LETTER TO THE EDITOR

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Microarray analysis of circular RNAs in HCT-8 cells infected with *Cryptosporidium parvum*

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Abstract

We read with great interest the article by Yin et al. (Parasit Vectors 14:238, 2021). The authors found that *Cryptosporidium* infection induced significantly aberrant expression of circular RNA profiles in HCT-8 cells, a finding which has farreaching implications. However, due to the high number of false positives caused by multiple comparisons, statistical methods for microarray analysis should be carefully selected. Accurate analysis results will provide a convincing basis for subsequent experiments. In addition, we recommend several more appropriate methods in this article.

Keywords: Cryptosporidium parvum, Statistics, Bioinformatics, Microarray

To the Editor,

With the development of high-throughput microarray and RNA sequencing technology, an increasing number of genes have been identified to be associated with parasitology [1, 2]. We read with great interest the article by Yin et al. [3]. These authors, using microarray, found that *Cryptosporidium* infection induced significantly aberrant expression of circular RNA (circRNA) profiles in HCT-8 cells. Their findings provide a fundamental basis to develop effective strategies against *cryptosporidiosis* and, consequently, have far-reaching implications.

In our opinion, it is necessary to further clarify the data analysis strategy of this study. Based on the authors' description, they appear to use unadjusted *p*-values and fold change of expression values to define significantly differentially expressed circRNAs. However, due to the high number of false positives caused by multiple comparisons, statistical methods for microarray analysis should be carefully selected. Accurate analysis results will provide a convincing basis for subsequent experiments.

We suggest that the authors can adjust the *p*-values by the Benjamini-Hochberg correction to solve the problem of multiple comparisons, such as in the study of Atoyebi et al. [1]. Another option, as reported in a previous study, is linear modeling with empirical Bayes moderation, which provided good control of the false discovery rate as well as reasonable sensitivity when defining differentially expressed non-coding RNAs [4]. Therefore, we would like to suggest using Limma (linear models for microarray analysis) [5], which is an R/Bioconductor software package that uses linear models to analyze microarray and high-throughput PCR data [6]. Based on the output, the choice of the appropriate expression fold changes and false discovery rate < 0.05 as the cutoff is a conservative method to analyze changes in gene expression. A number of parasitological studies have used this statistical method and obtained satisfactory results [2]. We believe that this statistical method can provide a good technical support for parasitological studies.

We welcome further explanation of the data analysis strategy by the authors, which will make the results of this study more rigorous.

CircRNAs: Circular RNAs; Limma: Linear models for microarray analysis.

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Abbreviations

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Authors' contributions

YW and HZ conceived and prepared the first draft of the manuscript. LY and YZ critically reviewed the draft. All authors read and approved the final manuscript.

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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