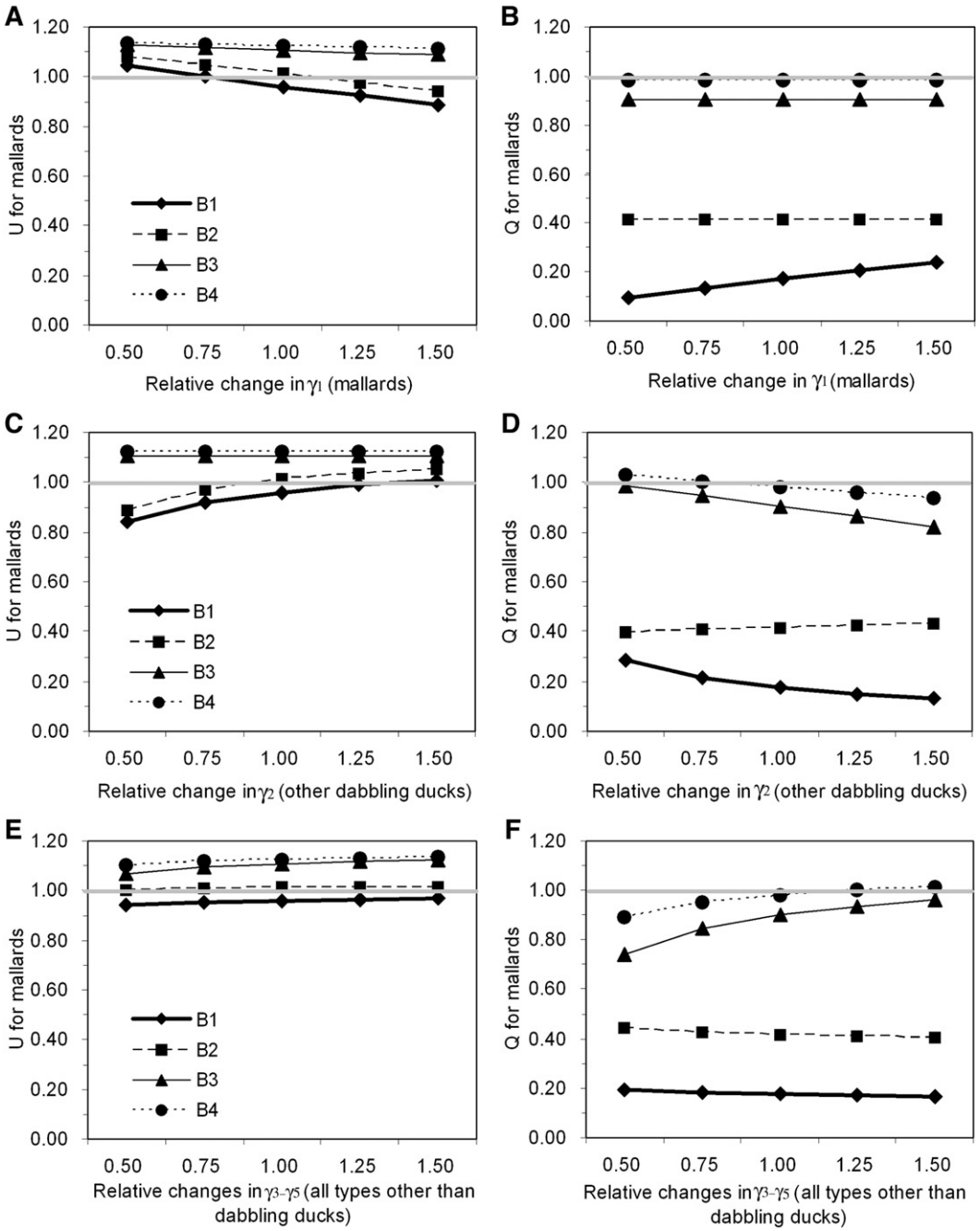

Fig. 4. Sensitivity of threshold quantities for mallards to different infectious periods. Sensitivity of the maximum likelihood estimates of the host-specific reproduction number (U; panels A, C and E) and the host-excluded reproduction number (Q; panels B, D and F) for mallards are examined by different infectious periods of mallards (A and B), other dabbling ducks (C and D) and all types other than dabbling ducks (E and F). The horizontal axis measures the relative change in the mean infectious period to the baseline value. The horizontal grey line indicates the threshold value 1.

population. For other host combinations (all combinations of two types which do not include type 1), Q was greater than 1 except for combinations of type 2 with types 3, 4 or 5 under the separable mixing assumption. It should be noted that only the combination of types 1 and 2 yielded the expected values of Q sufficiently smaller than unity for all four contact structures.

Sensitivity of U and Q for dabbling ducks to different infectious periods

Fig. 4 shows the sensitivity of U and Q for mallards (type 1) given different infectious periods γ. If the mean infectious period of mallards is shorter than we assumed, U for mallards may well become smaller than unity but Q for mallards never exceeded unity. If the mean infectious period of dabbling ducks other than mallards (type 2) is longer than we assumed, an abrupt decline in U for mallards was observed for the contact structures $\mathbf{B}_1$ and $\mathbf{B}_2$. For the other two contact structures, $\mathbf{B}_3$ and $\mathbf{B}_4$, U was less sensitive to the relative change in γ_2, which most likely reflects more frequent within-species transmissions compared to inter-species transmissions under these assumptions. On the other hand, Q was sensitive to γ_2 under contact structures $\mathbf{B}_3$ and $\mathbf{B}_4$, and its estimate may exceed unity when a long infectious period is assumed in type 2 hosts. None of the four different contact structures satisfied both $U<1$ and $Q>1$ if a long infectious period for type 2 was assumed, indicating that dabbling ducks other than mallards may play some role in the persistence of influenza under this scenario. Figs. 4E and F show the sensitivity of U and Q for

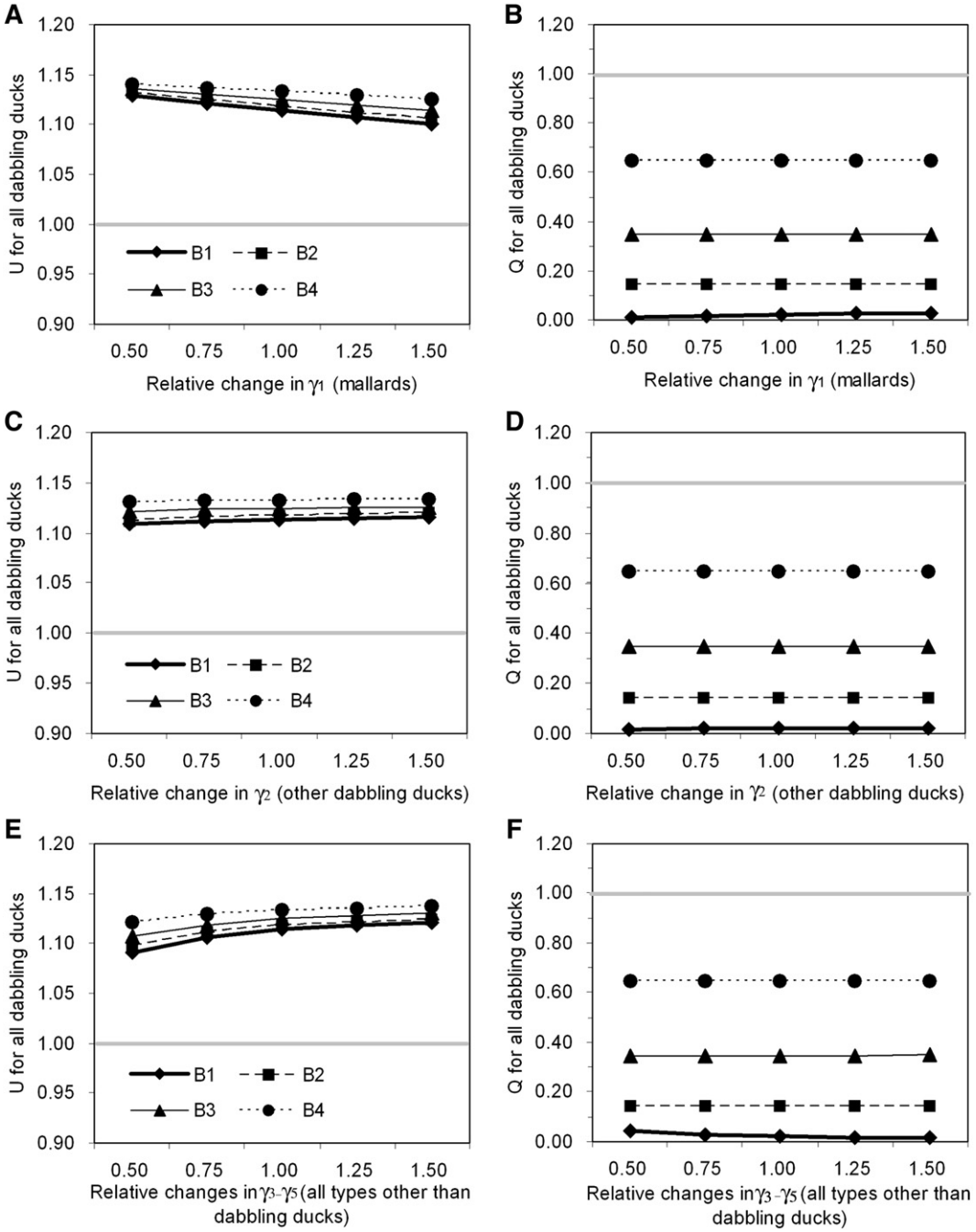

Fig. 5. Sensitivity of threshold quantities for dabbling ducks to different infectious periods. Sensitivity of the maximum likelihood estimates of the host-specific reproduction number (U; panels A, C and E) and host-excluded reproduction number (Q; panels B, D and F) for all dabbling ducks (i.e. types 1 and 2) are examined by different infectious periods of mallards (A and B), other dabbling ducks (C and D) and all types other than dabbling ducks (E and F). The horizontal axis measures the relative change in the mean infectious period to the baseline value. The horizontal grey line indicates the threshold value 1.

mallards to relative changes in γ_3, γ_4 and γ_5. In response to the increase in these infectious periods, U declined slightly for all assumptions of contact structure, while Q appeared to vary differently according to the assumed contact structure. Under contact structures $\mathbf{B}_3$ and $\mathbf{B}_4$, Q exceeded unity if the infectious periods of types 3–5 are 2/3 times shorter than we initially assumed.

Similarly, Fig. 5 examines the sensitivity of U and Q for all dabbling ducks (i.e. types 1 and 2) to different infectious periods. In all cases (except for very long infectious periods among types 3–5), we observed $U>1$ and $Q<1$. In particular, it should be noted that Q for all dabbling ducks remained below unity and was almost independent of the length of the infectious periods in different types of hosts, indicating that influenza transmission cannot be maintained in the absence of dabbling ducks. Unless the infectious periods of types 3–5 are assumed to be considerably longer than those we assumed based on the literature (Table 1), it is essential that types 1 and 2 are present to allow persistence of influenza A in this multi-host population.

Discussion

Although heterogeneous patterns of transmission in multi-host populations have been discussed in mathematical studies (Diekmann and Heesterbeek, 2000), they have rarely been applied to estimate threshold quantities. Some rare examples include age-related heterogeneity of transmission (Anderson and May, 1985; Coen et al., 1998; Farrington et al., 2001; Whitaker and Farrington, 2004a; 2004b; Sfikas et al., 2007) and heterogeneous social contact patterns of transmission (Edmunds et al., 2006; Wallinga et al., 2006; Mossong et al., 2008). However, all of these only considered human infectious diseases and thus, a single type of host species. Therefore, despite the theoretical development in the definition of the type-reproduction number (Roberts and Heesterbeek, 2003; Heesterbeek and Roberts, 2007), the quantitative usefulness of the next-generation matrix for examining infectious diseases in a multi-host population has not been sufficiently emphasized in statistical terms.

Motivated by the practical need to develop a methodological basis for the identification of reservoir hosts, we here proposed a simple method for the statistical estimation of the host-specific and the host-excluded reproduction numbers based on an epidemiological approach, defining a condition of being maintenance host as $U>1$ and that of reservoir community as both $U>1$ and $Q<1$. We applied this method to a dataset of influenza A in populations of wild birds and attempted to determine whether different waterbirds might constitute a reservoir community of influenza A virus in a natural population. If the separable mixing is assumed, a combination of mallards and dabbling ducks constitutes the reservoir community and neither of them is regarded as a maintenance host. If one of the other three contact structures ($\mathbf{B}_2$–$\mathbf{B}_4$) is imposed, mallards alone would constitute the reservoir community alone and hence be regarded as the sole maintenance host (and thus the unique reservoir species). Until today, this issue has been discussed only from a virological perspective, through examination of the susceptibility and/or disease-severity in inoculation experiments (Wood et al., 1985; Stallknecht and Shane, 1988; Ito and Kawaoka, 2000; Neumann and Kawaoka, 2006). Our method instead exploits endemic equilibria, and although simplifying assumptions (e.g. SIS-type dynamics and frequency-dependent contacts) were required for this wildlife infection, the endemic prevalence data were successfully used to examine whether mallards and other dabbling ducks are essential to the persistence of influenza infection in wild birds. In further explorations of influenza or future applications to other infectious diseases, the assumption of density-dependence can also be employed whenever the population density can be estimated for each type. To our knowledge, the present study is the first to theoretically demonstrate that mallards and other dabbling ducks are most likely constituting the reservoir community of influenza in the waterbird population.

Given our simplifying assumptions still sufficiently capture the fundamental features of reality (see below), two ecological conclusions can be drawn from our exercise. First, dabbling ducks (i.e. combination of types 1 and 2) satisfied both $U>1$ and $Q<1$, and thus, are regarded as essential host populations for influenza A virus infection within the waterbird community. That is, our method identified, at least, a "family" of importance. In particular, if we assume separable mixing, dabbling ducks are regarded as the reservoir community. In the case of the remaining three contact structures mallards may form the reservoir community alone and are referred to as the unique reservoir species. Our finding agrees with suggestions based on field observations in northern Europe (Munster et al., 2005; Olsen et al., 2006; Fouchier et al., 2007; Munster et al., 2007; Wallensten et al., 2007). The result also justifies surveillance specifically targeted at dabbling ducks (Munster et al., 2006). Second, it seems most likely that mallards are the unique reservoir species whereas the other dabbling ducks can be considered non-maintenance hosts only, meaning that they can be infected and cause secondary transmission but cannot sustain the pathogen by themselves. However, the estimates of U and Q for mallards appeared to be very sensitive to the assumptions on contact structure (and especially separable mixing did not satisfy $U>1$ for mallards). The indefinite results of threshold quantities for mallards (i.e. differing conclusions for $U>1$ or ≤ 1 by contact structure), the very coarse type classifications and the temporal and spatial aggregation of prevalence data that we used call for a further clarification on the role of mallards as well as other dabbling ducks within more detailed future studies.

Two other crucial aspects of influenza A virus infection, which we believe are critical to fully clarify the dynamics in the future, must be discussed. First, accounting for temporal and spatial patterns is deemed essential for migrating hosts. We ignored seasonal oscillations in prevalence, which may result from various factors such as seasonal variations in population dynamics, contact structures and contact rates. Whereas spatially differing patterns in prevalence have been discussed previously (Olsen et al., 2006; Munster et al., 2007), we refrained from extending our method in this regard, mainly due to limited sample sizes for each geographic location — unfortunately, in surveillance studies, the prevalence tends to be measured to the order of <5% with, at most, a few hundred samples per species.

Second, the mechanisms of acquired immunity and varying pathogenicities among different subtypes need further/future clarification. We only had access to aggregated prevalence estimates for the different host types, ignoring the fact that different subtypes of influenza virus exist. We assumed SIS-type dynamics, following Kida et al.'s (1980) suggestion that subtype specific immunity is absent. Nevertheless, whether this subtype specific immunity is truly and fully absent, and no partially protective immunity exists for influenza A subtypes in birds, has yet to be fully clarified. Although the sample sizes are thus far too limited to draw a firm conclusion, the higher frequency of infections among juveniles compared to adult ducks indirectly supports the presence of a partial protection (Wallensten et al., 2007; Munster et al., 2007). If any cross-protective immunity exists in wild birds, the transmission dynamics of influenza in these hosts should not be regarded as if it were the transmission of a single pathogen. Instead, in such case, the host susceptibility and the pathogenicity of specific subtypes would each play a key role and would have to be considered to fully clarify the transmission dynamics of influenza (Garamszegi and Møller, 2007).

In light of our methodology/approach we cannot preclude the importance of investigating various species other than dabbling ducks. Indeed, despite the fact that past and current surveillance efforts have been immense (e.g. Munster et al., 2005; Munster et al., 2007; Wallensten et al., 2007), we must unfortunately admit that the sample sizes are still too small for performing explicit epidemiological analysis. In order to enable clearer quantitative conclusions on the identification of the natural reservoir hosts in the future, we

emphasize the critical importance of following firm epidemiological sampling methods and systematic surveillance efforts. For instance, surveillance efforts should also include species with small prevalence and those difficult to catch in the field. Also, sampling during the low transmission season, which typically yields small prevalence estimates, deserves epidemiological attention, since these data are necessary for the full clarification of the temporal and spatial characteristics of influenza transmission.

Summarizing our results, we agree with Francis (1947) who already discussed the reservoir concept for influenza before the mid-20th century, and argued, "*The recurrent epidemics of influenza A and B continue the question of how influenza virus persists in the intervals. … A precise demonstration of the mechanisms through which the reservoir of the disease functions would constitute a great advance in constructing the biological pattern of influenza.*" (pp. 351–353) Once the biological and epidemiological limitations (including data collection) are resolved, we will be ready to offer much clearer conclusions about the structure of the reservoir community in waterfowl population.

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