Estimating Reservoir Competence of *Borrelia burgdorferi* Hosts: Prevalence and Infectivity, Sensitivity, and Specificity

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**ABSTRACT** Most vector-borne zoonotic pathogens are transmitted among several host species, but different species vary considerably in their importance to pathogen transmission, at least partially because they vary in their propensity to infect feeding vectors. This propensity is often called realized reservoir competence. Realized reservoir competence is the product of (1) the probability the individual host is infected, i.e., infection prevalence, and (2) the probability that if the host is infected, it will transmit the infection to a feeding vector, or infectivity. Prevalence varies in space and time, whereas infectivity may be a property of the host species. Both prevalence and infectivity are ecologically and epidemiologically important, but measuring them simultaneously is difficult. We present a probabilistic model that separately estimates host infection prevalence and infectivity from data on the infection status of vectors collected from individual hosts, data generally used to measure realized reservoir competence. We then consider how imperfect diagnostic tests (i.e., false negatives and positives) influence these probabilities—estimates of prevalence and infectivity are fairly robust to false negatives, but not to false positives. We thus extend the model to estimate the rate of false positives in order to improve estimates of prevalence and infectivity. We illustrate these methods by reanalyzing data from LoGuidice et al. (2003; Proc. Natl. Acad. Sci. U.S.A. 100: 567–571) on the reservoir competence of ten vertebrate hosts of *Borrelia burgdorferi*, the agent of Lyme disease. We find that these vertebrate hosts vary both in prevalence and infectivity and that both values are highly, positively correlated among species.

**KEY WORDS** reservoir competence, prevalence, infectivity, *Borrelia burgdorferi*, *Ixodes scapularis*

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reservoir competence (Fig. 1). These studies produce an ecologically relevant metric, but they are difficult to generalize from because realized reservoir competence is partially determined by prevalence, which varies in space and time.

The second approach is to first experimentally infect hosts and then, after a given interval, feed xenodiagnostic ticks on these known-infected hosts (e.g., Markowski et al. 1998). These ticks are similarly tested for B. burgdorferi infection to provide a direct measure of the probability an infected host will transmit B. burgdorferi to a feeding tick (infectivity). Measured values of infectivity reflect host physiology, immunological response(s), and other factors that determine host permissiveness to B. burgdorferi. It seems reasonable to assume that infectivity is consistent among members of a species or, in the face of host immune responses, that it at least follows a consistent temporal trend. Intraspecific consistency makes this method useful for comparing the relative potential importance of different species to B. burgdorferi transmission. However, high estimates of infectivity when hosts are artificially inoculated cannot demonstrate that individuals of a given species play an important role in pathogen transmission in nature. To establish species-specific roles in natural B. burgdorferi transmission, one also must estimate natural infection prevalence in each host species.

Unfortunately, the diagnostic tests available for detecting B. burgdorferi in vertebrate tissues are not very sensitive, although improving (Eisendle et al. 2007, Wilske et al. 2007) and are thus liable to underestimate host infection prevalence. Serological tests only document exposure to a pathogen, not infection. The tests for tick infection status are thought to be better, although to our knowledge, no published information exists on sensitivity or specificity of any of the several commonly used diagnostic tests of B. burgdorferi in ticks—dark field microscopy (Junttila et al. 1999, Richter et al. 2000), direct fluorescent antibody assays, enzyme-linked immunosorbent assays (Burkot et al. 1994), and diagnostic and quantitative polymerase chain reaction (PCR) targeting the flagellin gene (Johnson et al. 1992, Schmidt et al. 1996, Junttila et al. 1999, Zeidner et al. 2001, Soares et al. 2006), the recA gene (Mommert et al. 2001, Wang et al. 2003), the p66 gene (Mommert et al. 2001), the outer surface protein A gene (Persing et al. 1990), and several targets among the ribosomal genes (Kurtenbach et al. 1998, Kurtenbach et al. 2002, Layfield and Guilfoile 2002, Courtney et al. 2004, Schulze et al. 2005, Ornstein and Barbour 2006). Although several methods (e.g., quantitative real time PCR; Courtney et al. 2004, Ornstein and Barbour 2006) have been shown to detect vanishingly small amounts of bacteria from culture, it is not clear that this analytic sensitivity translates into the ability to correctly identify infected ticks (sensitivity in the epidemiological sense). Moreover, without a "gold standard" for B. burgdorferi infection, it has been impossible to estimate rates of false positives (imperfect specificity). Even a few false negative or false positive host test results could negatively or positively bias estimates of prevalence, particularly when sample sizes are low because of difficulties sampling rare and/or trap-shy species. Similarly, falsely negative or positive ticks would tend to depress or inflate estimates of infectivity. Thus, in addition to the methodological difficulties producing ecologically relevant measures of host prevalence and infectivity, it seems
inevitable that inaccurate diagnostic tests will bias these estimates.

Our purpose in this article is to provide a method for estimating the contributions of different host species to disease transmission or risk by separating the effects of prevalence and infectivity, and a means of accounting for imperfect diagnostic tests. We present a probabilistic model that uses infection data on fed ticks (or other vectors) from field-caught animals to decompose estimates of realized reservoir competence into their component parts: prevalence and infectivity (Fig. 1). We reanalyze data from LoGiudice et al. (2003) on the realized reservoir competence of 10 species (or groups of species in some cases) to estimate the relative importance of prevalence and infectivity in determining ecological roles of host species in producing infected ticks. We then show how imperfect diagnostic tests of infection, particularly false positives, can bias metrics of reservoir competence, and then how false positive rates can be estimated from multispecies data to improve estimates of prevalence and infectivity.

Materials and Methods

Realized Reservoir Competence Data. LoGiudice et al. (2003) captured several \( n = 4-51 \) individuals of each of 10 vertebrate species (Table 1) from wild populations, brought them into the laboratory for 2–3 d to collect the ticks feeding on each animal after they had finished their bloodmeal. These ticks were allowed to molt and then screened for \( B. burgdorferi \) infection by using a direct immunofluorescent assay. Realized reservoir competence was then estimated as the number of positive ticks from an individual host divided by the number of ticks tested from that individual, averaged over all individuals of a species. If the number of ticks tested is the same for each animal, this amounts to dividing the number of positive ticks by the number of ticks tested. As is often the case for vector burdens on individual hosts (e.g., Woolhouse et al. 1997), the number of ticks successfully recovered, molted, and tested from each animal varied considerably, and was often just one or two. LoGiudice et al. (2003) reasoned that individual hosts from which fewer than five ticks were recovered and tested would unacceptably bias realized reservoir competence estimates, so these individuals were excluded from analyses. In addition to this loss of data, the relative importance of prevalence and infectivity to these estimates cannot be resolved. The problem is that it is impossible to determine whether an individual host is infected when all of the ticks collected from it tested negative. This problem, of course, becomes worse when there are fewer and fewer ticks collected from each individual. If we take a probabilistic approach, however, we can use all of the data available, even individuals from which a single tick is tested, and simultaneously estimate both prevalence and infectivity.

Statistical Analyses. We begin with the simplest case where we make the assumption that the test for \( B. burgdorferi \) is perfect, that is, it produces no false negatives or false positives (we relax this assumption below). We then simply need to understand the ways in which a tick can be infected by an individual host of a given species or remain uninfected (dashed box in Fig. 2). The probability that a single tick is positive is \( \pi \phi \), where \( \pi \) is the probability the host is infected (prevalence) and \( \phi \) is the probability that the individual host infects the tick, given that the host is infected (infectivity). There are two paths that can lead to an uninfected tick. The tick might feed on an infected host but remain uninfected, with probability \( \pi (1 - \phi) \), or its host might be uninfected, which occurs at probability \((1 - \pi)\). The sum of these two paths is the probability of observing a single uninfected tick.

Often, however, many ticks feed on a host at the same time. Of these \( n \) feeding ticks, \( k \) of them are infected, where \( k \) can range from 0 to \( n \). We can specify the probability that \( k \) of \( n \) ticks feeding on a given host
are infected in terms of prevalence ($\pi$) and infectivity ($\phi$) with the binomial as

$$
\pi \binom{n_i}{k_i} \phi^k (1 - \phi)^{n_i - k_i} + (1 - \pi) \binom{n_i}{k_i} 0^k 1^{n_i - k_i}.
$$

If any of the $n$ ticks are infected (i.e., $k \geq 1$), the term to the right of the plus sign collapses to zero because the host must be infected in order for the tick to have been infected. If none of the $n$ ticks are infected ($k = 0$), then either the host was infected but did not transmit the infection, which is described by the term to the left of the plus sign, or it was uninfected, described by the term on the right. When $k = 0$ we cannot establish whether or not the host was infected and so the probabilities of both events must be taken into account.

Prevalence is a population-level parameter, specific to a given population of a particular host species. Infectivity, too, may be viewed as a population—if not species—specific constant, as discussed above. To estimate the population-level values of $\pi$ and $\phi$ for a given species we simply find the values of each that maximize the likelihood of observing the $k_i$ of $n_i$ B. burgdorferi-infected ticks on each host, $i$, for $i = 1, 2, 3, \ldots, N$, summed over all of the $N$ hosts in the sample. The likelihood of the parameter estimates of $\pi$ and $\phi$ given the data are

$$
\mathcal{L}(\pi, \phi \mid \text{data}) = \sum_{i=1}^{N} \left[ \pi \binom{n_i}{k_i} \phi^k (1 - \phi)^{n_i - k_i} + (1 - \pi) \binom{n_i}{k_i} 0^k 1^{n_i - k_i} \right]. \quad [1]
$$

Again, the second term on the right-hand side of equation 1 is zero if any of the ticks on host $i$ are positive. In the appendix we provide code for finding the maximum likelihood estimates of $\pi$ and $\phi$ using R (R Development Core Team 2005).

This method of estimating prevalence and infectivity from infection data for fed ticks makes two important assumptions. First, we assume all larval ticks feeding on hosts are uninfected. Previous studies have found little or no vertical transmission of B. burgdorferi (Piesman et al. 1986, Patrican 1997), supporting this assumption. Second, we assume that the diagnostic test is perfectly specific and sensitive, that is, there are no false positives or false negatives, respectively. If these values were known, they could be incorporated into the likelihood. As noted in the introduction, without a gold standard it is not possible to assess rates of false negatives and false positives. We therefore investigated the effects of false negative and false positive tests of tick infection status on estimates of prevalence and infectivity by simulating 1,000 “data sets” at each of nine levels of sensitivity (Fig. 3a and c) with specificity held constant at 1, and at nine levels of specificity (Fig. 3b and d), with sensitivity held constant at 1, for a total of 18,000 data sets. Each data set consisted of 20 hosts, each of which was randomly “infected” (or not) with probability = Prevalence. The 20 hosts were then assigned burdens of feeding vectors ($V$) by random draws from a Poisson distribution with mean = 10. The infection status of these feeding vectors was then determined by random draws from a binomial distribution with $n = V$ trials and the probability of success (i.e., infection) in each trial equal to Infectivity $\times$ Sensitivity + (1 - Infectivity) (1 - Specificity) if the host was infected and (1 - Specificity) if the host was not. We then estimated the parameters $\pi$ and $\phi$ for each data set by using equation 1 and compared these estimates with the “true” values. The importance of imperfect sensitivity and specificity was explored with several levels of true Prevalence and Infectivity, but we present only the results for Prevalence and Infectivity = 0.5.

Studies of realized reservoir competence often gather data from multiple host species at the same time. If we assume that false positives are a product of the diagnostic test rather than specific to a given host species, we can then use such multihost species data sets to estimate a common test specificity ($= 1 - \text{rate of false positives}$). This assumption seems reasonable, particularly when the fed larvae are allowed to digest the host bloodmeal and molt, retaining little if any host-specific factors that might interfere with the diagnostic test, as was the case in the LoGiudice et al. (2003) data.

The logic to estimating specificity in addition to prevalence and infectivity is the same as in the simple case above except that now we 1) estimate species-specific prevalence and infectivity parameters $\pi_i$ and $\phi_i$ from a data set of infection status of ticks fed on several different species; and 2) there is now a common diagnostic test-specific parameter,
Sp, that allows for some false positives. The probability that a tick that fed on host species j (for species j = 1, 2, 3, … S) becomes infected and tests positive is \( \pi_j \phi_j \), again assuming the test is perfectly sensitive, although estimates are fairly robust to violations of this assumption (see Fig. 3). The probability that a tick that feeds on an infected individual of host species j, does not become infected, but still tests positive is \( \pi_j (1 - \phi_j) (1 - Sp) \). Lastly, the probability that a tick feeds on an uninfected host of species j and still tests positive is \( (1 - \pi_j) (1 - Sp) \). The likelihood becomes

\[
\mathcal{L}(\pi_j, \phi_j, Sp \mid data) = \\
\sum_j \sum_k \sum_{i=1}^{N} \left[ \pi_j (\frac{n_{ij}}{k_{ij}}) [\phi_j + (1 - \phi_j)(1 - Sp)]^{k_i} \times [(1 - \phi_j) - (1 - \phi_j)(1 - Sp)]^{n_i - k_i} + (1 - \pi_j) \times \left( \frac{n_{ij}}{k_{ij}} \right) [1 - Sp]^{k_i} Sp^{n_i - k_i} \right].
\]

[2]

Results

The maximum likelihood estimates of prevalence and infectivity, and their product, realized reservoir competence, vary considerably among species (Table 1). Some species tend not to be infected (e.g., white-tailed deer and striped skunks), whereas others are frequently infected (e.g., white-footed mice, eastern chipmunks). Species vary even more in terms of infectivity. White-footed mice have a tremendous propensity to transmit B. burgdorferi to feeding ticks, whereas all others species have intermediate (e.g., eastern chipmunks and shrews) or low levels of infectivity (e.g., white-tailed deer and squirrels), which is in general agreement with previous studies (Donahue et al. 1987, Mather and Mather 1990, Mather et al. 1990, Telford et al. 1990, Norris et al. 1996, Ouellette et al. 1997, LoGiudice et al. 2003, Ullmann et al. 2003, but see Lane et al. 2005). The actual parameter estimates, however, can be strongly biased if we do not account for imperfections in the diagnostic test of tick infection status.

The simulation results demonstrated that false negatives are not as great a problem as false positives. Estimates of prevalence and infectivity were fairly robust to imperfectly sensitive diagnostic tests. The bulk of prevalence estimates clustered around the true value with as many as one in five positive vectors testing negative (Sensitivity = 0.8; Fig. 3a). (It is worth noting that the apparently wide range of prevalence estimates is a result of the simulation methods. Each simulated sample was drawn from a population with true prevalence = 0.5, but the actual prevalence within the sample varied. Simply scoring individual hosts as infected or not based on whether any ticks tested positive would have yielded similar levels of variability.) Infectivity became biased slightly low only when rates exceeded one in 40 false negatives (Sensitivity = 0.975; Fig. 3c).

These estimates are much more sensitive to imperfect specificity (false positives; Fig. 3b and d). Esti-
imated prevalence is biased high when the false positive rate exceeds \( \approx 1 \) in 100 (Specificity = 0.99) and become wholly inaccurate by one in 20 (Specificity = 0.95). Essentially false positives cause uninfected hosts to seem positive—just one positive vector is evidence that the host it fed upon was infected. As such, the apparent prevalence is biased high. Conversely, infectivity is biased low with increasing rates of false positives. Falsely positive hosts do not, of course, transmit the (apparent) infection to many vectors, so only one or two of potentially many ticks test positive. These rare “transmission events” seem to be the result of low infectivity and thus diminish estimates of \( \phi \). In essence, it becomes impossible to distinguish whether a host species is frequently infected, but rarely transmits the infection to feeding vectors or whether vectors feeding on uninfected hosts falsely test positive.

These biases are most pronounced when true prevalence is low and true infectivity is high (simulation results not shown). (In the case where false positives rates are higher than actual infectivity, the estimate of \( \phi \) will instead be biased upward.)

Incorporating false positives into our analyses of the LoGiudice et al. (2003) data dramatically improved our model fit (\( \Delta \text{Akaiake’s Information Criterion adjusted for small sample size [AICc] = 250} \)), supporting the conclusion that the direct immunofluorescent assay is not perfectly specific. We estimate specificity at \( \approx 0.97 \), meaning that three of 100 negative ticks are incorrectly scored as positive. Allowing for these false positives had the effect of reducing estimates of prevalence for almost all species, and estimates of infectivity generally increased. For two species, raccoons and opossums, the estimates are uninformative (Table 1). Individuals of these two species tended to have a single tick test positive; thus, they seemed to have high prevalence and low infectivity. With an imperfectly specific diagnostic test, however, these results may have instead been due to false positives. This suggests a limit to our ability to estimate prevalence and infectivity when realized reservoir competence converges on the estimated false positive rate (1 – Specificity). An experimental infection study of raccoons found that infectivity is, in fact, at or very near zero (Norris et al. 1996).

Discussion

Realized reservoir competence—the chance that a tick feeding on a particular free-ranging host species will become infected—is an ecologically and epidemiologically relevant metric. Species vary considerably in their realized reservoir competence (e.g., LoGiudice et al. 2003; Table 1), which in turn strongly influences the dynamics of and risk from \( B. \) burgdorferi. We can gain insights into why species vary in their ability to infect larval ticks by decomposing realized reservoir competence into prevalence and infectivity.

Mammal species have a wide-range of levels of \( B. \) burgdorferi prevalence (Table 1). This may reflect differences in exposure to infected nymphs (but see Mather et al. 1989) or species-specific differences in susceptibility to \( B. \) burgdorferi infection. Low infection prevalence in some host species, such as white-tailed deer and striped skunks, that likely are repeatedly inoculated by infected nymphs (Magnarelli et al. 2004) suggest an innate immunity to \( B. \) burgdorferi. Immunity based on the complement pathway has been described for mule deer (\( Odocoileus hemionus \)) and western fence lizards (\( Sceloporus occidentalis \)) (Ullmann et al. 2003). What is clear is that not all species are equally infected. Nor are they equally infectious—we found considerable variability in infectivity. Infected white-footed mice are very likely to transmit \( B. \) burgdorferi to feeding larvae, whereas other hosts, such as squirrels, are generally dead-end hosts for the bacterium. Thus, even if infection prevalence is extremely high for all species, community composition will still be important to overall incidence of Lyme disease (LoGiudice et al. 2003; Keesing et al. 2006).

Interestingly, prevalence and infectivity are strongly positively correlated across the host species (\( r = 0.88, t_{\text{ cfg }} = 4.1, P = 0.009 \)). This correlation suggests that both quantities are influenced by inherent physiological, likely immunological, features of each host, or by their rate of exposure to infected nymphs. Whatever keeps hosts infected and bacteria-mia high in the host—low rates of clearance by the immune system or high rates of (re)infection—will tend to increase the probability that the host transmits the infection to feeding larvae. Put another way, a cleared infection cannot be transmitted. The ability of infected white-footed mice to infect larval ticks decays with time, since infection (Donahue et al. 1987, Shih et al. 1995, Lindsay et al. 1997), although infections are not necessarily cleared (Hofmeister et al. 1999). The infectivity of meadow voles (Markowski et al. 1998, Anderson et al. 2006) and rice rats (Levin et al. 1995) also declines with time, suggesting a common pattern. Moreover, infectivity seems to differ between geographic locations (e.g., our estimate of \( \phi \approx 0.9 \) for white-footed mice in New York, compared with a maximal estimate of \( \phi \approx 0.3 \) in Ontario; Lindsay et al. 1997), in a way that is at least broadly consistent with differences in exposure to infected ticks. Thus, we would suggest that studies examining both the duration and dose-dependence of infectivity across species are likely to be fruitful and important.

We also must stress the importance of considering the accuracy of the diagnostic test. If we naively assumed that the direct fluorescent antibody (DFA) test correctly identified all negative ticks (i.e., no false positives), our estimates of prevalence would have been artificially high and our estimates of infectivity artificially low (Table 1). Indeed, when we allow for false positives we find that we cannot distinguish between low rates of transmission to ticks (low infectivity) and falsely positive test results for two species (i.e., raccoons and opossums). False positives are not unique to DFA, nor is the bias in parameter estimates unique to this likelihood-based method of estimation. Rather, these results demonstrate the limits of our knowledge when dealing with imperfectly specific tests. They also highlight an urgent need for data on the specificity of diagnostic tests. In the absence of such data, we urge scientists to be conservative when scoring infection status. It is encouraging to note, however, that if
the true test specificity is known and included in the likelihood calculations this bias is erased (Fig. 3b and d, dashed lines).

Last, we would like to emphasize that our reanalyses of the data of LoGiudice et al. (2003) strongly support their conclusions: different species have the potential to play dramatically different roles in *B. burgdorferi* transmission and Lyme disease risk. We find in general that those species most often infected are also the species with the greatest propensity to infect naïve larvae. It will be enlightening to see how widely this pattern holds in other vector-borne disease systems. The likelihood-based technique we presented should help researchers address this question.

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Appendix

R Code for Estimating Prevalence and Infectivity. We begin with the simple case, where there are no false positives. In this case we can estimate prevalence (π) and infectivity (ϕ) from data on a single species. The function EstPrevInf() calculates a vector of probabilities of observing Pos-infected vectors out of Tested vectors tested on each individual host, given P = π and I = ϕ.

EstPrevInf = function(P, I, Pos, Tested) {
  LL = log(P * dbinom(Pos, Tested, prob = I)) + (1 - P) * dbinom(Pos, Tested, prob = 0))
  #to prevent the function from #returning -inf, which causes #errors in the optimization #routine, those rows with -inf #are replaced with a very low value
  LL[is.infinite(LL)] = -99999
  return(LL)
}

We then find the values of P = π and I = ϕ that maximize the log likelihood of EstPrevInf(). For simple problems with few parameters, the optim() function of R is sufficient, but for more complex optimization problems we use a simulated annealing routine (Goffe et al. 1994) implemented in R (R Development Core Team 2005) by L. Murphy and C. Canham found in the Likelihood package at http://www.ecostudies.org/lme_R_code_tutorials.html. This routine, anneal(), also has the advantage of calculating support intervals on the parameter estimates. It sends a vector of predictions to a probability density function to calculate the probability of observing each. Because our predictions are actually probabilities, we use PDF . wrapper() to accept and send back these probabilities.

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The anneal() function also requires a list of initial parameter estimates, pars, and lists of high (par_hi) and low (par_lo) boundaries for these parameters.

\[
\text{pars} = \text{list}(P = 0.5, I = 0.5, \text{Pos} = "\text{Pos}", \text{Tested} = "\text{Tested}", \text{predicted} = "\text{predicted}"
\]
\[
\text{par_lo} = \text{list}(P = 0, I = 0) \\
\text{par_hi} = \text{list}(P = 1, I = 1)
\]

The actual data (e.g., ExampleData) should be a data frame where each row refers to an individual animal and one column has the number of vectors Tested and another has number that test Pos.

\[
\text{ExampleData} = \text{data.frame(cbind(}
\text{Tested} = \text{c(3, 10, 25, 16, 15, 12, 7, 3, 3, 2),}
\text{Pos} = \text{c(3, 0, 13, 13, 8, 10, 7, 5, 0, 0, 0))}
\]

We then call the simulated annealing optimization routine with the command:

\[
\text{Results}\_\text{anneal} = \text{anneal(model = EstPrevInf,}
\text{par = pars, source_data =}
\text{ExampleData, pdf = PDF\.wrapper,}
\text{par_lo, par_hi, dep_var =}
\text{"Pos", initial_temp = 1}
\]

The parameter values of Results\_best\_pars should be \( P = 0.661 \) and \( I = 0.689 \), and the AICc found in Results\_aic\_corr, should equal 48.48.

In the case where specificity is allowed to vary, we need to estimate its value across multiple species. The code is only slightly more complex, representing the \( \pi_j \) and \( d_j \) parameters for each of the \( j \) species. Here, we present a simple example for just three species.

\[
\text{EstPrevInfSpec} = \text{function(I1, P1, I2, P2, I3, P3, Spec, Pos, Tested, Spp)}
\]

\[
\text{LL} = \text{numeric(length(Pos))}
\]

The parameter lists include more parameters, but are otherwise the same:

\[
\text{pars} = \text{list(I1 = 0.5, P1 = 0.5, I2 = 0.5, P2 = 0.5, I3 = 0.5, P3 = 0.5, Spec = 0.5, Pos = "Pos", Tested = "Tested", Spp = "Spp", predicted = "predicted")}
\]

The data are also organized very similarly. There is just one additional column of data with the species names (here, species One, Two, and Three):

\[
\text{MultiSppData} = \text{data.frame(cbind(}
\text{Tested} = \text{c(3, 10, 25, 16, 15, 12, 7, 3, 2, 15, 16, 22, 25, 18, 18, 16, 21, 23, 5, 9, 10, 12, 13, 12, 8, 9, 17, 13, 6, 5, 16, 3, 1, 1),}
\text{Pos} = \text{c(3, 0, 13, 13, 8, 10, 7, 5, 0, 0, 1, 5, 1, 6, 0, 2, 4, 3, 0, 0, 0, 1, 2, 1, 1, 7, 6, 6, 7, 4, 3, 4, 7, 2, 0, 1))}
\]

The resulting solution should have an AICc of 154.58, with parameters: I1 = 0.674, P1 = 0.737, I2 = 0.191, P2 = 0.554, I3 = 0.505, P3 = 0.827, and Spec = 0.970.