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Specific detection of peste des petits ruminants virus antibodies in sheep and goat sera by the luciferase immunoprecipitation system

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ABSTRACT

Peste des petits ruminants (PPR) is a contagious and often fatal transboundary animal disease affecting mostly sheep, goats and wild small ruminants. This disease is endemic in most of Africa, the Middle, Near East, and large parts of Asia. The causal agent is peste des petits ruminants virus (PPRV), which belongs to the genus *Morbillivirus* in the family *Paramyxoviridae.* This genus also includes measles virus (MV), canine distemper virus (CDV) and rinderpest virus (RPV). All are closely related viruses with serological cross reactivity.

In this study, we have developed a Luciferase Immunoprecipitation System (LIPS) for the rapid detection of antibodies against PPRV in serum samples and for specific differentiation from antibodies against RPV.

PPR and rinderpest (RP) serum samples were assayed by PPR-LIPS and two commercially available PPR cELISA tests. The PPR-LIPS showed high sensitivity and specificity for the samples tested and showed no cross reactivity with RPV unlike the commercial PPR cELISA tests which did cross react with RPV. Based on the results shown in this study, PPR-LIPS is presented as a good candidate for the specific serosurveillance of PPR.

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1. Introduction

Peste des petits ruminants (PPR) is an increasingly alarming, highly contagious, viral disease primarily affecting sheep, goats and wild small ruminants (Libeau et al., 2014). Due to its high mortality and morbidity, PPR is responsible for severe economic losses in the countries where it is endemic (Libeau et al., 2014). This disease, which can cause 50–90% mortality in naïve populations, is widely spread throughout most of Africa, the Middle and Near East, South and Central Asia and China. Most of these areas rely on subsistence farming (Nanda et al., 1996; Libeau et al., 2014; Wang J et al., 2015). Thus, the control and global eradication of PPR, which are currently being undertaken by the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO), are seen as relevant aspects of poverty alleviation policies (Perry B.D. et al., 2002).

The causal agent of PPR, the peste des petits ruminants virus (PPRV), belongs to the genus *Morbillivirus* in the family *Paramyxoviridae* along with measles virus, canine distemper virus, rinderpest virus, phocine distemper virus, dolphin morbillivirus and feline morbillivirus (Diallo and Libeau, 2014; Gibbs et al., 1979; Libeau et al., 2014).

PPR is characterized by nasal and ocular discharges, gastroenteritis, necrotic stomatitis, pyrexia, and erosion of the pulmonary tract mucosa (Wolhsein and Saliki, 2006; Roeder and Obi, 1999). Death occurs primarily by bronco-pneumonia or extreme dehydration due to acute diarrhoea (Banyard et al., 2010). Apart from the broncho-pneumonia, symptoms of PPR are similar to those of rinderpest. Although rinderpest was officially declared eradicated in 2011, it remains imperative that PPR surveillance is conducted with laboratory assays that are specific to this disease, and do not cross-react with other pathogens. Such assays are available for nucleic acid detection by polymerase chain reaction (PCR) and antigen detection by monoclonal antibody-based immunocapture ELISA (Diallo and Libeau, 2014; Diallo et al., 1995). For detection of

Abbreviations: PPR, peste des petits ruminants; LIPS, luciferase immunoprecipitation System; RPV, Rinderpest.

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antibodies directed against PPRV, two commercial assays are available, the PPRV haemagglutinin-protein-based cELISA (Anderson & McKay, 1994) and the PPRV nucleoprotein-based cELISA (Libeau et al., 1995). The haemagglutinin protein of PPRV is a protective antigen capable of generating neutralizing antibodies (Barrett et al., 2006). The nucleoprotein of PPRV generates a strong immune-response, most of the antibodies for morbilliviruses are directed against it, although these antibodies are not protective (Diallo et al., 1995). Proteins such as the fusion protein of PPRV (FPPRV) are not used for serological assays. FPPRV is a protective antigen and generates a strong cellular immune response, but it has a weak humoral immune-response (Barrett et al., 2006).

Both ELISA tests, the PPRV haemagglutinin-protein-based cELISA and the PPRV nucleoprotein-based cELISA, detect PPR serum antibodies in a similar fashion (Couacy-Hymann et al., 2007). However, they present some cross reactivity with rinderpest sera (Anderson and McKay, 1994; Couacy-Hymann et al., 2007).

In this paper, we describe the development and characterization of the Luciferase Immunoprecipitation System (LIPS) for the specific detection of antibodies against PPRV using a specific peptide of the PPRV nucleoprotein. This fragment showed high specificity to PPRV and adequate antibody response (Bodjo et al., 2013).

In general, LIPS functions by detecting luciferase activity from the interactions of a crude extract containing a fusion protein of luciferase and a target antigen, serum and protein A/G beads (Burbelo et al., 2009). Whenever antibodies against the target antigen are present in the test serum, they bind to the antigen portion of the fusion protein. Protein A/G beads bind to the antibodies in sera and in doing so precipitate the luciferase-target-antigen fusion protein. Luciferase substrate is then added and light is measured. The amount of light emitted is proportional to the amount of fusion protein precipitated which in turn is proportional to the amount of antibody present in the serum (Burbelo et al., 2009).

LIPS which has been used in the past for antibody profiling of Epstein-Barr virus (Sashihara et al., 2009), hepaciviruses (Burbelo et al., 2012) and Lyme disease (Burbelo et al., 2015; Burbelo et al., 2010b), was adapted in this study for the detection of antibodies against PPRV.

2. Materials and methods

2.1. Generation of plasmid constructs for the expression of renilla luciferase fused to the N protein fragment 420-525 of peste des petits ruminants virus

The cytomegalovirus (CMV) promoter-driven Renilla luciferase vector, pGL4.75 (Promega) was modified by PCR to generate a new plasmid, pRFX, in which the stop codon of the luciferase gene was eliminated (primers shown in Table 1). The nucleotide region encoding amino acids 420 to 525 of the nucleoprotein of PPRV vaccine strain (Nigeria 75/1) was amplified by RT-PCR from purified RNA extracted from virus-infected cells using the One Step RT-PCR kit (Qiagen). The primers used were NP-F and NP-R (Table 1) which contained *Xba*I and *Fse*I restriction enzyme sites. The amplified product was digested with *Xba*I and *Fse*I, purified and inserted into the plasmid pRFX, which had been previously digested with the same enzymes. The PPRV nucleoprotein gene fragment was inserted downstream of the Renilla luciferase gene in order to obtain a Renilla luciferase–NPPR 420-525 fusion gene, resulting in the plasmid pRNP420 (Fig. 1).

Table 1
Primers used in the construction of pRNP420.

Primer	Sequence and Modification
FB-1	5′Phosphate-TTCTAGAGTCGGGGCGGCCGGCCG
FB-2	5′Phosphate-TCTGCTCGTTCTTCAGCACGCGCTCC
NP-F	ATCTGCTCTAGAGCTCCAGCACAAAACGGGAGAG
NP-R	ATATACGGGCCGGCCTTAGCCGAGGAGATCCTTGT

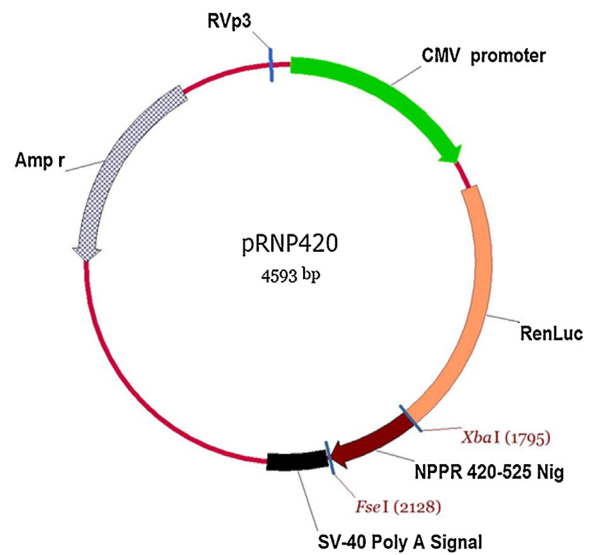

Fig. 1. Structure of the pRNP420 Mammalian Expression Vector.
Some of the features indicated are the Cytomegalovirus (CMV) promoter, the Renilla luciferase-NPPR 420-525 from Nigeria 75/1 fusion protein gene and the position of the two restrictions enzyme sites used for cloning.

2.2. Production of renilla luciferase–NPPR 420-525 fusion protein

The plasmid pRNP420 containing the gene for the fusion protein RLuc-NPPR 420-525, was purified using the HiSpeed Plasmid Midi Prep kit (Qiagen). Vero cells were grown in DMEM (Invitrogen) supplemented with 10% foetal bovine serum in 10 cm tissue culture plates. Cells were transfected with the plasmid at a ratio of 1:4 (DNA to transfectant reagent), using the standard Fugene 6 protocol (Promega). Two days after transfection, media was removed and the cells were washed with 6 ml of phosphate buffered saline (PBS), followed by the addition of 1.4 ml of cold lysis buffer [(50 mM Tris, pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$, 1% Triton X-100, 50% glycerol and protease inhibitor (2 tablets of complete protease inhibitor cocktail (Roche) per 50 ml of lysis buffer)]. Cells were then harvested with a cell scraper and the lysate transferred to a 5 ml polypropylene tube on ice.

Cells were ruptured using a sonicator (Vibra-Cell VCX 750, Sonics and Materials Inc., Newtown, CT USA) set at an amplitude of 50% using four pulses of 5 s each. The samples were then centrifuged at 16,000 g for 4 min at 4 °C and the supernatants collected and stored at −80 °C until required.

2.3. Western blot

Extracts of RLuc-NPPR 420-525 fusion-protein-transfected cells (20 μl) were heated at 80 °C for 10 min in 4X LDS Sample Buffer (Invitrogen). The samples were subjected to SDS-PAGE (NUPAGE 10% (v/v) gel, Invitrogen) and transferred to a 0.2 μm PVDF membrane (Invitrogen) using standard techniques. Baculovirus expressing recombinant full length NPPR in SF21 insect cells and baculovirus alone (Bodjo et al., 2008) were used as controls. The membrane was then probed for 1 h at room temperature with anti-PPR specific monoclonal antibody (Mab) P4G5 diluted in PBS containing 0.5% (v/v) Tween and 5% (w/v) powdered milk (Sigma). P4G5 was generated and previously characterized in our laboratory (Bodjo et al., 2008). The membrane was washed three times

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