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Unknown age in health disorders: A method to account for its cumulative effect and an application to feline viruses interactions

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ABSTRACT

Parasite interactions have been widely evidenced experimentally but field studies remain rare. Such studies are essential to detect interactions of interest and access (co)infection probabilities but face methodological obstacles. Confounding factors can create statistical associations, i.e. false parasite interactions. Among them, host age is a crucial covariate. It influences host exposition and susceptibility to many infections, and has a mechanical effect, older individuals being more at risk because of a longer exposure time. However, age is difficult to estimate in natural populations. Hence, one should be able to deal at least with its cumulative effect. Using a SI type dynamic model, we showed that the cumulative effect of age can generate false interactions theoretically (deterministic modeling) and with a real dataset of feline viruses (stochastic modeling). The risk to wrongly conclude to an association was maximal when parasites induced long-lasting antibodies and had similar forces of infection. We then proposed a method to correct for this effect (and for other potentially confounding shared risk factors) and made it available in a new R package, *Interatrix*. We also applied the correction to the feline viruses. It offers a way to account for an often neglected confounding factor and should help identifying parasite interactions in the field, a necessary step towards a better understanding of their mechanisms and consequences.

1. Introduction

Epidemiological, clinical, or biological outcomes are often studied considering each parasite separately. Nevertheless, parasites rarely exist in isolation. Hosts are exposed to numerous parasites (from micro- to macro-parasites, pathogenic or not) simultaneously and multiple infections of hosts are more frequently encountered than infections by a single parasite (Petney and Andrews, 1998; Cox, 2001).

Interactions between members of the intra-host community of parasites, e.g., competition for resources or interactions mediated by host immune responses, have been widely evidenced in animal models and experimental conditions (e.g., (Cox, 2001; Behnke et al., 1978; Christensen et al., 1987; Frontera et al., 2005)), with a main focus on human pathogens such as HIV, tuberculosis, malaria, sexually transmitted infections, and helminths (e.g., (Bentwich et al., 1999; Corbett et al., 2002; Celum, 2004; Druilhe et al., 2005; Abu-Raddad et al., 2006)). There is now strong evidence that parasites are affected by the presence of other parasites, their interactions altering the rates of co-occurrence, levels of infection and disease severity (e.g., (Abu-Raddad et al., 2006; Read and Taylor, 2001; Weiss and McMichael, 2004; Telfer et al., 2010; Ives et al., 2011)), as well as the success of parasites management measures (Pedersen and Fenton, 2007; Harris et al., 2009; Koch and Schmid-Hempel, 2011) and possibly disease (re)emergence (Pontier et al., 2009; Keesing et al., 2010).

Numerous studies on parasite interactions have been led in experimental conditions but are much rarer in natural host populations, with some exceptions for macroparasites (e.g., helminthes) communities (e.g., (Telfer et al., 2010; Dezfuli et al., 2001; Lello et al., 2004; Behnke et al., 2005; Jolles et al., 2008)). Field studies are however essential because experimental systems are oversimplified and because lab studies require an existing suspicion of interaction between the parasites. In addition, only studies in natural populations can give access to infection and co-infection probabilities. In other words, before studying their mechanisms in the lab, interactions of interest must be identified in the field.

Main difficulties encountered with studies in natural populations are methodological. Numerous confounding factors can create

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statistical associations, i.e., 'false interactions', between pathogens. For instance, as the risk to be infected by sexually transmitted parasites is strongly influenced by sexual behaviors (e.g., (Anderson et al., 1992)), those infections may appear associated even if they do not biologically interact, i.e., even if there is no 'true interaction'. In general, if several parasites share common risk factors the same host individuals are simultaneously at risk for those parasites and 'false interactions' are generated.

When confounding factors can be identified and recorded, true parasite interactions can be searched for using statistical tools such as log-linear models (e.g., (Behnke et al., 2005; Howard et al., 2001)), or modified chi-square analyses (e.g., (Kuris and Lafferty, 1994; Hellard et al., 2012)). However, some confounding factors are difficult to measure in the field. Host age is a striking example. Estimating the age of individuals is difficult for many wildlife species, leading to potentially strong errors in age determination or in the classification of individuals into age classes. Nonetheless, there are surprisingly very few statistical methods enabling to adjust for misclassification errors (Heisey et al., 2006; Conn and Diefenbach, 2007) and none in the framework of parasite interactions.

Host age is yet an absolutely crucial covariate to take into account as it is a risk factor for many infectious diseases. Among other examples, younger animals generally harbor fewer species of helminthes and lower worm burdens (Montgomery and Montgomery, 1989; Abu-Madi et al., 1998), and the seroprevalence increases with age for various parasites (e.g., Toxoplasmosis: (Jones et al., 2001); Hepatitis: (Murrill et al., 2002); Feline viruses: (Hellard et al., 2011)). This age-dependence is due to two additive phenomena. First, age has a 'biological' effect as host behaviors and immune defenses may evolve with age (e.g., (Anderson et al., 1992; Gasparoni et al., 2003; Levy, 2007; Bogaards et al., 2010)). Second, older individuals are more likely to be seropositive because of a longer exposure time, mechanically creating a cumulative effect of age.

When host age cannot be precisely estimated, it is crucial to deal at least with its mechanical effect. This is particularly important when studying microparasites in natural populations as such studies are most of the time cross-sectional and serological. Contrary to macroparasites whose follow up can be done quantitatively (i.e., fecal or blood counts), microparasites are often detected using indirect signs such as specific antibodies. Many infections are short (i.e., acute infections) and shedding times too brief (few hours or days) to make the search for the microparasites themselves efficient. This would require capturing hosts exactly when they are infectious. Most field data are thus limited to observed frequencies of seronegative, seropositive, and doubly seropositive individuals, with no information on the exact time of infection or on its intensity. In this context, the search for potential interactions between pairs of parasites consists in determining whether they are more often associated than expected by chance. The use of serological data reinforces the cumulative effect of age because antibodies may persist for months or years within the host. Aged individuals have a higher probability to have been exposed to the parasite and to have acquired the specific antibodies. The number of double seropositive individuals should also increase with age, thereby creating statistical associations between parasites.

In this paper, we address the problem of the detection of parasite interactions in cross-sectional data when there is no individual-specific age information or when only age-classes can be determined (e.g., juveniles *versus* adults). We focus on the impact of the cumulative effect of age in the search for interactions in serological data as this is an obvious application. However our approach is applicable for any disease, infectious or not (e.g., exposure to toxic chemicals, air pollutants, environmental agents, or toxic substances that may lead to health disorders later in life), as long as they are detectable for a certain period of time. We first use a dynamic model to investigate the impact of the cumulative effect of age in the detection of pairwise parasite interactions. Second, we propose a method to correct for this mechanical effect. We adapt a statistical method previously proposed to account for identified confounding factors in the search for parasite interactions ('the corrected chi-square', (Hellard et al., 2012)). This approach is applied to a real serological dataset of four feline viruses obtained in natural populations of domestic cats.

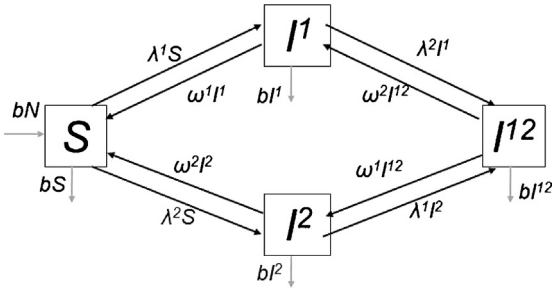

Fig. 1. Compartmental representation of the model of circulation of two independent parasites. The host population is divided in four compartments: susceptible (S), positive to parasite 1 (I^1), positive to parasite 2 (I^2) and coinfected (I^{12}) individuals. λ^x and ω^x are the force of infection and the antibodies' disappearance rate of parasite x, respectively. Birth and mortality rates (grey arrows) are considered to be constant, of equal value (b) and similar whether hosts are susceptible, singly or doubly positive.

2. Material and methods

2.1. The cumulative effect of age and false parasite interactions

A dynamic compartmental model in continuous time was used to model the circulation of two independent parasites sharing age as a common risk factor. In this model, the population is split into four classes, representing the frequency of individuals in each serological state: susceptible individuals, S, individuals positive to parasite 1, I^1, individuals positive to parasite 2, I^2, and doubly positive individuals, I^{12} (Fig. 1). Hosts are infected by parasite x with a force of infection λ^x, whereas the specific antibodies elicited after infection by parasite x disappear at a rate ω^x. Birth and mortality rates are assumed to be constant, both equal to b and not influenced by infections. Parasites are independent, i.e., the force of infection and antibodies' disappearance rate of a given parasite are independent from those of the other parasite.

2.2. Parasite characteristics favoring false interactions: a theoretical approach

The deterministic version of the model was used to determine how the cumulative effect of age can generate false interactions depending on the parasite characteristics. We considered the rate at which individuals acquire the infection (λ^x) and the antibodies' disappearance rate (ω^x) (Table 1).

Table 1
Parameters of the deterministic model and their values used in the theoretical approach.

Parameter	Definition	Value
b	Birth and mortality rate (both are considered equal)	0.25
ω^x	Antibodies' disappearance rate of parasite x	[0; 2]
λ^x	Force of infection of parasite x	[0.1; 20]