Methods for estimating parasite-induced mortality from intensity data and their limitations

Mark Wilber, Sara Weinstein, and Cherie Briggs

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Abstract

TODO

Introduction

Infectious agents can have major impacts on animal populations through changing population dynamics and stability (Dobson & Hudson 1992), altering predator-prey interactions (Joly & Messier 2004), and even causing species' decline and extinction (De Castro & Bolker 2005; McCallum 2012). Accurately estimating the impact of these infectious agents in wildlife is critical to understanding what regulates host and parasite populations, making predictions about disease transmission, and managing disease outbreaks (Langwig *et al.* 2015). The impact of pathogens, such as rabies (Coyne et al. 1989) [get citation], bovine TB (Cox *et al.* 2005), and rinderpest (Tillé *et al.* 1991), is typically quantified based on the presence or absence of disease, and does not account for the number of infectious agents present. This method is sufficient for many bacterial and viral agents that reproduce within a host, however for macroparasites pathology is linked to the intensity of infection and hosts cannot be simply categorized as infected and uninfected (Anderson & May 1979; Lafferty & Kuris 2002). Helminths exhibiting this intensity dependent pathology have significant impacts on human health (Brooker *et al.* 2004), domestic livestock economics (Roeber *et al.* 2013), wildlife survival (Kirk

2003; Logiudice 2003). While it is generally assumed that some fraction of wild host populations must succumb to parasitic infections, it is notoriously difficult to actually quantify parasite-induced host mortality (PIHM) in wild animal populations (McCallum 2000).

Ideally, parasite-induced host mortality is quantified by experimentally infecting and tracking individual hosts in the wild population; however, for logistical and ethical reasons this method is rarely feasible (McCallum 2000). Data on parasite intensity is much easier to collect and has often been used to identify the presence of PIHM (Crofton 1971; Lester 1977, 1984; Lanciani & Boyett 1989; Royce & Rossignol 1990; Ferguson *et al.* 2011) and quantify the relationship between infection intensity and host mortality (Adjei *et al.* 1986).

Crofton (1971) first proposed that PIHM could be identified by comparing the observed parasite distribution in the host population to the distribution predicted in the absence of parasite-induced host mortality. We briefly introduce the Crofton Method here and provide a more detailed explanation of its implementation in *Supplementary Material (SI)* 1. This method assumes that, prior to host mortality, infection intensity in the host population follows a negative binomial distribution and the tail of the distribution is truncated as intensity dependent pathology removes the most heavily infected hosts. Assuming mortality occurs only in these heavily infected hosts, evidence of this parasite-induced mortality should then be detectable by iteratively fitting a negative binomial distribution to hosts with lower and lower parasite loads, and comparing the tail ends of these predicted distributions to the observed parasite data.

The Crofton Method may be able to detect the presence of PIHM however, but it does not quantify the relationship between infection intensity and host survival probability. Adjei *et al.* (1986) suggested that this relationship could be calculated by first using the Crofton Method to estimate the pre-mortality parasite distribution and then using those distribution to calculate the probability of host survival with increasing parasite intensity. To do this, Adjei *et al.* (1986) modeled host survival as a

logistic function and then used a generalized linear model (GLM) to estimate the logistic parameters (see *SI* 2 for a technical description of the Adjei Method). These methods appeared to provide an estimate for the parasite intensity at which 50% of hosts exhibit PIHM (LD_{50}), as well as the unmeasurable fraction of the population that was lost (*SI* 2). However, to implement this method the observed data must be modified to fit the GLM framework and subjectively binned when mean infection intensity is high or sample sizes are small.

After 30 years, and despite clear limitations (McCallum 2000), these methods (particularly the Crofton Method) are still discussed among parasitologists and are the primary techniques for examining population level impacts of parasitism using parasite intensity data. In these methods, PIHM can only be identified by visually examining plots and, with no clear decision rule, it can be difficult to determine the significance of PIHM across different host-parasite systems. The survival function produced by the Adjei Method offers one solution; however, this method requires manipulating the original data and has never been tested.

Intensity data should only be used to estimate parasite impacts on host populations if unbiased and accurate methods exist. In this study, we first propose a novel method for calculating PIHM. We next use simulations to compare our method with the previous Adjei Method to test the ability of both methods to (1) detect occurrence of PIHM and (2) estimate the lethal parasite load (LD_{50}) and the associated survival function. We then apply both methods to real datasets previously used in PIHM analyses and compare the results. Finally, we discuss the limitations of inferring PIHM from intensity data and whether any method for inferring PIHM has a place in quantitative parasitology.

Methods

A novel, likelihood-based method for estimating PIHM

Here we propose a novel, likelihood-based method (henceforth Likelihood Method) that does not require binning or data alteration, reduces the number of parameters to be estimated, and uses standard statistical techniques to determine PIHM significance. We provide Python code for implementing the Likelihood Method in *SI* 4.

As with all previously proposed methods for estimating PIHM, the Likelihood Method first assumes that prior to mortality the parasite distribution is described by a negative binomial $g(x; \mu_p, k_p)$, where μ_p and k_p are the mean parasite intensity and aggregation before mortality, respectively (smaller k_p indicates more aggregation). Previous methods required calculating the total number of hosts before mortality (N_p) (Crofton 1971; Adjei *et al.* 1986), however this parameter is not needed in the Likelihood Method.

The Likelihood Method then assumes that the host survival function, which specifies the probability of a host surviving with x parasites, follows the logistic curve given by

$$h(x; a, b) = \frac{e^{a - b\log(x)}}{1 + e^{a - b\log(x)}} \tag{1}$$

With these two explicit assumptions, the Likelihood Method estimates four parameters: μ_p, k_p, a, and b by first defining a probability distribution that gives the probability of having a parasite load of x parasites conditional on host survival. Using standard rules of conditional probability this distribution can be written as

$$P(x|\text{survival}) = \frac{P(\text{survival}|x) * P(x)}{P(\text{survival})} \tag{2}$$

One can see that $P(\text{survival}|x)$ is the survival function $h(x; a, b)$, $P(x)$ is the pre-mortality parasite distribution $g(x; \mu_p, k_p)$ and $P(\text{survival}) = \sum_{x=0}^{\infty} P(\text{survival}|x) * P(x) =$

$\sum_{x=0}^{\infty} h(x;a,b) * g(x;\mu_p,k_p)$. Therefore equation 2 can be written as

$$P(x|\text{survival}) = \frac{h(x;a,b) * g(x;\mu_p,k_p)}{\sum_{x=0}^{\infty} h(x;a,b) * g(x;\mu_p,k_p)} \tag{3}$$

Using this probability distribution, one can then find the parameters μ_p, k_p, a, and b that maximize the likelihood of an observed host-parasite dataset. The equation $\exp(a/b)$ can then be used to calculate the parasite LD_{50}, here defined as the infection intensity at which 50% of hosts experience PIHM.

To estimate the significance of PIHM in a host-parasite system, a likelihood ratio test is used in which the full model is given by equation 3 and the reduced model is given by a negative binomial distribution. If PIHM is not significant in the system, the resulting likelihood ratio statistic should approximately follow a χ^2 distribution with two degrees of freedom.

Evaluating the Adjei and Likelihood Methods

Question 1: Can we detect PIHM?

We tested the ability of the Adjei and the Likelihood Methods to identify the presence of PIHM on simulated data with known pre-mortality parameters. First, we created a pre-mortality host population by drawing N_p randomly infected hosts from a negative binomial distribution with parameters μ_p and k_p. Second, we chose values of a and b and calculated the probability of survival for all N_p hosts using equation 1. Then, for each host, we drew a random number from a uniform distribution between 0 and 1 and if the calculated host survival probability was less than this random number, the host experienced parasite-induced mortality. The parasite distribution in these simulated surviving hosts is equivalent to the observed parasite distribution in a wild host population that has undergone parasite-induced host mortality.

We used these simulated pre-mortality and post-mortality datasets to test the ability of both methods to correctly determine whether or not PIHM was occurring when

the parameters N_p, μ_p and k_p were known. For the Adjei Method, N_p, μ_p, and k_p are estimated using the Crofton Method, while μ_p and k_p in the Likelihood Method can be estimated jointly with a and b or via the Crofton Method. Although the parameters N_p, μ_p, and k_p are always unknown in real systems, a method that fails under these ideal simulation conditions will certainly also fail using less ideal, empirical data.

We used three different values of μ_p (10, 50, 100) and for each μ_p we examined three different survival functions that had graduate, moderate, and steep decreases in the host survival with increasing parasite intensity (Figure 1A). For a given μ_p, each survival function had the same LD_{50} ([$\mu_p = 10$, $LD_{50} = 7.39$], [$\mu_p = 50$, $LD_{50} = 35.57$], [$\mu_p = 100$, $LD_{50} = 77.3$]), but different values of a and b. We examined each μ_p-survival function pair at three levels of parasite aggregation, $k_p = 0.1$, 0.5, and 1 — realistic values of parasite aggregation in natural populations (Shaw *et al.* 1998). For each of these parameter combinations we simulated 150 datasets and tested the probability of each method correctly identifying PIHM in the post-mortality dataset (power) and incorrectly identifying PIHM in the pre-mortality dataset (Type I error). For each method, we used a likelihood ratio test to determine whether the full model with PIHM provided a significantly better fit than the reduced model without PIHM at significance level of 0.05. We tested each parameter combinations for pre-mortality population sizes of $N_p =$ [50, 100, 200, 300, 400, 500]. N_p is not technically the sample size on which the methods are being tested on the post-mortality data because PIHM reduces N_p for each simulated dataset. We therefore used the average number of surviving hosts over all 150 simulations for a given parameter combination as our measure of sample size in the power simulations.

Question 2: Can we estimate fatal parasite intensity and the host survival function?

To compare the ability of the Adjei Method and the Likelihood Method to recover the LD_{50} and the parameters a and b of the survival function, we used the same simulation procedure and parameter combinations described above. For each parameter combination we simulated 150 datasets, estimated a, b, and LD_{50} and calculated the standardized

bias and precision (Walther & Moore 2005) for these estimates over pre-mortality host population sizes of N_p = [300, 500, 1000, 2000, 5000, 7500, 10000]. We used the average number of surviving hosts over all 150 simulations for a given parameter combination as our measure of sample size. Because parameters a and b showed similar patterns of bias and precision, we only show the results for a.

Efficacy of the Likelihood Method with unknown pre-mortality parameters

In the final simulation, we test the ability of the Likelihood Method to correctly identify PIHM and estimate LD_{50} when the pre-mortality parameters are unknown. The previous simulations showed that the Likelihood Method effectively identified PIHM when μ_p and k_p were known with values of 10 and 1, respectively. In the best-case scenario where a host-parasite system has these these parameters, we test the power of the Likelihood Method to identify PIHM for gradual, moderate and steep survival functions when the pre-mortality parameters also needed to be estimated. We perform 500 simulations over a range of different samples sizes following the simulation procedure described above.

Application to real data

We tested the ability of the Adjei Method and the Likelihood Method to identify PIHM in 6 host-parasite datasets given in Crofton (1971) and 4 datasets given in Adjei *et al.* (1986) (Table 2). Crofton analyzed infection patterns in the snail *Gammarus pulex* infected with the acanthocephalan *Polmorphus minutus*. Adjei *et al.* analyzed males and females of two species of lizard fish *Saurida tumbil* and *S. undosquamis* that were infected by the cestode *Callitetrarhynchus gracilis*.

In both earlier studies, the authors reported PIHM in some of the datasets and we test whether the Adjei Method and/or the Likelihood Method also predicted PIHM. For the 6 datasets from Crofton (1971), we truncated the data at 4 parasites, applied the Crofton Method to estimate the pre-mortality distribution, and then ran the Likelihood

Method and Adjei Method using these pre-mortality parameters. For the Adjei *et al.* (1986) datasets, we followed the same procedure as the authors and first truncated the data at 2 parasites and then fit the Crofton Method for the female fish of both species. Then, following the original authors' methods, we parameterized the male pre-mortality distributions for each species with the results from the females. Finally, we applied the Adjei Method and the Likelihood Method to determine whether or not PIHM was significant for these species and compared our results to those given by the authors. All fitting to data was done with the code provided in *SI* 4.

Results

Question 1: Detecting presence of PIHM

The power of the Adjei Method to detect PIHM in a system was close to unity for larger sample sizes and tended to decrease as sample size decreased (Figure 1B; *SI* 3 Figs 1-3). The Likelihood Method had a power close to unity for all parameter combinations and sample sizes considered. With gradual survival functions, the power of the Likelihood Method decreased slightly for small samples sizes (Fig. 1B, *SI* 3 Figs 1-3).

The Adjei Method showed highly inflated Type I error rates (i.e. falsely detected PIHM) for all parameter combinations that we considered (Fig. 1A; *SI* 3 Figs 1-3). This method also showed the unintuitive pattern of Type I error rate decreasing as sample size decreased. This pattern was due to the issue of binning discussed in the *Introduction* and *SI* 2. For small samples sizes, the applicability of the Adjei Method is compromised without binning the observed data in some way. In contrast, the Likelihood Method showed a Type I error rate at or near the pre-set level of 0.05 for all parameter combinations and sample sizes considered (Fig. 1A; *SI* 3 Figs 1-3).

Question 2: Estimating the LD_{50} and survival function

The Likelihood Method gave asymptotically unbiased estimates of the LD_{50} for all combinations of parameters examined in this study (Fig. 2, *SI* 3 Figs 4-6). Even for the smallest sample sizes we considered, the Likelihood Method's estimate of LD_{50} was largely unbiased, with small biases occurring for host survival functions that were gradual. The precision of the Likelihood Method's LD_{50} estimates decreased (increasing coefficient of variation) as sample size decreased for all parameter combinations we examined (Fig 2, *SI* 3 Figs 4-6).

The Adjei Method produced biased estimates of the LD_{50} across nearly all parameter combinations (Fig 2, *SI* 3 Figs 4-6)). For $\mu_p = 10$, the LD_{50} estimates from the Adjei Method were largely unbiased for large samples sizes, but as μ_p increased, the Adjei Method produced biased estimates of LD_{50} across all sample sizes, with bias increasing as sample size decreased (Figure 2, SI2 Fig 4-6). The LD_{50} estimates from the Adjei Method showed large decreases in precision with the steepest survival function across all values of μ_p (Figure 2, SI2 Fig 4-6).

In terms of the host survival function, the Likelihood Method gave unbiased estimates of survival function parameters when sample sizes were large, however as sample size decreased these estimates became severely biased (Fig. 2, *SI* Fig 7 - 9) The Adjei Method produced biased estimates of the host survival function across all sample sizes, with the bias consistently being larger when the survival function was steeper and μ_p was larger (Fig 2, *SI* 3 Figs 7-9).

Detecting PIHM with unknown pre-mortality parameters

When all pre-mortality parameters were jointly estimated, the Likelihood Method had a power of greater than 0.8 when the survival function was moderate and steep for host sample sizes of 424 and 83 respectively (Figure 3). When the host survival function was gradual, the Likelihood Method never had a power greater than 0.8 for any post-mortality

samples sizes we considered.

Application to real data

Of the 10 datasets we considered, the previous authors visually detected PIHM in 7 of them (Table 2). The Likelihood Method parameterized from the pre-mortality parameters of the Crofton Method detected significant PIHM in 6 of these 7 datasets at a significance level of 0.05. The only dataset in which the Likelihood Method did not detect a significant effect of PIHM was the Adjei dataset for female *S. tumbil*. For this dataset there was a marginally significant effect of PIHM ($\chi^2_{df=2} = 5.34; p = 0.069$). The Adjei Method detected PIHM in 9 of the 10 datasets (Table 2), consistent with our simulation results that the Adjei Method has a high Type I error rate.

Discussion

Quantifying the impact of parasitism on wild host populations is critical for managing wildlife populations and understanding parasite transmission. Ideally the relationship between infection intensity and host survival would be measured experimentally, but for logistical and ethical reasons, this is often impossible (McCallum 2000). Looking for evidence of mortality in parasite distribution data requires the least amount of information, but is notoriously difficult to implement. The methodological flaws in the Adjei Method limit its utility, so here we propose an alternative, likelihood-based, method to estimate host survival and the LD_{50} from observed parasite intensity data. This method is a significant improvement over the previous methods because it requires fewer parameters, provides a statistical decision rule for identifying PIHM and does not require any data manipulation.

Using simulated data, we found that the Likelihood Method always out performed the Adjei Method. For simply detecting the presence of PIHM, the Likelihood Method

was both more powerful and had fewer false detection events (Type I errors). When both methods were applied to published datasets previously used in PIHM analyses, the Adjei Method tended to detect PIHM where it had not previously been reported, consistent with the high Type I error rate observed in our simulations. The Likelihood Method was also more precise and less biased in calculations of both the parasite LD_{50} and host survival curve over the parameter values we considered. However, while only the Likelihood Method produced precise and unbiased LD_{50} estimates, neither method could provide unbiased estimates of the host survival function at realistic sample sizes. These simulations demonstrate that the Likelihood Method is more powerful and precise than the previously propose Adjei Method.

Although superior to the Adjei Method, the Likelihood Method is not universally applicable to real data. Our simulations showed when the when pre- mortality parameters were estimated directly, the Likelihood Method needed at least 83-424 samples to have 80% power and for steep to moderate survival functions and even more as the survival function became more gradual. While some of these sample sizes are reasonable for hosts such as invertebrates or small fish, even the smallest sample sizes are completely unfeasible for many vertebrates, particularly the species of conservation concern where addressing the impact of parasitism would be most important. An even larger sample size would be required to identify PIHM when parasites are highly aggregated, mean infection intensity is high, or parasite prevalence is low, all of which are common in many parasitic helminths. Moreover, our results are in agreement with previous work that has shown that as host-survival functions become progressively more linear, PIHM becomes all but impossible to detect (Lanciani & Boyett 1989). This result, however, does not preclude the use of this method as non-linear survival functions are not uncommon in empirical host-parasite systems (Benesh 2011). Finally, while linear functions make PIHM undetectable, at the other extreme, steep, non-linear survival curves produce severely biased estimates of the survival function. Give the interaction between all of these different factors, the Likelihood Method is probably limited to detecting PIHM in systems where greater than 100 hosts

can be collected, parasites are common and only moderately aggregated, and substantial host mortality occurs at relatively low parasite intensity.

While we have improved on the existing methods for quantifying PIHM from parasite intensity data, all such methods require several fundamental, and potentially problematic assumptions. Nearly all current methods derive from Crofton (1971) (but see Ferguson *et al.* 2011) and assume that, prior to any PIHM, parasites are distributed in the host population following a negative binomial distribution. But, it is fundamentally impossible to know what the pre-mortality parasite distribution was in a wild host population and it is widely recognized that different processes can lead to a variety of parasite distributions in hosts (Anderson & Gordon 1982; Duerr *et al.* 2003). However, the negative binomial is extremely flexible and there is substantial empirical and theoretical evidence to support the assumption that, prior to any PIHM, parasite distributions can be fit by a negative binomial distribution (Shaw & Dobson 1995; Shaw *et al.* 1998; Wilson *et al.* 2002).

Unfortunately, this flexibility in the distribution may also reduce our ability to detect PIHM. If a negative binomial can be fit to the observed post-mortality parasite distribution then, regardless of how lethal the parasite was, it will be impossible to detect PIHM because there is no need for a more complex model. Most observed parasite distributions are well fit by the negative binomial distribution (Shaw *et al.* 1998), suggesting that systems where these methods are applicable may be more the exception than the rule. Furthermore, even when truncation of the negative binomial distribution is detected, it may be caused by other processes such as within host density dependence, age dependent variation in host resistance and/or heterogeneous infection rates (McCallum 2000; Anderson & Gordon 1982; Rousset *et al.* 1996). This means that in the event that PIHM is detected, it may actually not be the result of PIHM.

Given these numerous caveats, is there a place in parasitology for methods that estimate PIHM from intensity data? We are in agreement with Lester (1984) that, at the very least, methods for estimating PIHM can provide preliminary insight into whether

or not PIHM is worth further exploration. However, we stress that these methods should only be used as an exploratory tool when assessing the role of PIHM in a system and potential users should critically evaluate whether they think they have a large enough sample size and an appropriate host survival function/post-mortality distribution for the methods developed in this paper to be applicable. Even if they are applicable, inferring PIHM from distributional data is no substitute for field experiments and an in depth understanding of the natural history of the host-parasite system under consideration.

Acknowledgments

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Table 1: Definition of parameters and functions used in the main text

Parameter	Definition
μ_p	Pre-mortality mean parasite intensity
k_p	Pre-mortality parasite aggregation
N_p	Pre-mortality host population size
x	Number of parasites in a given host
$g(x;\mu_p,k_p)$	Pre-mortality negative binomial distribution parasites distribution
a	Parameter of the logistic host survival function
b	Parameter of the logistic host survival function
$h(x;a,b)$	The host survival function
LD_{50}	$\exp(a/b)$, Parasite intensity at which 50% of hosts die

Table 2: Comparison of the PIHM predictions of previously used host-parasite datasets to those given by the Adjei Method and the Likelihood Method. The first column specifies the identity of the dataset, the second column specifies whether or not the author indicated that PIHM was occurring in the system, the third column indicates whether or not the Likelihood Method with pre-mortality parameters estimated from the Crofton Method detects significant PIHM, and the final column indicates whether the Adjei Method with pre-mortality parameters estimated from the Crofton Method detects PIHM. If a method detected significant PIHM the predicted LD_{50} is given in parentheses

Data Set (sample size)	Author detected PIHM?	Likelihood Method?	Adjei Method?
Crofton, Station 1 ($n = 538$)	Yes	Yes (7.27)	Yes (9.33)
Crofton, Station 2 ($n = 507$)	Yes	Yes (6.92)	Yes (14.95)
Crofton, Station 3 ($n = 633$)	Yes	Yes (5.93)	Yes (5.98)
Crofton, Station 4 ($n = 486$)	No	No	Yes (7.99)
Crofton, Station 5 ($n = 276$)	No	No	Yes (10.58)
Crofton, Station 6 ($n = 191$)	No	No	No
Adjei, *S. tumbil* female ($n = 446$)	Yes (5.7)	No	Yes (6.37)
Adjei, *S. tumbil* male ($n = 452$)	Yes (3.4)	Yes (3.42)	Yes (3.66)
Adjei, *S. undosquamis* female ($n = 2573$)	Yes (3.2)	Yes (3.04)	Yes (3.11)
Adjei, *S. undosquamis* male ($n = 2440$)	Yes (1.8)	Yes (1.83)	Yes (1.78)

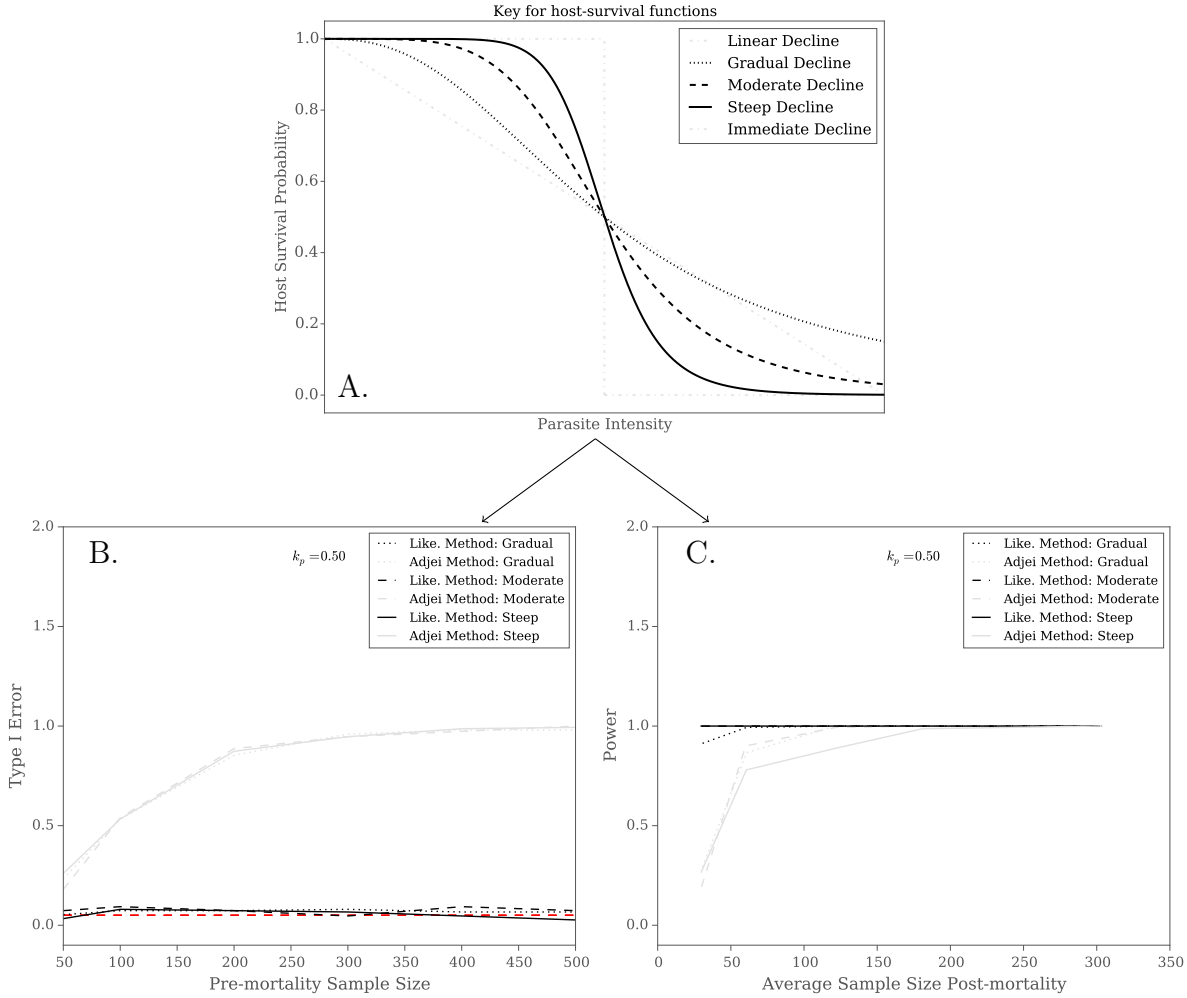

Figure 1: A) Five potential shapes for a host-survival functions. In the simulations we used a gradual survival function (dotted line), and moderate survival function (dashed line), and a steep survival function (solid line). The linear and immediate survival functions represent two potential extremes that we do not include in the simulations. For each of these survival functions and the parameter combinations described in the main text, we tested the Type I Error and Power of the Likelihood Method and Adjei Method. B) Gives the Type I Error of each method over a range of pre-mortality sample sizes with a pre-mortality mean parasite intensity (μ_p) of 50 and pre-mortality parasite aggregation (k_p) at 0.5. The red line shows the pre-set significance level of 0.05. C) Gives the Power of each method for detecting PIHM over a range of post-mortality sample sizes for $\mu_p = 50$ and $k_p = 0.5$. In general, the Likelihood Method has higher power and lower Type I Error than the Adjei Method. See the SI 3 Fig 1 - 3 for Type I Error and Power results for all parameter combinations.

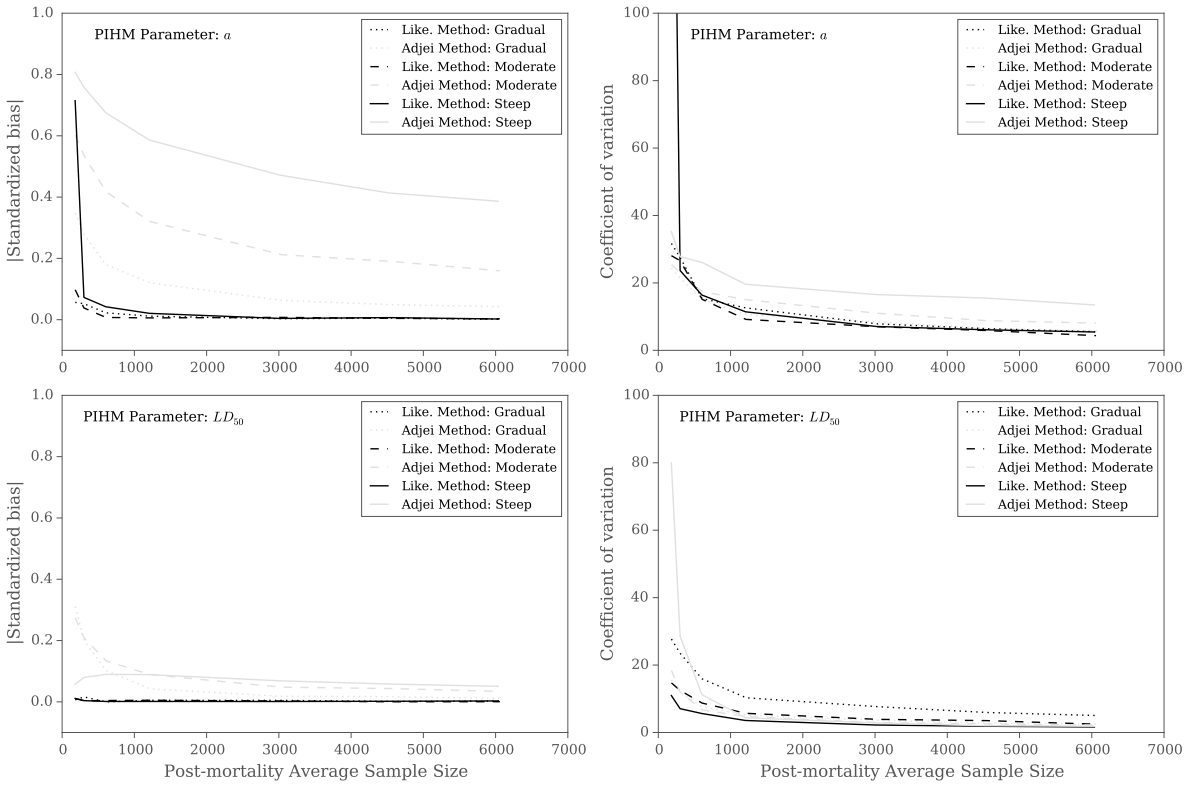

Figure 2: Bias and precision (coefficient of variation) for the Likelihood Method and Adjei Method estimates of the a parameter and the LD_{50} of the host survival function based on simulated PIHM data over a range of post-mortality sample sizes. The pre-mortality parameters for this simulation were $\mu_p = 50$ and $k_p = 0.5$. The figure shows the simulations for three different host survival functions (gradual, moderate, and steep), each with the same LD_{50}. Bias and precision results of LD_{50} and a for all other parameter combinations can be found in SI 3 Fig 4 - 9.

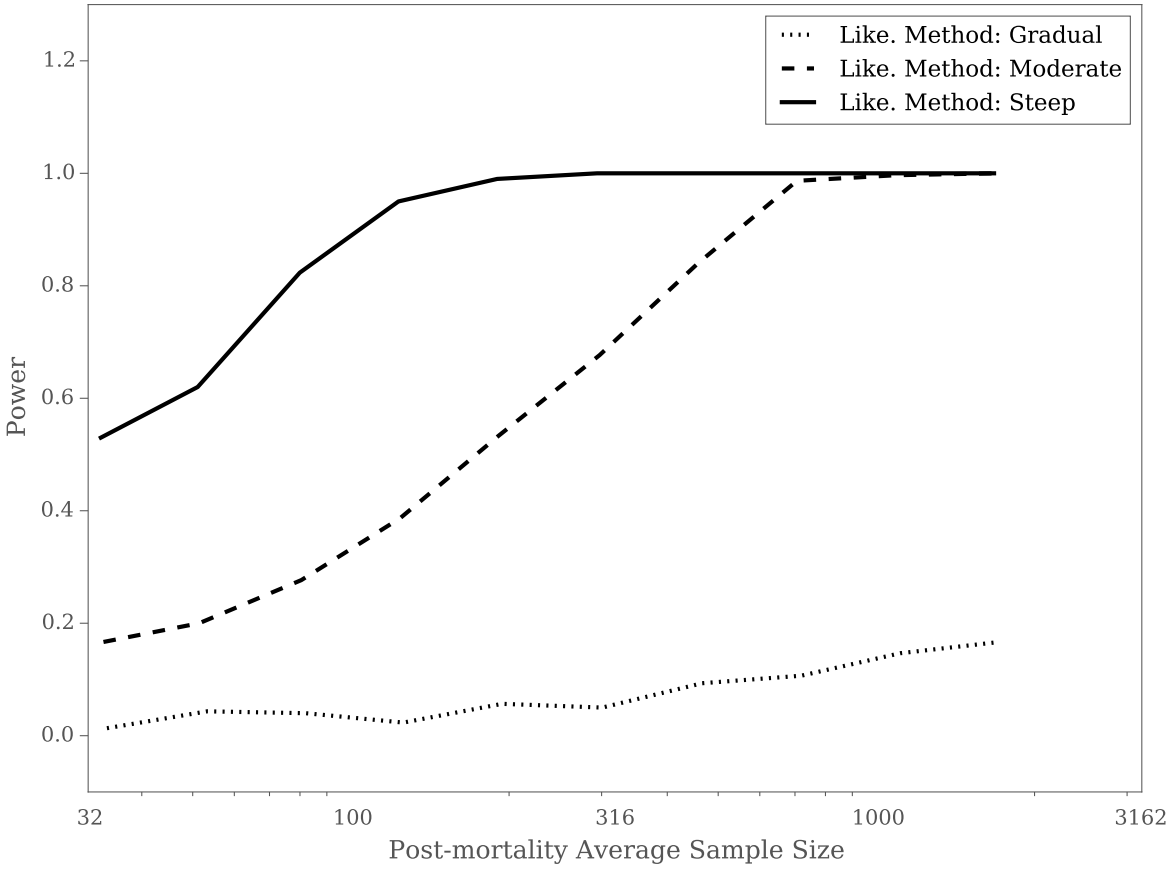

Figure 3: The power of the Likelihood Method to detect PIHM for gradual, moderate, and steep survival functions when all four parameters μ_p, k_p, a, and b were jointly estimated. The curves were generated from 500 simulations for 10 pre-mortality population sizes, N_p.