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A brief guide to *Quantitative Parasitology 3.0*

1. Introduction

Parasite individuals typically exhibit an aggregated (right-skewed) distribution among host individuals; most hosts harbour few if any parasites and a few hosts harbour most of them. This quantitative feature of parasitism renders many of traditional statistical methods obsolete and requires the use of advanced computer-intensive statistical methods. The distribution of parasites within a sample of hosts inherently exhibits a complex pattern that cannot be adequately quantified by a single statistical measure. Thus a single statistical test cannot reliably compare infections across different samples of hosts.

What statistical measures to apply for descriptions and comparisons? A few of the available statistical measures have markedly different biological interpretations, while others have more-or-less overlapping interpretations or no interpretations at all. We recommend applying measures that have clear and separate biological interpretations.

2. How to describe the parasitic infection of a sample of hosts

2.1. Always give the host sample size. In most cases, this is expressed as the number of host individuals examined. However, other meaning units may also be used, say, the number of host nests, the number of host faeces samples examined etc.

2.2. Describe prevalence. This is the proportion of infected hosts among all the hosts examined. Give the confidence interval (CI) of prevalence. QP3.0 offers two alternative methods for this purpose. This measure indicates how accurately the sample estimate of prevalence characterise the true population prevalence. It is advisable to use CIs belonging to the 95% probability in most cases.

2.3. Describe mean intensity. This is the mean number of parasite individuals found in infected hosts – the zero valus of uninfected hosts are excluded. Since sample size and prevalence are known, mean intensity defines the quantity of parasites found in a particular sample of hosts. Given the typical aggregated distribution of parasites, its actual value is highly dependent on a few extremely infected hosts. Provide the CI of mean intesity to indicate the accuracy of the estimation.

2.4. Describe median intensity. This is the median number of parasites found in infected hosts, again, the zeros of uninfected hosts are excluded. Median intensity shows a typical level of infection among the infected hosts. This measure is not affected by the few highly infected host individuals. Provide CI to indicate the accuracy of the estimation.

2.5. In particular cases one may prefer to use mean abundance instead of mean intensity. This is the mean number of parasites found in all hosts, involving the zero values of uninfected hosts as well. Provide its CI to indicate the accuracy of the estimation. Keep in mind that this measure unites two of the former ones: prevalence and mean intensity.

2.6. Crowding equals intensity as interpreted from the parasites' point of view. Thus intensity is averaged above host individuals, while crowding is averaged above parasite individuals. (For intensity values 1 and 3, mean intesity is (1+3)/2=2, while mean crowding is (1+3+3+3)/4=2.5.) Describing mean crowding and its CI is essential only when studying density-dependent characters of parasites.

2.7. Finally, it can be useful to quantify the skewness of parasite distributions. There are 3 indices widely used for this purpose; the variance-to-mean ratio, the exponent k of the negative binomial, and the index of discrepancy. Their interpretation is quite similar, they predict each other rather well and thus there is no need to provide each of them.

3. How to compare the parasite burdens across two or more samples

3.1. Compare prevalences by one of the three alternative methods.

> 3.1.1. If several samples are involved, the time need of more advanced tests may increase dramatically. In such cases, use the traditional Chi-Square Test.
>
> 3.1.2. Fisher's Exact Test is more preferable for the same purpose.
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> 3.1.3. Finally, it is advisable to choose an Unconditional Test to compare two prevalences. Its advantage over the Chi-Square is its being exact – i.e. it maintains the prescribed Type I error rate. Its advantage over Fisher's is that it is more sensitive in detecting differences (i.e. its statistical power is higher), in particular when samples are small (n1, n2 < 100). Note that the name "unconditional" is a general statistical term that would be difficult to explain here (the name is not at all self-explaining). See Reiczigel et al. (2008).

3.3. Compare mean intensities by a Bootstrap Test. This will show whether parasite quantities differ significantly between the infected proportions of the two samples.

3.4. Compare median intensities by Mood's Median Test. This will show whether the typical level of infection differs significantly between the infected proportions of the two samples. Also available for more than 2 samples.

3.5. One can also compare the frequency distributions of intensities by a stochastic equality test. It compares several random pairs of individual values taken from the two samples to test whether or not there is a significant tendency to get higher values from one sample than from the other.

3.6. In certain cases, one may also decide to compare mean abundances by Bootstrap Test. This will show whether parasite quantities differ significantly between two samples. This comparison unites two of the former ones: the comparison of prevalences and the comparison of mean intensities.

3.7. Finally, when analyzing frequency-dependent features of parasites (e.g. sex-ratio, body size), it can be useful to compare mean crowding across samples. This is a rather primitive, however, the only existing method: provided that the two 97.5% CIs are not overlapping, we conclude that the two values are different at a 95% level of significance.

4. Technical notes

Increasing the number of samples involved may increase the time need of Fisher's exact test dramatically. Therefore, QP3.0 stops if the estimated time need exceeds a particular time limit. This is 1 minute as a default, use WordPad to modify it in the file "FISHERX3.PAR".

Increasing the number of replications will improve the accuracy of Bootstrap Confidence Interval estimations, but the time need will also increase. The default value is 2000, use WordPad to change it in the file "BCACONF.PAR".

Increasing the number of replications will improve the accuracy of Bootstrap t-tests, while its time need will increase too. The default value is 2000. Use WordPad to modify it in the file "2SAMBOOT.PAR"

The "print" command will refer to your Windows default printer.

Some extreme sample values may cause certain modules to fail. As far as we know, our Chi-square test fails if you compare prevalences of samples both (or all) equally with 100% prevalence. Of course, such "tests" do not make sense, but can cause a fail if you run them accidentally. In case of no response use the "CTRL+ALT+DEL" keys and then close QP3.0 as usual under Windows. Some tests may also fail when the maximum value of intensity is very high (several thousands).

5. Avoid the followings

Do not use geometric means (not offered in QP3.0). This measure appears to have no clear biological interpretation.

Avoid the format "mean ± SD" (not offered in QP3.0). This is meaningful only for normal distributions, and definitely useless in case of aggregated (right-skewed) distributions so characteristic to parasites. Mean intensity or mean abundance measures looking like "5 ± 10" will make readers smiling. Rather use CIs to characterise the accuracy of your estimations; these will never overlap with the negative range.

Do not make overstatements when interpreting results. If Sample 1 exhibited a significantly higher prevalence than Sample 2, then we should not conclude that Sample 1 was "more infected" than Sample 2. We conclude that *one of the useful measures*, namely the proportion of infected-hosts, was higher in Sample 1.

6. Authorship and copyright notes

QP3.0 is free for distribution and use in education and science. Before using it for pharmaceutical, industrial, agricultural or any other economic purposes, please contact the authors. When using it for academic purposes please cite:

Reiczigel J, Rózsa L 2005. Quantitative Parasitology 3.0. Budapest.

Alternatively, as a theoretical background you can cite:

Rózsa L, Reiczigel J, Majoros G 2000. Quantifying parasites in samples of hosts. *Journal of Parasitology,* **86,** 228-232.

Some of the more recent methods offered by QP3.0 were described and analysed in the following papers:

Applying an unorthodox approach (Sterne's exact method or Wald's method) to test the exact confidence limits of prevalence:

Reiczigel J 2003. Confidence intervals for the binomial parameter: some new considerations. *Statistics in Medicine,* **22,** 611-621.

Stochastic equality:

Reiczigel J, Zakariás I, Rózsa L 2005. A bootstrap test of stochastic equality of two populations. *The American Statistican,* **59,** 156-161.

Crowding:

Reiczigel J, Lang Z, Rózsa L, Tóthmérész B 2005. Properties of crowding indices and statistical tools to analyze crowding data. *Journal of Parasitology,* **91,** 245-252.

Unconditional Test to compare Prevalences:

Reiczigel J, Abonyi-Tóth Z, Singer J 2008 An exact confidence set for two binomial proportions and exact unconditional confidence intervals for the difference and ratio of proportions. *Computational Statistics and Data Analysis,* **52,** 5046-53.

All but one (Aggregation indeces) statistical modules were compiled by J. Reiczigel.

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