Molecular detection of integrated reticuloendothelial virus genes in fowlpox virus field isolates and live vaccines of poultry

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ABSTRACT

Two field isolates (FWPV-W1 and FWPV-W2) obtained from unvaccinated backyard poultry chickens and four commercial live vaccines (FWPV-G, FWPV-V, FWPV-B and FWPV-H) of fowlpox virus origin were isolated on chorio-allantoic membrane (CAM) of embryonated chicken eggs. The CAM tissues infected with FWPV-W1, FWPV-W2, FWPV-G, FWPV-V, FWPV-B and FWPV-H individually were subjected to DNA isolation. The isolated DNA was tested for the presence of P4b gene by PCR to confirm FWPV. Then, each of the field isolates and commercial vaccines were screened for presence of reticuloendothelial virus envelope (REV-env) gene and long terminal repeat (LTR) region by PCR. FWPV-W1 isolate was positive for REV-env gene (807 bp) and REV-LTR region (370 bp), which confirmed presence of near full-length REV integration in its genome. Whereas, FWPV-W2 isolate was positive for LTR region and negative for REV-env gene. This suggested that full-length REV is not present in all FWPV field isolates. All the four commercial live vaccines, were negative for REV-env gene. This showed that full-length REV is absent in these commercial vaccines, ensuring safety of the usage of these vaccines in India. However, the commercial vaccines were positive for REV-LTR region, which does not affect the vaccine safety.

Key words: Envelope gene, Field isolate, Fowlpox virus, Live vaccine, LTR region, Reticuloendothelial virus
MATERIALS AND METHODS

Viruses: Two FWPV field isolates and four commercial vaccines were used in this study. The field isolates were collected as dried scab materials from unvaccinated backyard poultry chicken in Chennai (FWPV-W1) and Puducherry (FWPV-W2). The vaccines were obtained from Globion Pvt Ltd (FWPV-G), Venky’s Pvt Ltd (FWPV-V), BioMed Pvt Ltd (FWPV-B) and Hester Biociences Pvt Ltd (FWPV-H).

Virus isolation: FWPV-G, FWPV-V, FWPV-B and FWPV-H were re-suspended in sterile phosphate buffered saline (PBS) and 0.1 ml was inoculated onto chorio-allantoic membrane (CAM) of 13-day old specific pathogen free embryonated chicken eggs and was incubated at 37°C for 5 days (Tripathy and Reed 2003). Dried scab material of FWPV-W1 and FWPV-W2 were triturated separately in PBS containing 50 IU/ml penicillin and 50 μg/ml streptomycin. The suspension was clarified by centrifugation at 3,000 rpm for 10 min and 0.1 ml of the supernatant was inoculated onto CAM.

DNA isolation: Total DNA was isolated from CAM tissues infected with FWPV-W1, FWPV-W2, FWPV-G, FWPV-V, FWPV-B and FWPV-H respectively using QIAamp DNA kit (Qiagen, Germany) following manufacturer’s instructions. Briefly, the FWPV infected CAM tissue was triturated in mortar and pestle and was lysed using proteinase-K. The lysate was bound to the silica membrane which was washed twice using 75% ethanol and the total DNA was eluted in 50μl of elution buffer. The concentration of DNA was estimated in spectrophotometer using elution buffer as blank.

PCR amplification of P4b gene: Primers described in Table 1 were used for PCR amplification of P4b gene to detect the presence of FWPV in CAM infected with FWPV-W1, FWPV-W2, FWPV-G, FWPV-V, FWPV-B and FWPV-H. The reaction mixture comprised 5 μl of PrimeStar master mix (Clonetech, USA), 0.5 μM of each primer and 50 ng of total DNA in a final reaction volume of 10 μl. The reaction cycle involved an initial denaturation at 98°C/30 sec followed by 35 cycles of denaturation at 98°C/10 sec, 53°C/10 sec and extension at 72°C/30 sec with a final extension at 72°C/5 min. The amplified PCR product was separated by agarose gel (1.5%) electrophoresis stained with ethidium bromide and was viewed under ultraviolet light.

PCR amplification of reticuloendothelial virus genes: Primers described in Table 1 were used for PCR amplification of reticuloendothelial virus (REV) envelope (REV-env) gene and LTR region respectively. The reaction mixture of PCR comprised one unit of phusion DNA polymerase, 1.5 mM of MgCl₂, 200 mM of each dNTP, 0.5 mM of each primer and 50 ng of total DNA in a final reaction volume of 50 μl. The reaction cycle involved an initial denaturation at 98°C/30 sec followed by 35 cycles of denaturation at 98°C/10 sec, optimum annealing

Table 1. Primers used for P4b gene and REV gene amplification

| Gene     | Primer sequence (5’–3’) Ta (°C) | Product size (bp) | Reference          |
|----------|--------------------------------|-------------------|--------------------|
| P4b      | F: CAGCAGGTGC TAAACAACAA      | 53                | 578                |
|          | R: CGGTAGCTTA ACGCCGAATA      |                   | Binns et al. (1989)|
| REV-env  | F: TGACCAGGC GGCAAAAACC       | 52                | 807                |
|          | R: CGAAAGGGA GCCTTACACT       |                   | Wilhelmsen et al. (1984)|
| REV-LTR  | F: ACCTATGCC TCTTATCCAC       | 52                | 370                |
|          | R: CTGATGCTT GCCCTCAAC        |                   | Wang et al. (2006) |
temperature/10 sec and extension at 72°C/30 sec with a final extension at 72°C/5 min. The amplified PCR product was separated by agarose gel (1.5%) electrophoresis and purified using Nucleosep Gel and PCR cleanup kit (Machery-Nagel, Germany) as per manufacturer’s instructions. The PCR product was eluted in 20 ml elution buffer.

**Nucleotide sequencing:** The purified PCR elute of REV-env gene and REV-LTR region was subjected to nucleotide sequencing. The sequences were aligned using MEGA7.0 software (Kumar et al. 2016) and were subjected to BLAST analysis in NCBI.

**RESULTS AND DISCUSSION**

In present study, two field isolates and four commercial live vaccines of FWPV that was propagated on CAM were positive for P4b gene, which was amplified at 578 bp. P4b gene is the conserved gene of FWPV that is used as a molecular detection tool to confirm the presence of FWPV in a sample (Lee and Lee 1997). Upon confirmation by P4b gene PCR, the field isolates and commercial live vaccines of FWPV under this study were screened for the presence of REV gene by molecular methods. The integration of REV genes into FWPV genome is a naturally occurring recombination event that had occurred in FWPV presence of REV gene by molecular methods. The vaccines of FWPV under this study were screened for the P4b gene PCR, the field isolates and commercial live in a sample (Lee and Lee 1997). Upon confirmation by molecular detection tool to confirm the presence of FWPV gene is the conserved gene of FWPV that is used as a positive for P4b gene, which was amplified at 578 bp. P4b live vaccines of FWPV that was propagated on CAM were processed in 20 ml elution buffer.

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