Development of a Capillary Electrophoresis-Based Competitive PCR for On-Site Detection of Infectious Precocity Virus in *Macrobrachium rosenbergii*

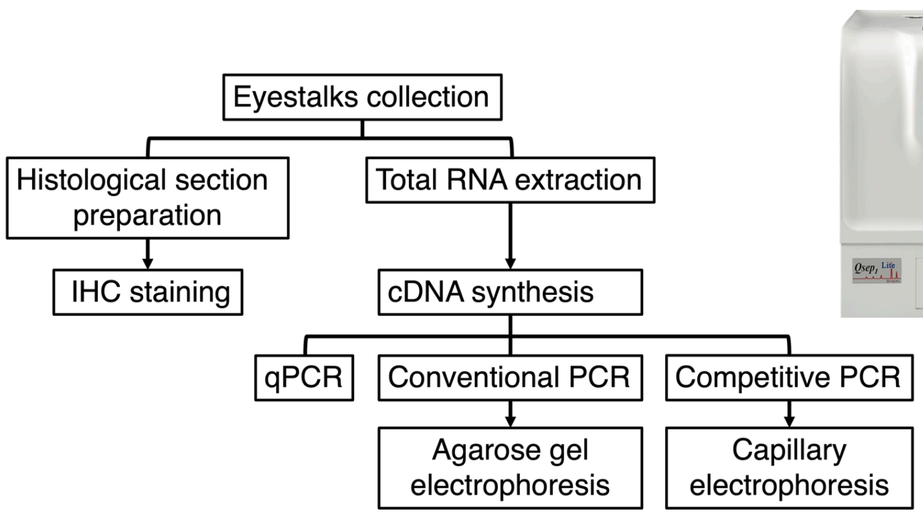
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Abstract

Macrobrachium rosenbergii (Prawns) is a large freshwater species widely cultured in the global aquaculture industry. Recently, precocious maturation has become a prevalent issue associated with Infectious Precocity Virus (IPV) infection. This virus can lead to Iron Prawn Syndrome (IPS), resulting in sexual precocity and stunted growth. Conventional PCR is limited by cycle constraints and cannot accurately quantify viral load or reflect the extent of eyestalk damage, restricting its use in on-site aquaculture settings. Therefore, this study establishes a capillary electrophoresis-based competitive PCR (CE-cPCR) method to enable rapid, on-site viral detection and quantification, while simultaneously reflecting the extent of histopathological damage.

Keywords: *Macrobrachium rosenbergii,* Infectious precocity virus (IPV), Iron prawn syndrome (IPS), CE-cPCR, Viral quantification, Aquaculture disease

Materials and Methods





Results

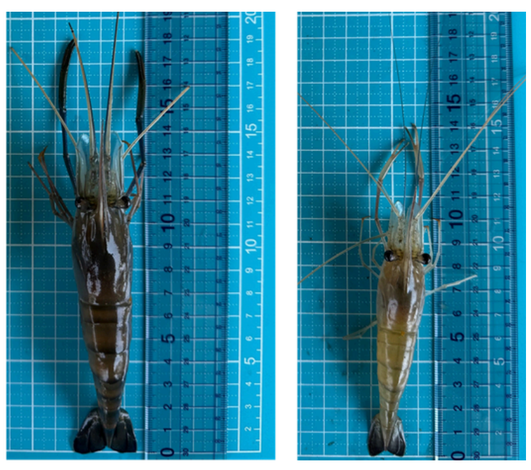


Fig 1. Gross Signs of Infected Prawns. Field sampling revealed significant size disparity among infected individuals, indicative of stunted growth.

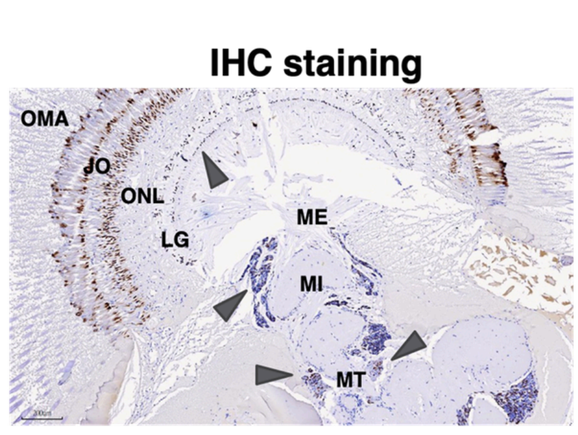


Fig 2. IHC staining. IHC analysis revealed positive immunostaining in the eyestalks (arrows), confirming the presence of IPV.

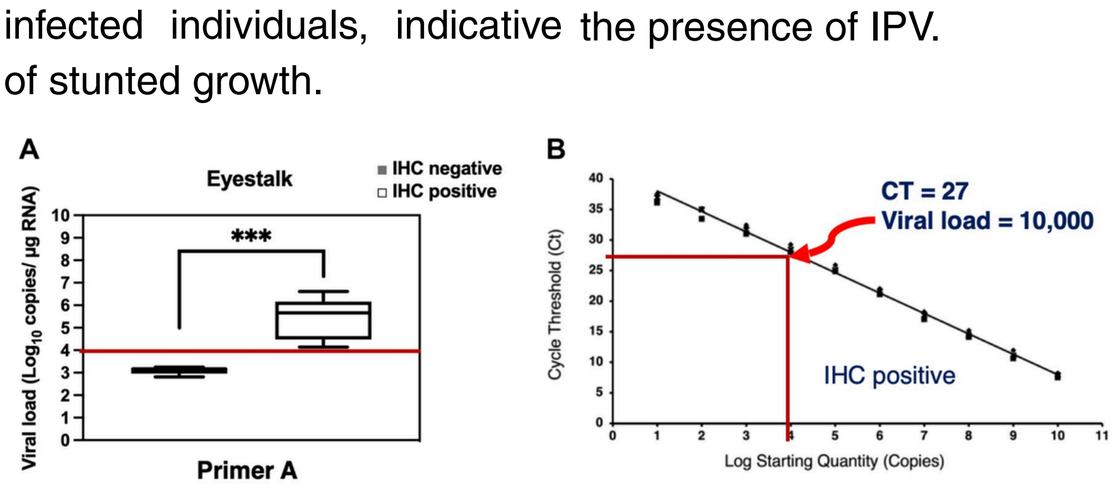
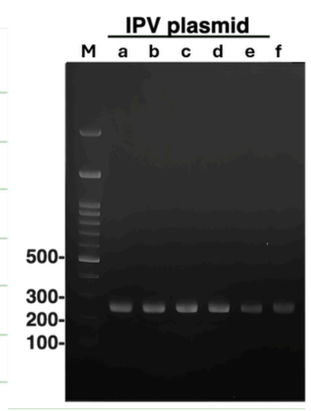


Fig 3. Correlation between RT-qPCR and IHC results for IPV detection.
(A) The detection limit of IHC staining is about $1.95*10^4$ viral copies. Samples with viral loads above this level are considered IHC positive, while those below are IHC negative.
(B) The RT-qPCR cycle threshold (CT) cutoff for IPV infection is 27. Samples with CT ≤ 27 have higher viral loads (≥ $1.95*10^4$ copies) and are considered IPV positive, while samples with CT > 27 are considered IPV negative.

Lane	Sample	CT value
a	$1×10^{10}$	11.2
b	$1×10^{9}$	15.78
c	$1×10^{8}$	20.75
d	$1×10^{7}$	24.99
e	$1×10^{6}$	30.57
f	$1×10^{5}$	36.66



Lane	Sample	CT value
1	12-1	24.41
2	12-2	24.25
3	12-3	19.72
4	12-4	22.89
5	12-5	26.14
6	12-6	27.54

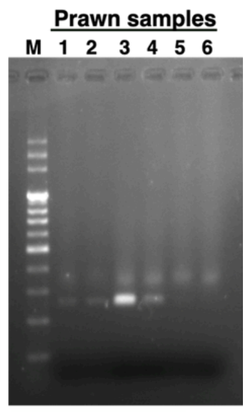


Fig 4. Detection limitation of conventional PCR using IPV plasmid serial dilution and field samples.
(A) IPV plasmid serial dilutions were analyzed by conventional PCR and agarose gel electrophoresis. Band intensity did not correlate with plasmid concentration, indicating limited quantitative capability.
(B) Randomly collected field samples showed no visible bands when Ct values exceeded 26, indicating limited sensitivity for low viral loads.

IPV plasmid	competitive
$7.63x10^1$	10^4
$3.05x10^2$	10^4
$1.22x10^3$	10^4
$4.88x10^3$	10^4
$1.95x10^4$	10^4
$7.81x10^4$	10^4
$3.13x10^5$	10^4
$1.25x10^6$	10^4
$5.00x10^6$	10^4

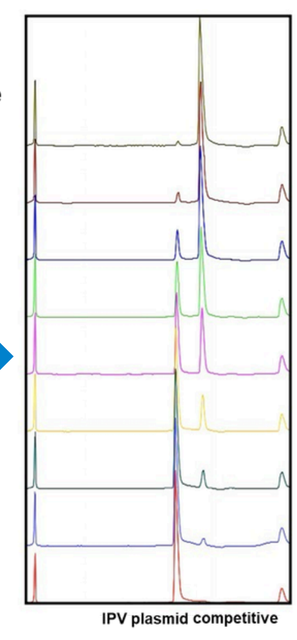


Fig 5. Calibration of the CE-cPCR system based on a predefined pathology-relevant reference point ($1.95*10^4$ copies).
The electropherograms display the IPV target peak on the left and the fixed competitor peak on the right. Comparable peak intensities between the target and competitor were achieved at this predefined reference level. In practical application, a higher IPV peak indicates tissue damage, whereas a lower peak implies undamaged tissue.

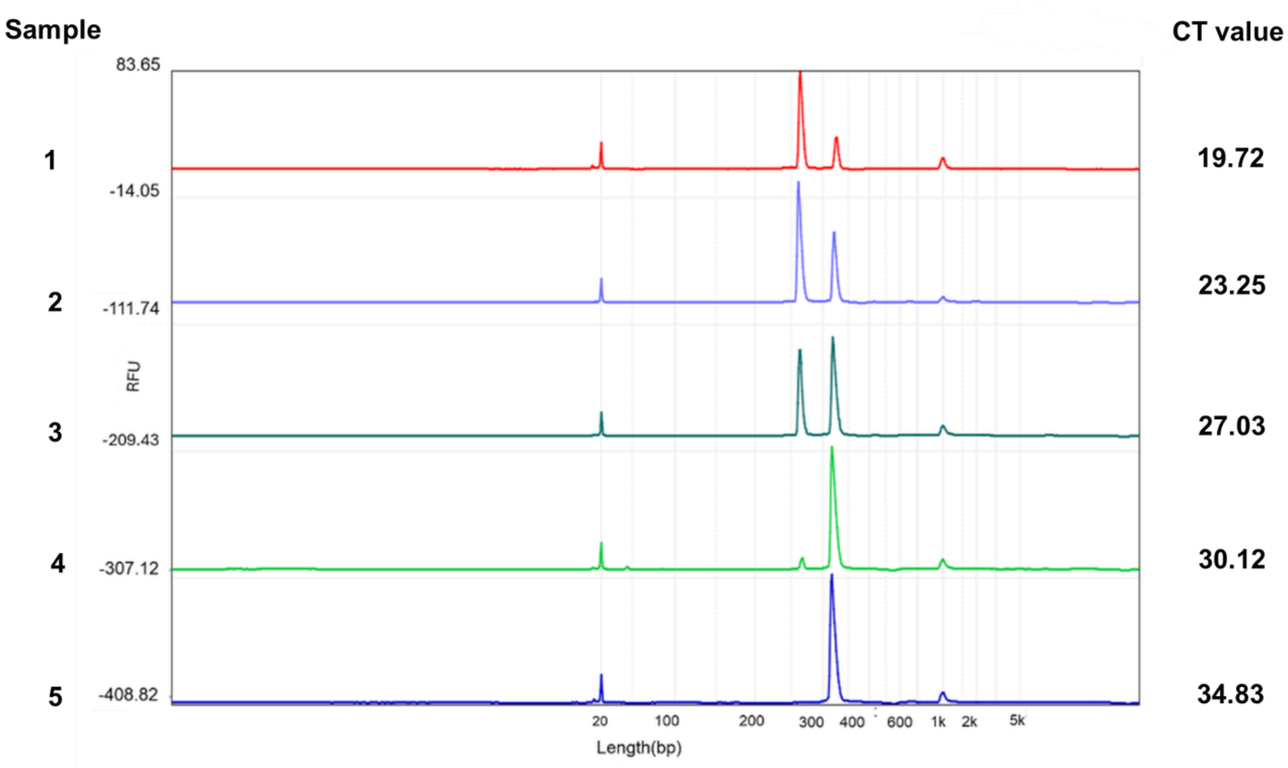


Fig 6. CE-cPCR analysis of randomly collected field samples.
CE-cPCR display the IPV target peak on the left and the competitor peak on the right. A higher IPV peak indicates tissue damage. These CE-cPCR results demonstrated that signal intensity perfectly correlated with tissue damage, consistent with the pathological threshold defined by IHC ($1.95*10^4$ copies) and the RT-qPCR cutoff (Ct = 27), indicating its capability to reflect disease status in field samples.

Conclusions

1. **Limitation of Current Methods**: Conventional PCR lacks the sensitivity to reliably quantify IPV viral loads, particularly at low-level infections.
2. **Establishment of a Diagnostic Baseline**: By cross-validating RT-qPCR and IHC, we established a precise pathological threshold (1.95×10^4 copies; Ct = 27), enabling accurate interpretation of disease severity.
3. **A Novel Field-Ready Solution**: The developed CE-cPCR method provides rapid semi-quantitative detection aligned with this pathological threshold. Its strong correlation with histopathological damage highlights its potential as a practical field-deployable tool for IPV management in aquaculture.