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Objectives

Avipoxvirus sp. is a serious threat for the success of reinforcement programs of some endangered birds species. In the endangered Houbara bustard (*Chlamydotis sp.*), avipoxvirus infections can lead to high morbidity in captivity and compromise the survival of wild release birds. However, up to now, avipoxvirus infections in houbara bustards were poorly studied. In order to better understand its epidemiology and pathogenesis, a quantitative detection method of viral DNA using SYBR-green real-time PCR was developed as a tool for further studies. Quantitative detection using this method was successfully applied in houbara bustard lesions.

Methods

From a houbara bustard lesion, the avipoxvirus 4b core protein gene was amplified and sequenced. Primers were designed from this sequence. After cloning, scalar dilutions of the plasmid constructed were made and concentration and purity were determined through spectrophotometric analysis at 260/280 nm. Dilutions were then used for a SYBR-green real-time PCR assay to validate the method and determine its sensitivity. Finally, DNA extracted from houbara bustard lesions was used for a SYBR-green real-time PCR assay to test the method on biological samples.

Results

Primers were designed from the houbara bustard sequence to have 100% identity with published houbara bustard sequence of 4b core protein gene and with the complete genome of canarypox virus. The theoretical size of the amplicon is 109bp. The chosen primers were very effective in most of plasmid dilutions and the assay encompassed at least six orders of magnitudes (from 10^7 to 10^2 copies) with a high linear relationship ($r^2>0.99$) between CT values and plasmid copies. Quantitative detection in houbara bustards lesions with the assay allowed viral DNA detection in clinically diagnosed lesions.

Conclusion

The method developed here showed its efficiency for detection and quantification of avipoxvirus DNA in biological samples. We expect that this method will be a valuable tool either for epidemiological studies like viral detection in potential insect vectors or for in-vivo and in-vitro infection studies.

Description

A SYBR-green real time-PCR assay was developed for quantitative detection of avipoxvirus DNA. The method was successfully applied for detection and quantification of viral DNA in houbara bustard lesions.

Keywords

Avipoxvirus; 4b core protein gene; SYBR-green real time-PCR; Houbara bustard; *Chlamydotis sp.*

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