Reviewer's report

Title: A Quantitative PCR (TaqMan) Assay for Pathogenic Leptospira spp.

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Reviewer: Dr M Guiver

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Unable to decide on acceptance or rejection until the authors have responded to the compulsory revisions

This paper describes the evaluation of a Taqman PCR assay for the detection of pathogenic Leptospira spp. The application of this assay may be useful in early detection of infection before a serological response is produced, however there have been many published PCR assays for the detection of Leptospira spp.

Discretionary points
1. The sensitivity of the assay was assessed by adjusting the concentration of a cultured leptosporial cells using a counting chamber. These estimates appear optimistic and considering the inherent errors in estimating numbers can the difference between the detection limit of serum and urine be considered significant? The authors do not state the volume of eluate used to extract the samples using the Qiagen kit however, if it is assumed the extracted sample is recovered in 50μl then from an original sample volume of 200μl, the addition of 5μl of this extract represents 1/10 of the original 200μl sample (20μl). A minimum of 1 genome copy is obviously required for the PCR reaction to proceed. An absolute theoretical limit of 10 cells per 200μl or 500 per ml is therefore required and it seems unlikely the Qiagen column would reliably recover 1 or 2 cells. It is acknowledged that the ribosomal gene sequence is present in multiple copies which assists in detection, however the authors may like to review the stated lower limits of detection.

Compulsory revisions

2. The authors state "we employ real-time (quantitative) PCR using Taqman chemistry to detect leptospires in clinical and environmental samples", however no data is presented to support this statement. This should be removed to suggest that it may be useful for detection from such samples but will require evaluation.
3. Evidence to support the value of this assay in detection of leptospires from clinical samples was unconvincing. Presentation of the results should be made clearer with more relevant information. It is not stated what class of antibody the ELISA is detecting. If IgM is detected this may explain the difference in results with the MAT. More detail regarding the sera tested should be included. In particular, of the 66 samples tested how many were acute and how many were convalescent samples? It seems disappointing that not more of the samples were positive by PCR and of the 4 that were positive all were also culture positive and very high Ct values were recorded indicating very low levels of DNA.

4. Considering whole blood was used for culture and only a small volume (2-5 drops) was able to recover viable leptospires, the authors might like to contemplate in the manuscript the possibility of increasing sensitivity by extracting DNA for PCR from whole blood samples.

5. The authors claim this assay can be used for detection of leptospires from urine, although this has not been demonstrated from clinical samples and this should be made clear.

6. Overall the evidence to support the view that this assay is useful for detection of leptospires from clinical samples is not convincing. It should be stated that much more work in evaluating clinical material needs to be undertaken.

**Competing interests:**

None declared.