Molecular evaluation of Peste des petits ruminants (PPR) vaccine

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ABSTRACT

Peste des petits ruminants (PPR) is a highly contagious, economically important viral disease of sheep and goats with high morbidity and mortality rates. A live attenuated PPR vaccine has been produced in Veterinary Serum and Vaccines Research Institute (VSVRI), Abbasia. It has and still been utilized to control PPR disease in endemic areas of the Arabian Gulf. In this study, the identity of four batches of PPR vaccines was tested as per the Office International des Epizooties (OIE) guidelines and Couacy-Hymann et al., 2002 using RT-PCR technique that based on the amplification of fragments of N-protein. All batches are also subjected to sterility tests for detection of bovine viral diarrhea virus as possible extraneous virus contaminant using RT-PCR and detection of mycoplasma as possible bacterial contaminant using PCR technique. All of the four batches were positive when tested using specific primers and they were free from BVDV or mycoplasma contamination. Results in this study showed that molecular techniques could be used for rapid evaluation of PPR vaccine including RT-PCR for identity testing and for detection of BVDV as adventitious contaminant and PCR assay for detection of bacterial contaminants as mycoplasma.

Key words: PPR vaccine, Identity test, RT-PCR

Abbreviations: PPR: Peste des petits ruminants, RT-PCR:reverse transcriptase polymerase chain reaction, VSVRI: Veterinary Serum and Vaccines Research Institute, OIE: Office International des Epizooties, VERO: African green monkey cell line, BVDV: Bovine viral diarrhea virus.

INTRODUCTION

Peste des petits ruminant (PPR) is a highly contagious viral disease of sheep and goats characterized by high morbidity and mortality (Khan et al., 2007; Asim et al., 2009 and Dhar et al., 2002). The disease was first reported in Ivory Coast in West Africa in 1942 (Gargadennec et al., 1942) and later found in Sengal (Gilbert et al., 1962), Central Africa (Scott, 1981), Sudan (Taylor, 1984), India (Shaila et al., 1989) East Africa (Wamwayi et al., 1995), Saudi Arabia (Abu-Elzein et al., 1990), Jordan and Middle East (Lefevre at al., 1990) and in Egypt (Abd El-Rahim et al., 2010). It is even considered a threat to Europe after reaching Turkey, Morocco (Yesilba et al., 2005 and Banyard et al., 2010) Algeria and Tunis (De-Nardi et al., 2012 and Ayari-Fakhfakh et al., 2010). The causative agent of the disease; PPRV; is classified as a member of genus *morbillivirus* in the family *Paramyxoviridae* (Barrett et al., 2005). PPRV is antigenically closely related to rinderpest virus (RPV) (Hamdy et al., 1976; Taylor et al., 1990; Anderson et al., 1990 and Couacy-Hymann et al., 1995). It has a single strand negative sense RNA genome that encodes eight proteins in the order of 3'- N-P/C/V-M-F-H-L-5' (22, 23). Among them, the nucleocapsid protein (NP) is the major viral protein. It has been the target for developing diagnostic tests that can be used to identify PPRV. In 1989, nucleic acid technology was applied for detection of PPRV by using, as probes, the cDNA corresponding to the nucleocapsid gene of each virus and labelled with 32P nucleotide (Bailey et al., 2005). Although it was sensitive, such a test is not suitable for routine use due the health hazard linked to the radioactive labels. An alternative and very sensitive technique, the amplification of the viral nucleic acid by the reverse transcription-polymerase

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chain reaction (RT-PCR) is described for specific detection of PPRV (Haffar et al., 1999). For control of PPR disease in its endemic areas, a live attenuated PPR vaccine is produced. This study aims to use RT-PCR for identity testing in the evaluation of PPRV by the amplification of fragments of N-protein using NP3 and NP4 primers as they showed maximum sensitivity and specificity (OIE, 2013 and Couacy-Hymann et al., 2005). Sterility tests were performed using molecular techniques for detection of BVDV (Lopez et al., 1991 and Weinstock et al., 2001) and mycoplasma (OIE, 2013; McAuliffe et al., 2005 and Muyzer et al., 1993)

MATERIAL AND METHODS

Vaccines

Four batches of PPRV vaccines that were produced in Veterinary Serum and Vaccine Research Institute (VSVRI) from the isolate Nigeria 75/1 are reconstituted and used in the current study.

Nucleic acid extraction

The nucleic acid extraction was carried out using QIAamp viral RNA MiniKit following the manufacturer's guidelines for detection of PPRV and BVDV and using QIAamp DNA extraction kit for detection of mycoplasma. Extracted nucleic acid was kept briefly at 4°C pending molecular assays.

Primers:

Primers for PPRV identity test

Sequences of primers used in this study are based on the initial protocol by Couacy-Hymann et al., 2002 and based on the N-gene amplification

Forward: NP3: 5'-TCT CGG AAA TCG CCT CAC AGA CTG-3' Indicating the locations 1583-1560

Reverse: NP4: 5'- CCT CCT CCT GGT CCT CCA GAA TCT-3' Indicating the locations 1292-1316

Primers for detection of BVDV

Sequences of primers used are based on the initial protocol by Weinstock, D. et al., 2001. Primers were designed from the highly conserved region of BVDV genome within the 5' UTR of strain NADL that share maximum homology to BVDV genome type 1, 2.

Forward: 5'-TAGCCATGCCCTTAGTAGGAC-3' Indicating the genomic locations 103-124

Reverse:5'-ACTCCATGTGCCATGTACAGC-3' Indicating the genomic positions 372-392

Primers for detection of mycoplasma species

Sequences of primers used are based on the initial protocol by McAuliffe, L. et al., 2005 using a universal primer for identification of mycoplasma species. GC-341F: 5`CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG3` and R543: 5`ACC TAT GTA TTA CCG CG 3`

RT-PCR:

RT-PCR for identifying PPRV

RT-PCR was performed according to iScript one step qRT-PCR kit, Biomatik. 5µl of RNA template were added to 15 µl of PCR master mix containing 2.6 µl nuclease free water, 10 µl of igreen master mix, 0.4 µl of qRT-PCR enzyme master mix(50X), 1µl of forward primer (6µM) and 1 µl of reverse primer (6µM) to obtain a final volume of 20 µl. the amplification was carried out according to the following program: initial denaturation at 94°C for 4 min followed by 34 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec with final extension of 72°C for 10 min.

RT-PCR for detection of BVDV

RT-PCR was performed according to iScript one step qRT-PCR kit, Biomatik. 5µl of RNA template were added to 15 µl of PCR master mix containing 2.6 µl nuclease free water, 10 µl of igreen master mix, 0.4 µl of qRT-PCR enzyme master mix(50X), 1µl of forward primer (6µM) and 1 µl of reverse primer (6µM) to obtain a final volume of 20 µl. the amplification was carried out according to the following final volume of 20 µl. The amplification was carried out according to the following program: initial incubation at 62°C for 30 min, denturation at 94°C for 120 sec and 40 cycles of 94°C for 60 sec, 62°C for 60 sec and 65°C for 10 min.

PCR for detection of mycoplasma

PCR was carried out according to Geneaid ultra-pure taq PCR master mix. 5 µl of DNA template is added to 1 µl of forward primer, 1 µl of reverse primer, 5 µl ultra-pure taq PCR master mix and sterile water to obtain afinal volume of 25 µl. The amplification was carried out according to the following program:initial denaturation at 94° for 5 min followed by 30 cycles of denaturation at 95° for 1 min, annealing at 56° for 45 sec and extension at 72° for 1 min then final extension at 72° for 10 min.

Analysis of PCR amplified products

5 µl of each PCR product is analyzed by electrophoresis on 1.5% agarose gel that was stained with ethidium bromide and visualized by UV fluorescence.

RESULTS

Identification of PPRV in vaccines

Analysis of different PPR vaccine batches with the NP3 and NP4 primers yield an amplicon of the expected size of 351 bp "Fig. 1"

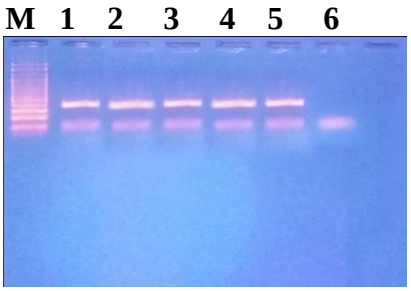

Fig. 1: PCR amplicon of the N- gene showing the band size of 351 bp. Lane M (molecular ladder), lane 1 (positive PPRV), lanes 2-5 (PPR tested vaccines), lane 6 (negative control)

Sterility tests

Sterility of vaccines was routinely tested to conform to OIE standards in evaluation of vaccine product. Vaccines were tested for detection of BVDV virus contamination and they were free from any adventitious BVDV (Fig. 2). The four batches were also tested for possible contamination with mycoplsma and were found to be free from mycoplasma species (Fig. 3)

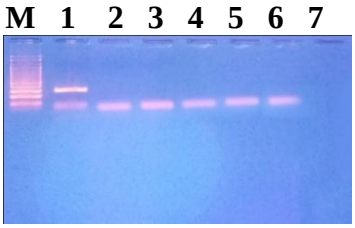

Fig. 2: RT-PCR evaluation of PPR vaccines for presence of BVDV. M (molecular ladder), Lane 1 (NADL strain of BVDV as positive control), lane2 (negative control), lanes 3-6 (PPR vaccines). Presence of a 290 bp band indicates positivity to BVDV

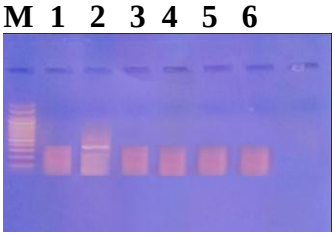

Fig. 3: PCR evaluation of PPR vaccines for presence of mycoplasma. M (molecular ladder), lane 1 (negative

control), lane 2 (positive control), lane 3-6 (PPR vaccines),. Presence of 340 bp band indicates positivity to mycoplasma species.

DISCUSSION

Peste des petits ruminants is a major transboundary disease of small ruminants in sub Saharan Africa that causes significant morbidity and socio-economic losses. Its expansion to Asia and other African countries is a great concern and challenge to the whole world.

Effective control strategies need to be contemplated in countries that rely on sheep and goat rearing and also to protect their marginal countries.

For the purpose of control of any viral disease, an effective vaccine is essential prerequisites. In Egypt, a specific vaccine against PPR is developed by attenuating the isolate Nigeria 75/1 in VERO cell culture system (Diallo et al., 1989).

Four batches of the locally produced attenuated PPR vaccine were tested for identity as per OIE guidelines (OIE, 2013) and according to Couacy-Hymann et al.,2002 using molecular biological techniques in agreement with Albayrak et al., 2009 who determined that RT-PCR is sensitive and reliable method for identifying PPRV.

The vaccines were tested also for detection of BVDV as extraneous virus contaminant by RT-PCR as per Weinstock et al., 2001 and for detection of mycoplsama species by PCR as per McAuliffe et al., 2005 and they were found to be free from any BVDV or mycoplsama contamination.

All vaccine samples were positive for detection of N-gene amplification and this agreed with Saravanan et al., 2010 (34) who used RT-PCR for identity testing of different vaccines against PPRV using primers against F, M, and N proteins .

Using primers to amplify N-gene for detection of PPRV was also supported by the results of Kwaiatek et al., 2010 (35) who mentioned that the N protein is a good candidate for differential diagnosis between PPRV and PRV These results also agreed with Kerur et al.,2007 (36) who used N gene sequences in classification of PPRV into linages as they yield better pictures of molecular epidemiology of PPRV. The used NP3 and NP4 primers use in this study were also proved to be the most sensitive and specific primers for detection of PPRV as per Mahajan et al., 2014 (37) who used kappa value to compare different primers based RT-PCR and found that NP3, NP4 primers show almost 100% agreement.

There was difference in the intensity of the bands observed in N gene amplification between samples and this may be due to various amount of templates found in each vaccine preparation subjected to the identity test in agreement with Saravanan et al., 2010 (34) who mentioned the difference in bands intensity with using different sets of primers .

All the four vaccine batches were free from BVDV which is known to be a contaminant of serum and subsequently vaccines (38, 39). These results agree with Brock, 1991(40) who used RT-PCR for identification of BVDV in serum and buffy coat. Results also agree with Belak and Ballagi-Podany, 1991as they used RT-PCR for detection of BVDV in cell cultures and reported that it was a specific and sensitive method. Also we agree with Lopez et al., 1991who mentioned that it was a rapid method for detection of BVDV in cell cultures and biological products. Our work is also supported with the results of Hiroshi et al., 2002; Nakamura et al., 2002 and Dong et al., 2005 who used it as a rapid, simple, specific and sensitive method for evaluation of live viral vaccines. We also agree with Weinstock et al., 2001 who used RT-PCR for detection of BVDV in serum samples and reported

that it is a rapid and cost-effective method for detection of BVDV. But our results disagree with Hiroshi et al., 2002 who reported that general RT-PCR cannot detect active extraneous BVDV in animal vaccines as they used a novel RT-PCR method which detect only the active virus.

PPR vaccines are tested for presence of mycoplasma contamination as per OIE guidelines (OIE, 2013). All vaccine batches are found to be free from contamination with mycoplasma species and this agree with Sasaki et al., 1996 and Feberwee etal., 2005 who used PCR as sensitive and specific technique to detect mycoplasma in the process of vaccine evaluation.

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