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Long term immunity against Peste Des Petits Ruminants mediated by a recombinant Newcastle disease virus vaccine

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ABSTRACT

Peste des Petits Ruminants (PPR) is a highly contagious and often fatal disease of sheep and goats. Conventional live vaccines have been successfully used in endemic countries however, there are not completely safe and not allowing differentiation between vaccinated and infected animals (DIVA). In this study, a recombinant Newcastle disease virus (NDV) expressing the hemagglutinin of PPRV (NDV-PPRVH) was evaluated on small ruminants by serology response in sheep and goats, experimental infection in goats and immunity duration in sheep. The NDV-PPRVH vaccine injected twice at 28 days' interval, provided full protection against challenge with a virulent PPR strain in the most sensitive species and induced significant neutralizing antibodies. Immunological response in goats was slightly higher than sheep and the vaccine injected at $10^{8.0}$ 50 % egg infective dose/mL allowed anti-PPRV antibodies that lasted at least 12 months as shown by antibody response monitoring in sheep. The NDV vector presented a limited replication in the host and vaccinated animals remained negative when tested by cELISA based on PPRV nucleoprotein allowing DIVA. This recombinant vaccine appears to be a promising candidate in a free at risk countries and may be an important component of the global strategy for PPR eradication.

1. Background

Peste des petits ruminants (PPR) is a highly contagious disease of small ruminants that causes considerable clinical and economic losses (Albina et al., 2013; Buczkowski et al., 2014; Parida et al., 2016). The aetiological agent is the PPR virus (PPRV), which is an enveloped negative-strand RNA virus belonging to the *Morbillivirus* genus of the *Paramyxoviridae* family, classified as *Small ruminant morbillivirus* species (Amarasinghe et al., 2019). Since the first outbreak in Ivory Coast in 1942, PPR distribution expanded to North and Austral Africa, Middle East, Central Asia and China. More than 70 countries confirmed the presence of the disease and many countries are at risk of introduction (OIE, 2015).

Vaccination remains the main tool for PPR prevention and the use of appropriate vaccines should be carried out according to the epidemiological context of the region. The live attenuated vaccine confer solid

Abbreviations: BEFV, bovine ephemeral fever virus; BHV-1, bovine herpesvirus; BSL3, biosafety level 3; CEF, chicken embryo fibroblast; CDV, Canine Distemper Virus; DIVA, Differentiating Infected from Vaccinated Animals; D, day; dpi, days post infection; dpv, day post vaccination; EID_{50}, 50 % egg infective dose; ELISA, enzyme-linked immunosorbent assay; NDV-PPRVH, recombinant NDV vectoring H gene of PPRV vaccine; H, hemagglutinin; F, fusion protein; ICPI, intracerebral pathogenicity index; IFA, immunofluorescence assay; IM, intramuscular; IN, intra-nasal; IV, intravenous; IVPI, intravenous pathogenicity index; MDT, mean death time; M.O.I, Multiplicity of Infection; NA, neutralizing antibody; ND, Newcastle disease; NDV, Newcastle Disease Virus; NiV, Nipah virus; PPR, Peste des petits ruminants; PPRV, Peste des petits ruminants virus; qRT-PCR, quantitative real-time reverse transcriptase-polymerase chain reaction; RV, rabies virus; RVFV, Rift Valley Fever virus; SC, subcutaneously; SGP, Sheep and goat pox; SPF, Specific Free Pathogen; VN, virus neutralization; VSV, vesicular stomatitis virus; WNV, West Nile virus.

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protection, but the vaccine is heat-sensitive, does not allow to differentiate infected from vaccinated animals (DIVA) and present a risk of transmission of extraneous agents (Diallo et al., 2007; Silva et al., 2011). Efforts towards developing new generation vaccines based on DIVA concept are of great interest in the final phase of disease eradication, making possible epidemiological surveillance in areas where both vaccination and stamping out are applied (FAO and OIE, 2015). The experimental use of sheep and goat poxvirus (SGPV) vaccine vectoring PPR virus (PPRV) genes has been reported (Adama Diallo et al., 2002; Chen et al., 2010; Fakri et al., 2018). Those recombinant vaccines can be used only in SGP endemic areas and the mammalian vector virus is replicating in vaccinated animals. In this study we evaluated protection with a recombinant vaccine using a worldwide distributed avian vector.

Newcastle disease virus (NDV, aka avian paramyxovirus 1) is an enveloped, non-segmented, negative-stranded RNA virus, belonging to the *Orthoavulavirus* genus of the *Paramyxoviridae* family, classified as avian paramyxovirus serotype 1 (APMV-1, species avian orthoavulavirus 1) (Rima et al., 2019). Initially applicable only to DNA viruses, reverse genetic technology was then applied to a large group of negative-strand RNA viruses, to which NDV belongs (Kim and Samal, 2018). NDV recombinant viruses, were first developed for influenza then extended to other viruses like bovine herpes 1, canine distemper, ephemeral fever, infectious bronchitis, Nipah, rabies, Rift Valley fever, vesicular stomatitis, West Nile viruses and recently for PPRV (Ge et al., 2015, 2011, 2010; Huang et al., 2004; Khattar et al., 2010; Kong et al., 2012; Kortekaas et al., 2010; Murr et al., 2020; Nakaya et al., 2001; Pepin et al., 2010; Wang et al., 2016; Zhang et al., 2017, 2016; Zhao et al., 2017). NDV strains are classified as low-virulent (lentogenic), moderately virulent (mesogenic) and highly virulent (velogenic) (Alexander, 1997). Currently, lentogenic strains, such as LaSota and B1, are used for the production of live attenuated vaccines in poultry farming as well as a vaccine vector.

In this study, LaSota strain has been used as a vector to express the structural hemagglutinin (H) glycoprotein of the PPR virus, which is essential for cell attachment and virus penetration. The objective of this study is to evaluate conferred protection in target animals by serology monitoring and challenge of the recombinant NDV-PPRVH vaccine.

2. Material and methods

2.1. Cells and viruses

Human epithelial-2 cells (HEp-2, ATCC No. CCL-23) and baby hamster kidney cells (BHK-21, ATCC No.CCL-10) cells were grown in dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10 % fetal bovine serum (FBS, Gibco). African green monkey kidney cells (Vero, ATCC No.CCL-81) were cultured in the same medium containing 5% FBS. Chicken embryo fibroblasts (CEF) were prepared from 10-day-old specific-pathogen-free (SPF) embryonated chicken eggs (ValoBio-Media, Germany) and grown in DMEM containing 10 % FBS. All media were supplemented with L-glutamine, NaHCO3 and sodium pyruvate.

The NDV vaccine strain LaSota (GenBank accession no. AY845400.2) used as vector was originally received from the China Veterinary Culture Collection. Live attenuated PPRV vaccine strain Nigeria75/1 (N75/1) was obtained from the China Institute of Veterinary Drug Control, propagated and titrated on Vero cells. Recombinant NDV strains were grown and titrated in 9-day-old SPF embryonated chicken eggs by inoculation of the allantoic cavity. Recombinant NDV was also grown and titrated in BHK-21 or HEp-2 cells in Opti-MEM (Invitrogen). CEF were used for propagation and characterization of recombinant NDV. The infection of NDV in BHK-21 cells was detected by using an indirect immunofluorescence assay (IFA) and observing the cells under an immunofluorescence microscope. The modified vaccinia virus strain Ankara (MVA) expressing the T7 RNA polymerase was grown in primary CEF (Wyatt et al., 1995). All viruses were stored at −80 °C before use.

2.2. Establishment and characterization of the recombinant virus

The recombinant NDV expressing the hemagglutinin (H) of PPRV N75/1 strain, was generated by reverse genetics in Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences as described previously (Ge et al., 2007). Briefly, the hemagglutinin (H) of PPRV open reading frame cDNA was amplified from RNA genome by reverse transcription RT-PCR. The H gene was introduced into the NDV genome contained in pLa through PmeI site in the P-M intergenic region at nucleotide position 3165 of the NDV genome (Fig. 1). The resultant plasmid designated pLa-PPRVH, was used for virus rescue as described by Ge. et al. (Ge et al., 2015, 2011, 2007). HEp-2 cells were infected with MVA-T7 at a multiplicity of infection (MOI) of 1 and then transfected with 1 mg of pLa-PPRVH together with expression plasmids. After 16 h of incubation at 37 °C, the medium was replaced with 2 mL of fresh OptiMEM containing 0.5 mg of the protease inhibitor N-tosyl-phenylalanine chloromethylketone trypsin, and cells were incubated for another 3 days at 37 °C. The supernatant was first propagated in 9-day-old embryonated SPF eggs and the allantoic fluid, was used to infect CEF cells. The recovered virus was confirmed by sequencing the entire viral genome.

Master seed viruses were propagated and passed in SPF embryonated eggs for 10 passages. The virus titration was carried out for the 1th, 5th and 10th passage virus. The expression of the PPRV H protein in the 10th egg-passage virus infected BHK-21 cells were confirmed by immunofluorescence. The master seed virus was also passed in SPF chicken for five generations. For each generation, oral-laryngotracheal swabs were collected at 3 days post inoculation. The viruses were recovered by inoculation in 9-day-old SPF chicken embryo eggs. The virus recovered from the 5th chicken-passage was used to test in vitro the presence of H gene (by PCR), the H protein expression (by IFA) and in vivo for pathogenicity testing by determination of the mean death time (MDT), the intracerebral pathogenicity index (ICPI) and the intravenous pathogenicity index (IVPI) in embryonated SPF eggs or in SPF chickens as described previously by authors (Alexander, 1989; OIE, 2018; Swayne et al., 2003).

The immunofluorescence assay was performed as described previously (Ge et al., 2011). The primary antibodies used were specific polyclonal sera against PPRV from inactivated PPRV 75/1-immunized mice and against NDV from NDV LaSota immunized chicken. Secondary antibodies used were fluorescein isothiocyanate-conjugated rabbit anti-chicken IgGs (Sigma). Cells were analyzed with a fluorescence microscope.

2.3. Recombinant vaccine production

NDV-PPRVH was grown and titrated in 9-day-old SPF embryonated eggs. The use of SPF eggs, not containing antibodies against NDV, is an important parameter for safety and to increase the harvested virus titre. The virus was inoculated into the allantoic cavity and the harvest of the allantoic fluid was performed 96 h later when the infectious titre of the virus was the highest. Viral titration was performed by inoculation of serial dilutions on 9-day-old embryonated eggs, viral presence detected by haemagglutination and results expressed as median egg infective doses (EID_{50})/mL, using the endpoint method (Reed and Muench, 1938). The virus was stored at -80 C before use. The live vaccine was prepared with the virus suspension by addition of a stabilizer (peptone in phosphate buffer) at 50 % followed by lyophilisation, with freezing at -60°c during 4 h, a sublimation step at −12 °C during 24 h and a desorption phase at 24 °C for 14 h. The final product was tested according to the OIE Terrestrial Manual for sterility, identity, purity and infectious titre (OIE, 2017).

2.4. Vaccination

We used 40 Alpine goats and sheep of local known sensitive breed

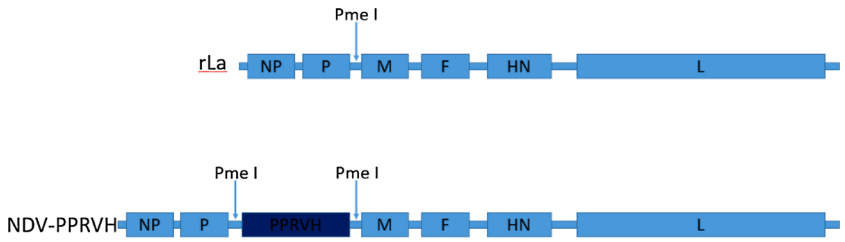

Fig. 1. Generation of recombinant NDV expressing the PPR H gene. Schematic representation showing the rLaSota genome with the restriction endonuclease Pme I site introduced between the P and M genes and the PPR H gene inserted into the Pme I site.

(Fakri et al., 2017), 6–9 months old, from an experiment farm in Morocco NW. Animals were first tested negative for PPRV by cELISA. Three groups of sheep and goats were vaccinated for dose escalation and challenged as shown in Table 1. Vaccination consists on a 1 mL primary injection followed by a booster 28 days later by intramuscular route. A group of two sheep and two goats (G4) was kept unvaccinated as control.

2.5. Vaccination monitoring and DIVA evaluation

Sera were collected weekly or monthly before and after vaccination. Antibody response was monitored for PPRV by virus neutralization (VNT) and enzyme-linked immunosorbent assay (ELISA). VNT was performed in 96-microwell plates. The test is based on a serial ¼ dilutions of heat inactivated sera mixed with infectious virus (100 TCID50). Sera were tested in replicates (four wells per dilution) for neutralization activity against PPRV strain Nigeria 75/1. The neutralizing antibody titre was calculated in accordance to Reed and Muench method (OIE, 2016, 2013). cELISA kit based on nucleoprotein (N) (PPRC-4 P ID-VET) was used to check DIVA property (Libeau et al., 1995). bELISA kit based on H protein was used to detect kinetic of PPR antibody response (Bodjo et al., 2018). Serology response for goats was monitored during two months after vaccination except challenged animals, for sheep until 12 months' post vaccination for G1, vaccinated by the lowest dose ($10^{8.0}$ EID_{50}). Serology was also checked to detect NDV replication in animals using Newcastle Disease Virus Antibody Test Kit (ProFLOK™ NDV Ab, Zoetis) and detection by HRP conjugated, sheep IgG antibody (A130−100 P, Bethyl) and goat IgG antibody (A50−104 P, Bethyl).

2.6. Challenge for vaccine potency

The vaccine potency was evaluated by experimental infection of goats. Goats appeared to be very sensitive to the PPR disease comparatively to sheep. Sheep produced mild symptoms making difficult to assess vaccine protection under experimental conditions (Fakri et al., 2017). Also, in the field, during PPR outbreaks in Morocco, the overall rates of morbidity and mortality levels in sheep remained very low compared to what is typically reported in the literature (Fakri et al., 2016; Hammouchi et al., 2012). The model of challenge has been conducted on goats as described by two authors (El Harrak et al., 2012; Hammouchi et al., 2012) and validated in our previous studies to assess vaccine protection against PPR (Fakri et al., 2018, 2015).

Experiments was performed under biosafety level 3 (BSL3) in accordance with the guidelines described for the care and handling of experimental animals by the Laboratory Committee for Control and Supervision of Animal Experimentation. Two weeks after the second vaccination, goats of G1 and goats of G4 were challenged with virulent PPRV strain. The used PPR virulent strain of lineage IV was isolated during the 2015 outbreak in Morocco from a lamb showing characteristic clinical signs of PPR (Fakri et al., 2016). This strain is known to induce characteristic symptoms of the disease.

Alpine goats were challenged by intravenous (IV) injection (1 mL) and intra-nasal (IN) spray (1 mL) of PPRV virulent strain (El Harrak et al., 2012). The titre of the inoculated virus was $10^{5.4}$ $TCID_{50}$/mL. Monitoring was based on a daily observation of hyperthermia and clinical signs from 0 to 14 days' post infection (dpi) according to Elharrak et al. (2012) and Hammouchi et al. (2012). Clinical scores were used to evaluate the disease severity and to allow comparison between animals and groups. The clinical scoring was followed with a ranking from 0 to 4 based on the severity of: general behavior, hyperthermia, alimentation, diarrhea, nasal discharge, salivation, respiratory symptoms including dyspnea, coughing, sneezing and mortality. A total cumulative score of the assessed signs per animal per day were then calculated. Animals that showed clinical symptoms of the disease were euthanized when the clinical score reach 15–18 according symptoms severity (dyspnea, diarrhea). Blood on dry tubes, lacrimal, nasal and rectal swabs were collected from goats every 3 days post infection. All surviving animals were euthanized at the end of the experiment. Specific post mortem (PM) samples were taken from lung, mesenteric nodes, pulmonary nodes, trachea and liver for virus or RNA detection.

Table 1
Animals group distribution. Description of animal number per group and the administrated dose.

Group number	Animals		Dose ($logEID_{50}$/mL)
	Sheep	Goats	
G1	8	4	8.0
G2	8	–	8.7
G3	8	8	9.5
G4	2	2	unvaccinated

2.7. qPCR screening

PPRV genome detection was performed on PM samples and swabs using quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) (Batten et al., 2011). RNA extraction was accomplished using a RNA kit (Bioline BIO-52075, isolate II RNA Mini kit). Amplification was carried out with the Invitrogen Superscript III Platinum R one step qRT-PCR system (Cat. no. 11,745-100).

2.8. Statistical analysis

Significant differences among groups regarding hyperthermia, protection index, clinical scoring and serological response were determined using Student's *t*-test. A P-value of ≤ 0.05 was considered statistically significant.

3. Results

3.1. Rescued recombinant virus

The growth titre of the 1th, 5th and the 10th of NDV-PPRVH in SPF eggs were determined. There was no significant difference in growth titres among different passages. The virus titres of the 1th, 5th and the 10th egg-passages were $10^{9.5}$, $10^{9.4}$ and $10^{9.5}$ EID_{50} per ml of egg fluid, respectively. The presence of PPRV H gene in the genomes of the 1th, 5th

and the 10th egg-passages of NDV-PPRVH were confirmed by PCR detection. The expression of PPRV H protein of the 1th, 5th and the 10th egg-passages of NDV-PPRVH in BHK-21 cells were also confirmed by immunofluorescence assay (IFA).

For each generation of master seed passed in SPF chicken, oral-laryngotracheal swabs were collected at 3 days post inoculation. The viruses were recovered after SPF chicken embryo eggs inoculation. The virus recovered from the 5th chicken-passage was used to the PCR detection for presentation of H gene in viral genome and IFA for expression of H protein in BHK cells were confirmed. The MDT, ICPI and IVPI were ≥120 h, 0 and 0 respectively.

These results indicated that the master seed NDV-PPRVH kept its protein properties (gene and expression) and low pathogenicity to poultry after egg and chicken passages.

3.2. Vaccine Safety on target species

During 14 days following first vaccination and booster, all vaccinated animals remained healthy, without any effect on their appetite and behavior. No abnormal reaction in the injection site and no hyperthermia exceeding 1 °C were reported.

3.3. Vaccine immunogenicity

Results of sheep dose escalation study showed a significant difference ($p < 0.05$) in the neutralizing antibody response between the high and the low dose although the 3 groups of sheep have a minimal average antibody titre of 2.0 (equivalent to a serum dilution of 1/100) one week after the second vaccination and 1.7 (equivalent to a serum dilution of 1/60) three months pv (Fig. 2). After the first vaccination, percentage of seroconversion reach 37.5 % for G1 and G2, and 50 % for G3. After the second vaccination, 100 % of animals seroconverted with no dose effect. There was a significant increase of antibody response ($p < 0.05$) after the booster for all groups and the average antibody titer for each group could be considered positive only after the second immunization with average values greater than the threshold of 1 in log.

Comparative study of the response to vaccination between goats and sheep at the same dose (G3) revealed 75 % of seroconversion of goats to the first injection VS 50 % in sheep and the average antibody titre was significantly higher ($p < 0.05$) in goats. 50 % of goats (G3) were antibody positive as soon as 14 dpv confirmed by bELISA, with an average antibody titer of 1.01 (equivalent to a serum dilution of 1/10) at 28 dpv (Figs. 3 and 4), to reach 100 % of positive animals after the booster with an average value of 3.08 (equivalent to a serum dilution of 1/1200). The antibody kinetic evaluation by bELISA confirmed the significant increase of the PPRV H antibodies ($p < 0.05$) following the booster for goats and sheep to reach an average inhibition percentage of 80.01 and 50.32 for goats and sheep (Fig. 4).

Regarding the NDV antibody detection evaluated on group 3, at 21 days post vaccination (dpv) 2/8 sheep seroconverted to NDV after one injection, at 28 dpv 3/8 sheep and 3/8 goats. All vaccinated animals seroconverted to NDV antigen two weeks after the second injection. The antibody kinetic evaluation showed a significant increase of the average NDV s/p ratio ($p < 0.05$) following the booster, from 0.189 to 0.623 and 0.170 to 0.632 for sheep and goats respectively (Fig. 3). The number of positive animals decreased to 2/8 sheep and none of goats two months after the booster. Sheep were tested negative for NDV at least from the fifth months after the booster.

3.4. DIVA evaluation and immunity duration

Unvaccinated animals remained negative during all the experiment period (before challenge for goats), no antibodies were detected by VNT of PPRV and ELISA of N and H by cELISA and bELISA respectively. For unvaccinated goats, one animal presented antibodies against N and H, at D9 after infection with inhibition percentage of 58.76 and 26.78 respectively. The second goat died at D7, no antibodies were detected in sera at D6 for both animals. When tested by cELISA based on the PPRV nucleoprotein, all vaccinated animals were detected negative comparatively to antibodies against H detected by bELISA as shown by Fig. 4. At the end of the 12 months, when tested by cELISA, all vaccinated sheep remained negative (Fig. 5).

All sheep of G1 still presented neutralizing antibody until 12 months pv to reach an average value of 1.5 (Fig. 5). A significant increase of the antibody response was observed after the booster to reach an average value of 2.0, to stabilize from the sixth month pv around 1.5.

3.5. Protection against virulent PPRV challenge on goats

After challenge the two unvaccinated Alpine goats (G4) showed specific clinical signs of PPR infection: dyspnea (2/2), nasal (1/2) and ocular discharges (1/2) followed by a respiratory syndrome with painful dyspnea (2/2) and profuse diarrhea (2/2) in the terminal stage. The two control goats died at D7 and D9 after infection with a clinical score of 17

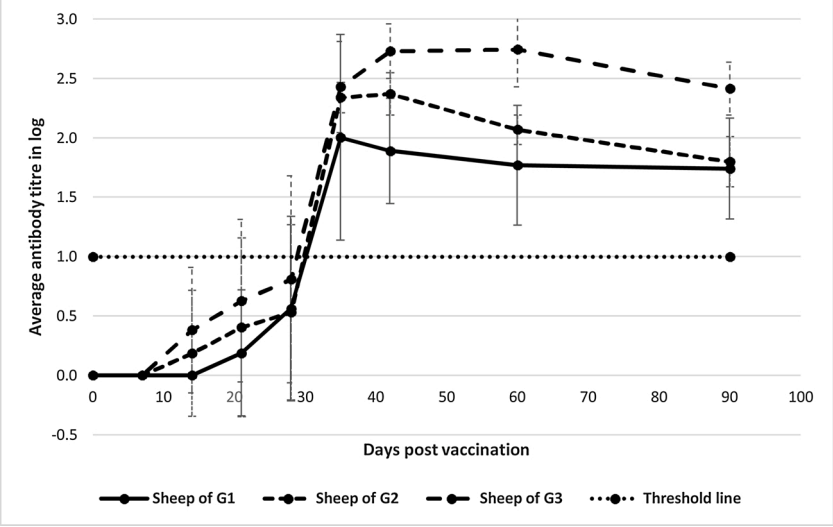

Fig. 2. Vaccine dose escalation evaluated on sheep (mean antibody titer). Neutralizing peste des petits ruminants virus antibody kinetic evaluation after vaccination of goats with the recombinant NDV-PPRH vaccine at $10^{8.0}$, $10^{8.7}$and $10^{9.5}EID_{50}$/dose for G1, G2 and G3 respectively. VNT titer >1.00 in log (equivalent to a serum dilution of 1/10) was considered positive (threshold line).

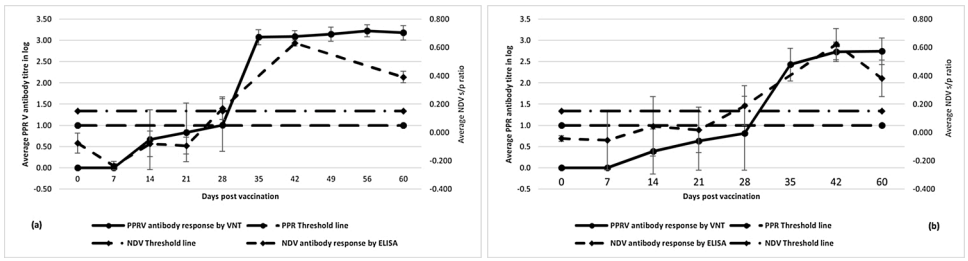

Fig. 3. Antibody kinetic evaluation against peste des petits ruminants and Newcastle viruses after vaccination of goats (a) and sheep (b) of group 3 (mean antibody titer). Vaccination was conducted with the recombinant NDV-PPRH vaccine at $10^{9.5}$ EID_{50}/dose. The virus antibody kinetic was performed by peste des petits ruminants viral neutralization test and by ELISA for antibodies against Newcastle virus. VNT titer >1.00 in log (equivalent to a serum dilution of 1/10) was considered positive. ELISA NDV antibody s/p ratio >0.150 was considered positive.

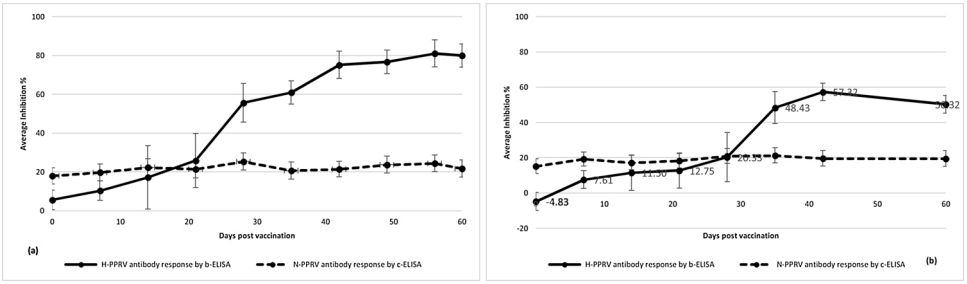

Fig. 4. Antibody kinetic evaluation against hemagglutinin (H) and nucleoprotein (N) of peste des petits ruminants virus after vaccination of goats (a) and sheep (b) of group 3. Vaccination was conducted with the recombinant NDV-PPRH vaccine at $10^{9.5}$ EID_{50}/dose. The antibody kinetic was performed b-ELISA for antibodies against H and by c-ELISA for antibodies against N.

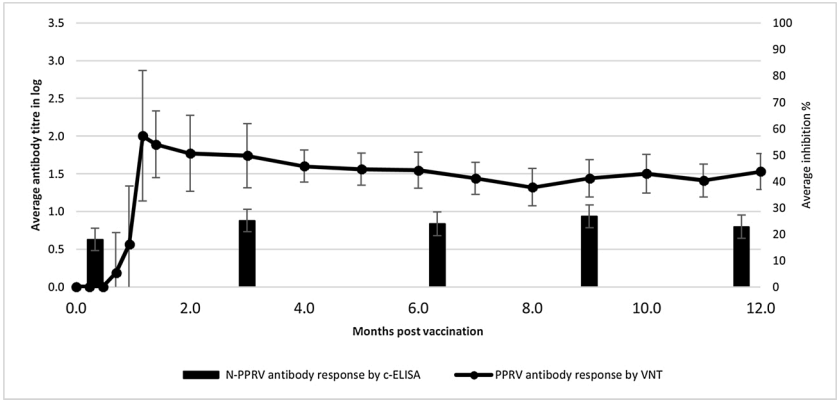

Fig. 5. Serology evaluation during 12 months after vaccination of sheep of group 1 with the recombinant NDV-PPRH vaccine at $10^{8.0}$ EID_{50}/dose. The virus antibody kinetic of sheep was performed by peste des petits ruminants viral neutralization (mean antibody titer) and by c-ELISA for antibodies against nucleoprotein (N).

and 18 respectively. For vaccinated goats (G1), no clinical symptom was reported during the 14 days of the observation period, body temperature remained normal and clinical scoring was nil. qPCR analysis showed a viral excretion detected in ocular (Ct 28.7 and 20.5) and rectal swabs (Ct 16.3 and 18.2) of unvaccinated goats. At PM examination, specific lesions of pneumonia and inflammatory nodules were observed in lung and digestive tract with enlarged and hypertrophied nodes. High viral concentration was detected by qPCR in mesenteric node (Ct 14.7 and 15.2) and in lung (Ct 17.2 and 19.4). No viral genome detection by qPCR in vaccinated animals swabs or tissues.

4. Discussion

Peste des Petits Ruminants is worldwide distributed with huge economical losses that justify the implementation of a Global Eradication Program (FAO and OIE, 2015). Live vaccines are currently used in endemic countries with satisfactory results despite presence of negatives aspects like the weak thermotolerance or risk of extraneous agents' transmission. New vaccine generation may avoid inconvenient and allow differentiation between infected and vaccinated animals.

Newcastle disease virus (NDV) has several advantages : the vector of avian origin has an abortive replication in mammalians, locally restricted to the site of inoculation (Bukreyev et al., 2005; Murr et al., 2020), our results showed that the vector virus can replicate but at a low level, no antibody against NDV was detected from the second months, for goats, after the booster comparatively to PPRV antibody.

LaSota is an attenuated strain, safe in avian and non-avian species as described by several authors (Choi, 2017) and demonstrated in our study. The use of non-mammalian virus vector represents also an important benefit in vaccine safety considering potential extraneous contaminant agent transmission through vaccination (Bumbarov et al., 2016; Pastoret, 2010; Rajko-Nenow et al., 2020) and mammals lack preexisting NDV immunity, which minimizes the risk of vaccination failure. Unlike herpesvirus and poxvirus vectors whose genome codes for a large number of proteins, NDV codes for only six structural proteins and is therefore less competitive for immune responses between the

vector and expressed recombinant proteins. NDV replicates in the cytoplasm, does not integrate the host cell DNA, potential recombination is then extremely rare (Conzelmann, 1998; Huang et al., 2003; Nakaya et al., 2001; Roberts and Rose, 1998).

PPRV strains are known to be heat sensitive which affects vaccine efficacy in the tropics. As it was demonstrated by Murr et al. (2020), NDV seems to be more thermotolerant than PPRV and the use of NDV as a vector could represent an advantage. Moreover, an author has been demonstrated that the used LaSota strain was more stable than Clone 30, B1 and VG-GA strains that could be an additional advantage (Boumart et al., 2016). A future investigation of the NDV-PPRH thermostability with incubation at different temperatures should be performed.

In this study, we evaluated the ability of the recombinant NDV-PPRVH vaccine expressing the H protein to induce an immune response to protect sheep and goats against PPR. The H gene was inserted in the P-M intergenic region of the NDV vector. Results found by an author showed that foreign genes can be inserted at different positions in the NDV genome without severely affecting replication efficiency or virus yield (Zhao and Peeters, 2003). Murr et al. (2020) obtained recently a protection of goats with the H gene inserted in the F-HN region of the NDV vector.

Results reported by Ge et al. (2015) suggested that the NDV vector of the H protein of the CDV has a more pronounced ability to induce antibody response against CDV in minks than the one with the fusion (F) protein and the NDV-CDV F fail to protected mink from virulent CDV challenge (Ge et al., 2015). The same authors demonstrated that the recombinant NDV vaccine can induce in dogs a long-term protective immunity against rabies (Ge et al., 2011). Our results also confirmed the role of H protein in the immunity process to induce PPRV neutralizing antibody production in sheep. Vaccinated sheep at a dose of $10^{8.0}$ EID_{50}/mL still present antibody against PPRV until 12 months after vaccination. The developed recombinant NDV-PPRV vaccine, at the recommended dose with double immunization, is likely to protect small ruminant for at least 12 months. In this study we focused also in post vaccination monitoring in sheep for immunity duration, comparatively to recent study in addition to evaluation of protection in goats, due to the important role of sheep as reservoir during a PPR control program (Fakri et al., 2017; Murr et al., 2020).

Our results demonstrated that the developed recombinant vaccine was protective for small ruminants at a dose of $10^{8.0}$ EID_{50}/mL. The vaccination with the recommended dose induced significant antibody response against PPRV in sheep and sensitive animal protection against challenge with a virulent PPRV strain. Goats are more sensitive than sheep to express the disease and the experimental infection is more reliable (El Harrak et al., 2012; Fakri et al., 2017; Hammouchi et al., 2012). Furthermore, in our experiment serological antibody titres are higher in goats at the same vaccine dose.

In dose escalation experiment, we injected three different doses and demonstrated a significant correlation with immunological response. As reported by other authors the protective dose for cattle, pig, horse and pets was $10^{7.0}$ to $10^{9.0}$ EID_{50}/dose in BHV-1, BEFV, RVFV, VSV, NiV, WNV, CDV and RV vaccines. Regarding PPRV, Murr et al. (2020) recommended a double injection at $2 \times 10^{6.5}$ $TCID_{50}$/mL, that seems to be efficient to protect goats against the disease, in our study the recommended dose was $10^{8.0}$ EID_{50}. Furthermore, serology testing showed that the second vaccination increased significantly the immune response, it's has been also confirmed by several authors testing other NDV recombinant vaccines (Chen et al., 2010; Gao et al., 2006; Murr et al., 2020; Weingartl et al., 2006). For instance, in calves, a single intramuscular recombinant NDV RVF vaccination induced neutralizing antibodies and this response was significantly enhanced by a booster (Kortekaas et al., 2010). A second vaccination was also necessary for the NDV Herpesvirus-1 bovine recombinant vaccine (Khattar et al., 2010). Vaccination with the NDV recombinant rabies virus vaccine has resulted in strong and lasting production of neutralizing antibodies against rabies virus in dogs and cats (Ge et al., 2011). In our study, at the recommended dose, the second immunization was necessary to reach 100 % of seroconverted sheep with an average antibody titre around 2.0 (equivalent to a serum dilution of 1/100) 14 dpv, also confirmed by Murr et al. (2020) with titers ranging between 1.3 (equivalent to a serum dilution of 20) and 1.9 (equivalent to a serum dilution of 1/80) in goats three weeks after the second inoculation. In our study the VNT were based on homologous neutralization while Murr et al. (2020) reported heterologous neutralization, however an author demonstrated that the presence of strong epitopes common to all lineages provides a mechanism for the broad cross-protection seen between PPRV strains (Hodgson et al., 2018).

For poultry, NDV based vaccines are generally administered by the respiratory route to induce local immunity (Alexander, 1997). DiNapoli et al. (2009) and Subbiah et al. (2008) demonstrated that intranasal route for delivery in mammals is not recommended because of poor replication of NDV in the upper respiratory tract of mammals due to the lower temperature when compared to birds. Those authors recommend intra-tracheal route (DiNapoli et al., 2009; Subbiah et al., 2008). The finding is in accordance with Kortekaas et al. (2010) who compared in calves the induced immunogenicity when the recombinant NDV RVF vaccine was administered by intranasal route versus intramuscular route. Results demonstrated that vaccination via IM route with live NDV vector vaccine was more effective. Hence we selected in this study the IM route, more practical for vaccination and can guarantee the dose administration. Subcutaneous route is more routinely used for ruminant vaccination, but IM route facilitate systemic reaction knowing that the NDV vector has a limited replication cycle in mammalians (Harmsen et al., 2011).

Development of a recombinant NDV-PPRVH represents in addition the possibility to differentiate between vaccinated and infected animals, only antibodies against hemagglutinin (H) protein should be detected in vaccinated animals with no antibodies against nucleoprotein (N) comparatively to infected animals. The developed recombinant NDV-PPRVH vaccine, can easily be differentiated with wild-type and attenuated PPRV strains circulating in the field. Vaccinated animals remained negative in serology when tested by cELISA based on the N protein and were positive to bELISA that detected antibodies against H protein, which confirm the DIVA potential of the recombinant vaccine, comparatively to unvaccinated animal with no antibody detection and infected animals with detection of both antibodies against N and H proteins.

5. Conclusion

In conclusion, the NDV-PPRVH recombinant vaccine induced on sheep and goats high level of neutralizing antibodies and conferred protection against PPR challenge in the most sensitive species. The vaccine conferred an immunity for at least 12 months as demonstrated in sheep and allowed DIVA. This recombinant vaccine appears to be a promising candidate in a free at risk countries and may be an important component of the global strategy for PPR eradication. In addition, the vaccine is safe for small ruminants with no risk of reversion, shedding or transmission of extraneous agents. Assessment of the vaccine candidate in field conditions should be carried out in the future.

Ethics approval

Animal experiments were carried out in accordance with the international guidelines for care and handling of experimental animals. The study protocol was approved by the Internal Ethic Committee.

Consent for publication

Not applicable

Availability of data and material

All data generated or analyzed during this study are included in this published article.

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Authors' contributions

WC and ZBu performed the construction of recombinant viruses. FF and ZBa performed the animal experiments. FF and SE performed the serology testing. ZE performed the molecular biology testing. ME, WC and Zbu designed and supervised the study. FF and ME analyzed the data. KT participated in the BSL3 containment research with respect of animal welfare standards. FF and ME drafted the manuscript. All authors have read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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