Parasites & Vectors

LETTER TO THE EDITOR

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# Response to comments on our article (Yin YL et al., Parasit Vectors, 10.1186/s13071-021-04739-w) by Yuqing Wang and colleagues

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**Abstract**

This letter responds to comments on our article (Yin YL et al., Parasit Vectors, 10.1186/s13071-021-04739-w) by Yuqing Wang and colleagues, who wrote a letter entitled "Microarray analysis of circular RNAs in HCT-8 cells infected with *Cryptosporidium parvum*" and discussed statistical procedures for microarray analysis during *C. parvum* infection. To further confirm our data, in this letter, a common R package for analyses of differentially expressed genes, namely DESeq2, with Benjamini-Hochberg correction, was used to analyze our microarray data and identified 26 significantly differentially expressed circRNAs using adjusted *P* value < 0.05 and | $Log_2$ (fold change [FC]) | ≥ 1.0, including our circRNA ciRS-7 of interest. Therefore, the protocol for selecting circRNAs of interest for further study in our article is acceptable and did not affect the subsequent scientific findings in our article.

**Keywords:** *Cryptosporidium parvum*, Statistical procedure, Microarray analysis, ciRS-7

## To the Editor

Cryptosporidiosis, caused by *Cryptosporidium* spp., is an important zoonotic parasitic disease, seriously threatening the health of humans and many animals [1, 2]. Considering that the severity of cryptosporidiosis is closely associated with host status, especially immunity, understanding the host response to infection is critical to effectively develop well-directed control strategies against cryptosporidiosis [3].

Microarray analysis is a sensitive tool to accurately investigate differentially expressed circRNAs during many disease processes [4–8]. It is based on hybridization and fluorescence detection and uses specific probes targeting the back-spliced junction of each circRNA. CapitalBio Technology Human CircRNA Array, which was used in our study, also has its own analyzing software, GeneSpring, and GeneSpring supports normalization processing and analysis of differential circRNAs of original data from microarray analysis. Therefore, we used this software to process our microarray data. Due to the generally mild impact of *Cryptosporidium* infection on host cell gene transcription compared to other pathogens [9–12], to obtain as many differentially expressed genes as possible, we chose fold changes > 2 combined with $P \leq 0.05$ as the threshold to preliminarily screen differentially expressed circRNAs in the microarray analysis in our article [3]. The criterion of $P < 0.05$ or $P \leq 0.05$ was also used by recent studies [13–16]. From the subsequent verification of the experimental results, circRNAs of interest obtained using the threshold of $P \leq 0.05$ are stable and accurate. Certainly, the unadjusted *P*-value will introduce false positives during multiple comparisons, as stated by Wang et al. [17], in their Letter to the Editor published commenting on our article. To reduce this

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possibility, expression of the circRNA of interest, ciRS-7, was further verified by using real-time PCR in multiple repetitions in our article. This protocol is also a standard method to select genes of interest for subsequent experiments [15, 16, 18, 19].

Nowadays, several software packages and models have been used to define significantly differentially expressed (DE) genes during multiple comparisons. Three R packages, namely limma, DESeq2 and edge R, are commonly used to analyze DE genes for microarray and RNA-seq data. Of these, both limma and DESeq2 are quite reliable and are not much different for multiple comparisons of these data, with > 90% of genes detected overlapping between the two methods [20]. It should be noted that limma can find the accurate DE genes better but obtains fewer significant DE genes because of its rigorous screening criteria, while DESeq2 is more suitable when more possible candidate DE genes are expected. However, both methods are preliminary screening tools that will inevitably introduce false-positive/negative results to a certain extent. Therefore, the convincing expression of candidate DE genes should be further confirmed by other more precise methods with multiple repetitions, such as the real-time PCR used in our article. Since *Cryptosporidium* infection has a mild impact on host cell gene transcription, here we further analyzed our microarray data by using DESeq2 for multiple comparisons using Benjamini-Hochberg correction and identified a total of 26 significantly DE circRNAs (Additional file 1: Table S1) using the standard of adjusted $P$ value < 0.05 and $|\ \mathrm{Log}_2$ (fold change [FC]) $|\ \geq 1.0$, including our circRNA of interest, ciRS-7 ($P = 0.030272316$ and $\mathrm{Log}_2$ FC = 2.497972579).

Collectively, the protocol for selecting circRNAs of interest for further study in our article is acceptable and did not affect the subsequent scientific findings. We welcome further discussions of our article.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13071-021-04996-9.

**Additional file 1**: **Table S1**. Significantly differentially expressed circRNAs between *C. parvum*-infected and -non-infected HCT-8 cells.

### Authors' contributions

GHZ and YLY conceived and prepared the first draft of the manuscript. GHZ, YLY and XY critically reviewed the draft. All authors read and approved the final manuscript.

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### Availability of data and materials

All datasets generated for this study are included in the article and its additional file.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing of interest.

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