Vaccine: X 15 (2023) 100384

Contents lists available at ScienceDirect

Vaccine: X

journal homepage: www.elsevier.com/locate/jvacx

Production of recombinant lumpy skin disease virus A27L and L1R proteins for application in diagnostics and vaccine development

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ARTICLE INFO

Keywords:
Lumpy skin disease virus
A27L
L1R
Subunit vaccines
Montanide™ Gel 01 PR

ABSTRACT

Vaccination using live attenuated vaccines (LAVs) is considered the most effective method for control of lumpy skin disease (LSD). However, this method is limited by safety concerns, with reports of adverse reactions following vaccination. This study evaluates A27L and L1R which are essential proteins for virus attachment and membrane fusion as recombinant sub-unit vaccines against LSD. These proteins were recombinantly expressed in *Escherichia coli* and purified using affinity chromatography. Purified proteins were formulated individually (A27L or L1R) and in combination (A27L and L1R) with 10% (w/w) Montanide™ Gel 01 PR adjuvant at a final antigen dose of 20 μg per protein. The safety and immunogenicity of these formulations were evaluated in rabbits in a 42-day clinical trial. Animals were vaccinated on day 0 and boost injection administered 21 days later. No reduced morbidity, increased temperature and any other clinical signs were recorded in vaccinated animals for all three vaccine formulations. The highest neutralizing antibody response was detected on day 42 post-primary vaccination for all formulations when using serum neutralising assay. The neutralisation data correlates with antibody titres quantified using a whole cell ELISA. Evaluating the combination of A27L and L1R as potential diagnostic reagents showed highest sensitivity for detection of antibodies against LSD when compared to individual proteins. This study reports the immunogenicity of recombinant A27L and L1R combination for successful application in LSD vaccine development. Furthermore, these proteins demonstrated the potential use in LSD diagnostics.

Introduction

Lumpy skin disease (LSD) is a contagious bovine viral disease that affect cattle of all ages and breeds with younger and lactating calves being more susceptible [1]. The etiological agent of LSD, lumpy skin disease virus (LSDV) is classified in the genus Capripoxvirus of the family Poxviridae along with sheeppox and goatpox viruses [2]. LSD was initially known as an African disease with no country in the sub-Saharan exempted from the outbreak [3–5]. The disease has rapidly spread and is considered transboundary following outbreaks in the Middle East, Europe, Russia, and most recently Asia which were previously considered disease-free regions [6–10]. LSD is a notifiable disease of high economic importance as it causes a severe reduction in milk production, reduced hide and meat quality, infertility, abortion, and sometimes death of infected animals [8].

Currently, there is no treatment for LSD, vaccination and active serosurveillance remain the most effective measures to control the spread of the virus [11]. The commercially available vaccines for prophylactic immunization of cattle are mostly live attenuated vaccines (LAVs) manufactured with the Neethling strain or its' derivatives. An indirect ELISA kit has been commercialized for serosurveillance and the diagnostic of LSDV and other Capripoxviruses. Though LAVs have shown efficacy, they are subject to safety concerns and are prone to adverse reactions following vaccination [12–15]. Most recently, research studies have explored LSD-inactivated vaccines as an alternative treatment for preventing and managing LSD infections [16–18]. These types of vaccines are considered safer than LAVs, however, studies have reported that short-term and partial protection was observed following vaccination with inactivated LSDV vaccines [16,17]. Hence, there remains a drive for the development of safer new generation LSD vaccines without residual pathogenicity and confer a higher level of protection. Advances in genetic technology and vaccine design

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https://doi.org/10.1016/j.jvacx.2023.100384
Received 24 June 2023; Received in revised form 4 September 2023; Accepted 5 September 2023
Available online 7 September 2023

strategies have promoted recombinant subunit vaccines as efficient and safer alternatives for disease management. Antigens used in subunit vaccine development can now be designed using nanotechnology while others display systems such as virus-like particles (VLPs) that improve subunit vaccine efficacy [19]. Moreover, antigens can also be used as diagnostic reagents for disease surveillance.

The LSDV ORF 117 and ORF 060 are orthologs of vaccinia virus (VV) proteins A27L and L1R, respectively. These proteins have been reported as potential antigens and are targets of neutralizing antibodies following their ability to elicit an immune response in other poxviruses such as sheeppox and VV [20–22]. The A27L protein is found on the surface of the intracellular mature virion (IMV) and is known to play an important role in virus-cell binding and membrane fusion [23,24]. Moreover, A27L participates in the transportation of the intracellular virus and the formation of the intracellular enveloped virion (IEV) [25,26]. L1R is a myristylated protein essential for the formation of infectious IMV [27]. The L1R protein also participates in cell entry and membrane fusion [27,28]. Immunization of mice with these recombinant proteins in VV resulted in complete protection from infection following a challenge with a lethal dose of virulent strain [29–32]. These studies have demonstrated the potential application of IMV recombinant proteins as suitable vaccine candidates. To our knowledge, there are currently no studies that have reported on the immunogenicity of the combination of LSDV A27L and L1R recombinant proteins. Therefore, in this study, we recombinantly expressed LSDV A27L and L1R for possible application in subunit vaccine development and assessed the potential use of these antigens in diagnostics.

Methods and materials

Virus propagation and genomic DNA extraction, and gene amplification

The propagation of the Neethling LSDV vaccine strain into Mardin Darby Bovine Kidney (MDBK) cells and the maintenance of cells was achieved as described in Matsiela et al., 2022 [18]. The LSDV genomic DNA was extracted using the QIA amp DNA mini kit (Qiagen) according to the manufacturer's instructions. The DNA fragment encoding for the LSDV genes A27L and L1R were amplified from the genomic DNA by PCR amplification using specific gene primers (Table 1). Amplification was conducted with the Phusion® HF DNA Polymerase (New England Biolabs) according to the manufacturer's instructions, specifically conducted with 30 cycles of amplification and the annealing temperature set at 60 °C for 30 s.

Cloning, expression, and purification of A27L and L1R

The amplified genes encoding for A27L and L1R were purified from agarose gel and cloned directionally into a pET28a vector (Novagen) using *BamH1* and *Sal1* (New England Bio labs) restriction enzymes. The genes were cloned with the his-tag on the C-terminus of the pET28a vector for affinity purification. The resulting recombinant plasmids pA27L and pL1R were transformed into chemically competent *E. coli* BL21 (DE3) (Invitrogen). The presence of target genes pA27L and pL1R was confirmed using colony PCR and restriction enzyme digestion. *E. coli* BL21 (DE3) cells carrying target genes were grown overnight in 2X YT medium supplemented kanamycin (34 mg/ml) at 37 °C/200 rpm shaking. The overnight culture was inoculated into 2X YT medium (1:100) and grown to OD_{600} nm of 0.8 at 37 °C/200 rpm shaking. The expression of the recombinant proteins was induced with 0.25 mM of Isopropylthio β-galactoside (IPTG) (Sigma-Aldrich) for 6 h. The cultures were harvested by centrifugation at 5000*g* for 10 min.

The expression of target proteins was observed in the soluble and insoluble fractions for A27L and L1R, respectively. Hence, protein purification was conducted using different methods. The native affinity His-tagged tagged purification of the A27L protein was conducted using a 5 mL HisTrap™ HP affinity column (GE health care) according to the manufacturer's instructions. The unbound proteins were washed off the column with a washing buffer (20 mM Na_2HPO_4, 0.5 mM NaCl pH 7.4) containing up to 100 mM imidazole. The A27L protein was eluted with 20 mM Na_2HPO_4 pH 7.4 containing 0.5 mM NaCl and 200 mM imidazole. The L1R protein was expressed in the inclusion bodies therefore, it was solubilized and purified as described in Schlager et al., 2012 [33]. Briefly, the L1R pellet was solubilized with a denaturation buffer (8 mM Na_2HPO_4, 286 mM NaCl, 1.4 mM KH_2PO_4, 2.6 mM KCl, pH 7.4) containing 1% SDS (w/v) and 1 mM 1,4 dithiothreitol (DTT), then sonicated and incubated in an ice water bath for 30 min. The sonicated cells were centrifuged, and the resulting supernatant was filtered using a 0.2 μM disk and loaded directly into the 5 mL HisTrap™ HP affinity column that had been charged with $NiSO_4$ and equilibrated with denaturing buffer containing 0.1% sarkosyl and 5 mM imidazole. The unbound proteins were washed off the column with a denaturing buffer containing 0,1 % sarkosyl and up to 50 mM imidazole. The L1R protein was eluted with a denaturing buffer containing 0.1% sarkosyl and 200 mM imidazole. The homogeneity of samples collected during purification steps for A27L and L1R was analyzed using 12.5% SDS-PAGE. Fractions containing pure proteins were pooled and dialysed into a phosphate-buffered saline (PBS) pH 7.4 and concentrated using an amicon ultra centrifugal filter device (Merk) with a 3 KDa cut-off size. The protein concentration of the purified A27L and L1R was determined using the Bradford assay [34] and the proteins were stored at −20 °C till further use.

Vaccine formulation

The formulation of LSDV recombinant subunit vaccines was done as described in Matsiela et al., 2022 [18]. Briefly, three vaccines containing LSDV antigens A27L and L1R and a combination of both antigens were formulated. All three vaccines were formulated with a final antigen dose of 20 μg of each protein and Montanide™ Gel 01 PR adjuvant (SEPPIC) at a final concentration of 10% (w/w). Formulations were conducted at room temperature using a homogenizer set at 1300 rpm. Controls were formulated in the same manner, briefly, the inactivated LSDV vaccine (10^6 $TCID_{50}$/mL) formulated with Montanide™ Gel 01 PR adjuvant was used as a positive control and the negative control (placebo) was formulated with adjuvant only 10% (w/w). The sterility of all vaccine formulations was confirmed by growth promotion tests on the three different media: soy media, and thioglycolate and trypsin agar with bovine blood. The stability of vaccine formulations was confirmed using centrifugation test to ensure no separation occurs.

Table 1
Primers used in this study.

Primer name	Sequence	Restriction sites	GC-content (%)	Melting temperature (°C)
A27_Forward	*5′-AAT***GGATCC**ATGGACAGAGCTTTATCAATCTTTC-3′	*BamH1*	40	61
A27L_Reverse	*5′-AAT***GTCGAC**TCATAGTGTTGTACTTCGGCC-3′	*Sal1*	48	59
L1R_Forward	*5′-CAAT***GGATCC**ATGGGAGCAGCCGC-3′	*BamH1*	68	64
L1R_Reverse	*5′ –CAAT***GTCGAC**TCATCCGTATCCCGAACTTTGAC-3′	*Sal1*	48	65
pET28a_Promoter	5′-ACGCCATATCGCCGAAAGG-3′	–	60	55
pET28a_Terminator	5′- GGCAGGGATCTTAGATTCTG-3′	-	44	54

* Inserted enzyme restriction sites in bold italics.

Animals, safety, and immunogenicity studies

The safety and immunogenicity of the five vaccine formulations were evaluated in rabbits at an experimental animal facility in Onderstepoort Biological Products (OBP) in accordance with the standard operating procedures of the institution and national animal research guidelines. New Zealand white rabbits of unspecified gender aged 9–14 weeks and, with weights ranging from 2.5 to 3 kg were acclimatized and monitored daily for general health observations seven days prior to the start of immunogenicity studies. Thirty rabbits were randomly assigned into five groups/cages corresponding to each vaccine group consisting of six animals. The animal cages were kept at a controlled-temperature environment (±22 °C) and the animals were fed Epol's rabbit chow and clean water ad libitum. Animal vaccination was performed at the back of the neck using the 26-gauge needles by subcutaneous (S/C) route (1 mL) at day 0 and secondary vaccination on day 21. For the first 14 days of the trial, the animals were monitored for clinical signs to evaluate vaccine safety before administering the second vaccination. No reduced mobility and mortalities, skin lesion, or any LSD symptoms were observed in all the test animals. Blood samples were collected at seven days intervals throughout the 42 days trial for serological monitoring. After day 42, the animals were sedated and subjected to euthanasia as described elsewhere [18]. Blood samples were processed through centrifugation at 2700 rpm for 30 min and heat inactivation (56 °C) to obtain serum.

Serological analysis

Serum neutralizing test (SNT)

Serum neutralizing tests were performed under sterile conditions using positive and negative sera as described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial animals [35]. Briefly, 50 μL of inoculation medium (GMEM with amphotericin B (0.5 %), and streptomycin (7.5%)) was pipetted in all the wells of 96-well plates. Sera (50 μL) were added in triplicates and serially diluted in an inoculation medium and the LSDV live antigen (50 μL) was added to the diluted sera at a final dose of 100 $TCID_{50}$/mL present in each well. The plates were pre-incubated at 37 °C for 1 h. Thereafter, 100 μL of bovine dermis (BD) cells (cultured using completed minimum essential medium (MEM) with 10 % bovine serum and 1 % glutamax) was added to each well at a final concentration of 4 X 10^5 cell/mL and the plates were placed at 37 °C under 5% of CO_2. The development of cytopathic effect (CPE) was monitored daily for seven days. Antibody titres were expressed as the reciprocal of the serum dilution that inhibited 50% of viral CPE and results were recorded after seven days post cell infection.

Indirect enzyme-linked immunosorbent assay (iELISA)

The SNT data was verified using a commercial ELISA kit, ID Screen® *Capripox Double Antigen Multi-species* (IDvet Innovative Diagnostics) with minor adjustment. Briefly, 96 well plates were coated with the LSDV antigen diluted 1: 100 in PBS [36]. The plates were incubated overnight at 4 °C and then washed five times with PBS containing 0.05% (v/v) Tween-20 (Sigma-Aldrich) (PBST). A buffer containing 5% (w/v) skim milk in PBST was added (250 μL/ well) to block non-specific binding and the plates were incubated for 1 h at 37 °C at 80 rpm and then washed five times with PBST. Positive LSDV sera (anti A27L, L1R and A27L/L1R combination) in a 1:1 dilution with a PBST containing 1% skim milk was added to the plates. The positive and negative controls provided in the kit were included. Plates were then incubated for 1 h at 37 °C and washed five times with PBST. The conjugate was diluted 1: 5000 in a diluent containing 5 % skim milk and added at 100 μL/ well before a 30-minute incubation. The plates were washed five times with PBST and 100 μL of TMB substrate was added to each well followed by a 10-minute incubation at 37 °C 80 rpm. The reaction was stopped by adding 100 μL of the stop solution to each well and the plates were read to obtain OD_{450} nm.

Diagnostic evaluation of LSDV-proteins based iELISA

The iELISAs were performed using the commercial IDVet diagnostic kit as described above in 2.4.2. Briefly, plates were coated with different concentrations (100 ng, 75 ng, 50 ng, 25 ng, 12, 5 ng, and 7.5 ng) of A27L, L1R, and a combination of both proteins serially diluted 1:1 with PBST. LSDV live attenuated vaccine-positive serum (titre of 8,75 $\log_2$) was diluted in 1:1 and used titrating the coated wells. The control wells were coated with rift valley fever virus (RVFV) (100 $TCID_{50}$/mL) and LSDV field isolate (100 $TCID_{50}$/mL) diluted in 1:1 and used as a negative and positive control, respectively and the plates were incubated overnight at 4 °C. The washing and detection of LSD antibodies were conducted following the manufacturer's instructions above.

Ethical consideration

The ethical clearance in experimental animals used in this research was approved by the National Department of Agriculture, Land Reform, and Rural Development under Section 20 of the Animal Diseases Act (Act 35 of 1984) in South Africa. The experimental protocol used in this study was approved by the OBP Animal Ethics Committee (South African Veterinary Council Facility Registration number: FR1514054) and the experiments in animals were carried out in accordance with the standards as set out by Experimental Division at OBP.

Statistical analysis

The LSDV neutralizing index and indirect ELISA raw data for the detection of positive and negative antibodies was evaluated using one way nova with the turkey multiple comparison tests. The one-way anova was used to compare vaccine treatments to each other and while the turkey was used to compare the different coating concentrations. Graph pad prism was used to graphically represent the data with error bars indicating the standard deviation from each data point.

Results

Expression and purification of A27L and L1R

Genes encoding for A27L and L1R were successfully amplified from the genomic LSDV DNA and cloned into an *E. coli* pET28a expression vector. For L1R, the transmembrane domain was removed and only the extravirion domains were used for increased protein expression. Recombinant proteins pA27L and pL1R were expressed and purified to obtain antigens for immunological investigations. Highest expression was achieved with induction of 0, 25 mM IPTG after 6 h. The expression of A27L was observed at an expected size of 21 kDa in the soluble fraction (Fig. 1, lane 4), while L1R was expressed at an expected size of 26 kDa in the inclusion bodies (Fig. 1, lane 6). Samples not induced with IPTG did not show any visible expression bands (Fig. 1, lanes 2 and 5). Our findings correlated with a previous study, where the overexpression of sheeppox A27L and L1R in the *E. coli* pET system was observed in the soluble fraction and in the inclusion bodies, respectively [22].

SDS-PAGE analysis showed that LSDV proteins A27L and L1R were purified using His-tagged affinity chromatography (Fig. 2). Final concentrations of A27L and L1R was determined at 6 mg/mL and 8,5 mg/mL, respectively. The yields obtained with A27L were similar to previously reported study [37].

Serological analysis

SNT

The humoral immune response in vaccinated animals was determined using SNT and ELISA. In the SNT data, serum dilutions ≥ 1:4 that inhibited 50% LSDV-induced CPE in cell culture were considered positive titres of virus-neutralizing antibodies. In all tested vaccines, no

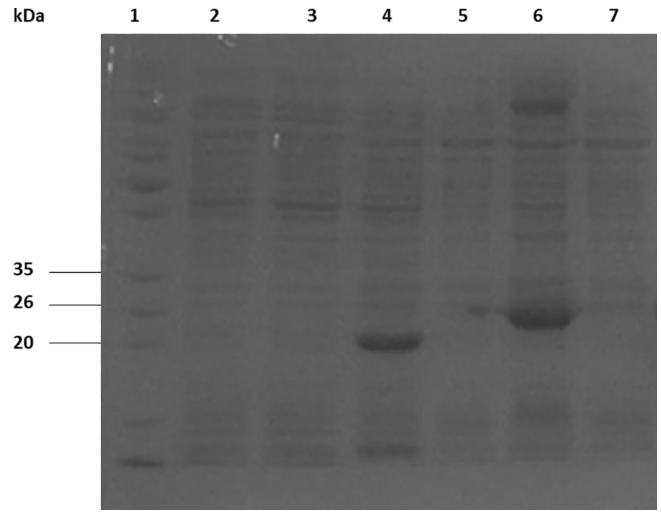

Fig. 1. SDS-PAGE analysis of the expression of A27L and L1R. Protein expression was induced with 0,25 mM IPTG at 30 °C for 6 h. The cultures were harvested, and the samples were obtained through centrifugation at 6000*g*. The samples were resuspended in an assay buffer, sonicated, and centrifuged to separate the pellet and the supernatant of each protein. Lane 1 represents the protein marker and Lane 2 is the crude sample of the A27L protein not induced with IPTG. Lanes 3 and 4 are the resuspended pellet, and the supernatant of the A27L protein, respectively. Lanes 5 is the extract of the crude sample of the L1R protein not induced with IPTG. Lanes 6 and 7 represent the resuspended pellet, and supernatant of the L1R protein, respectively. Samples were analyzed on a reducing 12.5% SDS-PAGE and stained with a 0.25% Coomassie blue solution.

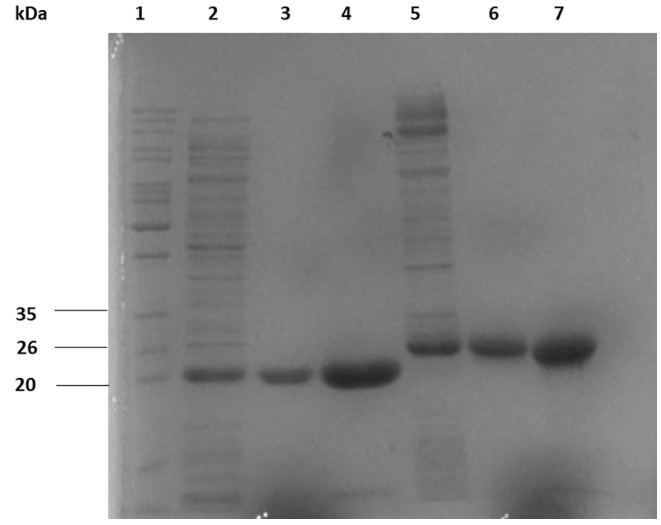

Fig. 2. Purification profiles of the his-tagged LSDV soluble and insoluble lysate using the 5 mL His Trap column. Protein purification following a 1L expression induced with 0,25 mM IPTG at 30 °C for 6 h. Lane 1 is the protein ladder, and lane 2 is the crude sample of the A27L protein prior to purification. Lane 3 is the pooled sample of purified A27L elutes. Lane 4 is the concentrated sample of the pooled A27L elution samples. Lane 5 is the crude sample of the L1R protein prior to purification. Lane 6 is the pooled sample of purified L1R elutes. Lane 6 is the concentrated sample of the pooled L1R elution samples. Analysis in a reducing 12.5% SDS-PAGE stained with a 0.25% Coomassie blue solution.

positive neutralizing antibody titres were reported on 0 and 7 days post-vaccination (dpv) (Fig. 3). Positive antibody titres were reported after the second vaccination on 21 and 28 dpv for anti-serum raised to the A27L, and on 21,28, 35 and 42 dpv for anti-serum raised to the L1R (Fig. 3). Positive neutralizing antibodies induced by the combination (A27L and L1R) vaccine were reported 14, 21, 28, 35 and 42 dpv with the highest average antibody titre of 21,3 observed at 42 dpv. All the tested vaccines showed a similar trend where antibodies were detected in 14 dpv and showed a relative increase on 21 and 28 dpv. This was followed by a decline at 35 dpv and an increase at 42 dpv. The A27L vaccine formulation resulted in lower immune response when compared to L1R and combination product. The statistical difference between the three test vaccines and the controls was observed on 35 and 42 dpv (Fig. 3).

iELISA

Antibodies raised to recombinant A27L, L1R and combination of A27L and L1R were verified and quantified using the commercial ELISA kit with modification. ELISA plates were manually coated with 100 $TCID_{50}$/mL LSDV field isolate and experiments were conducted using reagents contained in the kit. The threshold for ELISA positivity was set at OD_{450}nm 0.3 as instructed in the manual of the IDvet commercial kit. Positive seroconversion was observed from 21 up to 42 dpv for sera raised against the A27L vaccine (Fig. 4). These results were comparable with those obtained in the SNT where neutralizing antibodies against the A27L vaccine were also observed on 28 up to 42 dpv. Positive antibodies raised to the L1R vaccine were detected from 21 up to 42 dpv. Positive antibodies for the combination vaccine were detected from as early as 7 dpv up to 42 dpv. Comparable results were observed in the SNT data for neutralizing antibodies raised to the L1R vaccine and the combination of both proteins. Overall, antibodies raised against the combination showed higher antibody titres in the ELISA and SNT when compared to antibodies raised to individual vaccines.

Application of A27L and L1R proteins as diagnostic reagents

Recombinant A27L and L1R proteins were evaluated for application as diagnostic reagents using the IDvet commercial ELISA kit with slight modification, where plates were manually coated with different concentrations of proteins. Wells coated with 100 $TCID_{50}$/mL RVFV used as negative control resulted in no signal for positive LSDV-serum detection (Fig. 4). LSDV proteins (A27L and L1R) were able to detect antibodies against LSD at five out of six tested concentrations (Fig. 5A). The antibody detection decreased with a decrease in coating concentration. The 100 ng/mL coating concentration resulted in the highest detection whereas 5 ng/mL had the least detection. The A27L was more sensitive in detecting antibodies against LSDV compared to L1R. Detection profile of the A27L and L1R combination was similar to individual proteins (Fig. 5B). Studies that evaluated the diagnostic ability of goatpox virus also reported that A27L had higher detection ability over L1R and A33R [37,38]. A comparison of the coating proteins was found to be statistically significant (p-value $< 0{,}0001$).

Discussion

LSD is endemic in most African countries and in the past 10 years the disease has rapidly spread to several regions which were previously considered disease-free [11]. To date, the few countries that have no reports of LSD include New Zealand, America, Western Europe, and Australia. Recent reports in Asia constitutes the largest cattle-producing areas affected by LSD outbreaks [39,40], and the re-emergence of the disease in Israel [41], suggests there is a substantial risk of LSD incursion in countries with no previous reports of the disease. Successful control and possible eradication of LSD relies on early detection of the index case, followed by rapid and widespread vaccination campaigns. In Balkans Europe, both retrospective serosurveillance and active vaccination have led to the no recent reports of LSD cases [41].

There are continuous efforts towards the development of safer and effective vaccine alternatives for prophylactic immunization of animals against LSD. The current study evaluated the safety and immunogenicity of recombinant A27L and L1R for development of LSD subunit vaccines.

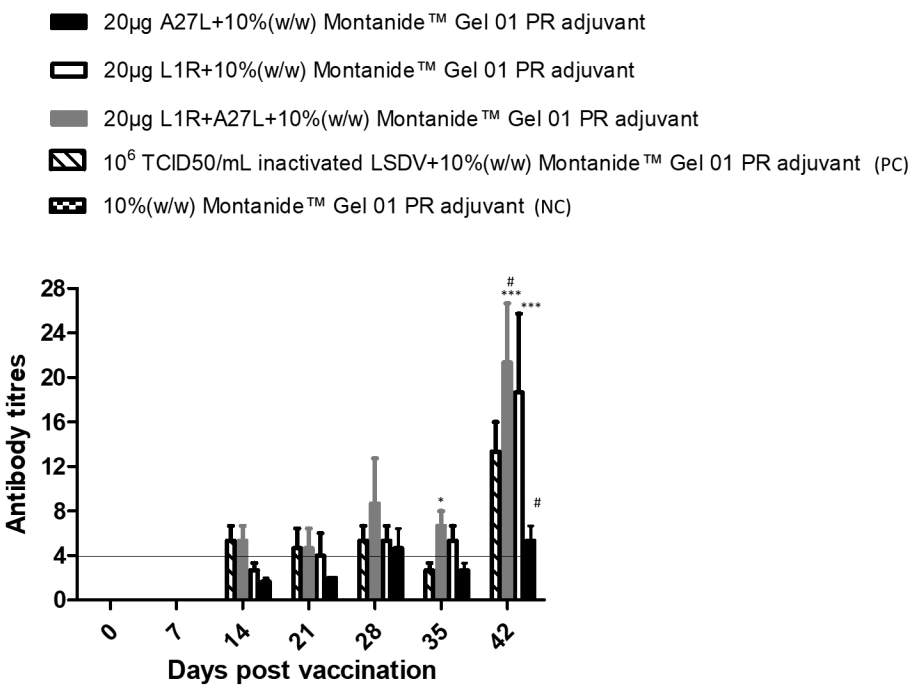

Fig. 3. SNT analysis of serological response induced by LSD recombinant sub-unit vaccines. Data represents average antibody titres of serum obtained following two vaccinations on days 0 and 21 on rabbits with five vaccines. The inactivated LSDV vaccine represents the positive control, and the placebo (Montanide™ Gel 01 PR adjuvant) represents the negative control. The statistical significance between the controls and the test vaccines is represented by # (vaccine vs PC ($P < 0,05$)), ***(vaccine vs NC ($P < 0,001$)), and *(vaccine vs NC ($P < 0,05$)). The grey separates the LSDV negative (below) and positive (above) antibody titres.

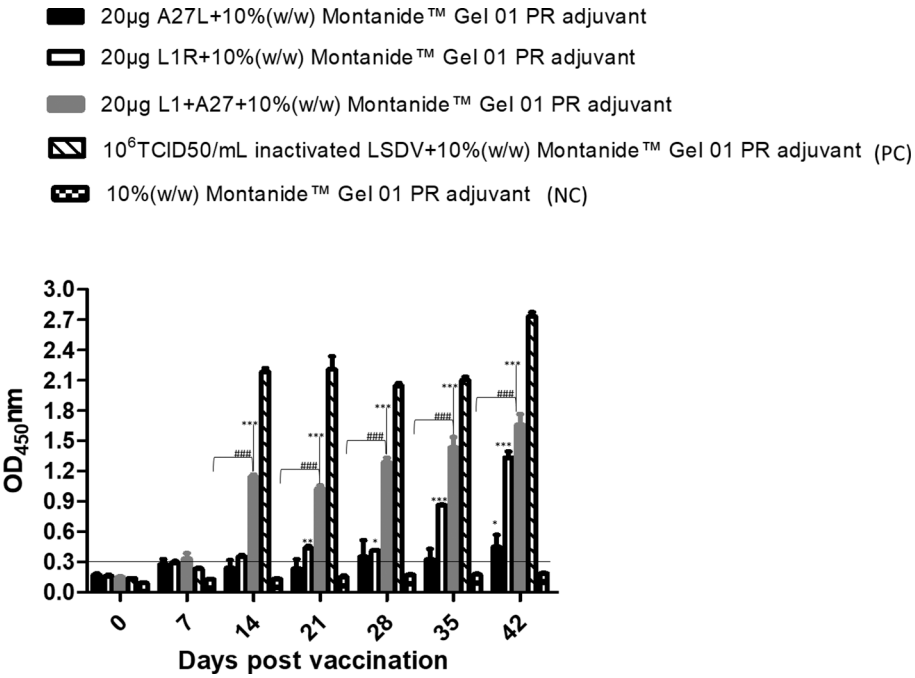

Fig. 4. ELISA analysis of serological response induced by LSD recombinant sub-unit vaccines. Data represents average antibody titres of serum obtained following two vaccinations on days 0 and 21 on rabbits with five vaccines. The inactivated LSDV vaccine represents the positive control, and the placebo (Montanide™ Gel 01) represents the negative control. The statistical significance between the controls and the test vaccines is represented by ### (vaccine vs PC ($P < 0,001$)), ***(vaccine vs NC ($P < 0,001$)), **(vaccine vs NC ($P < 0,01$)), and *(vaccine vs NC ($P < 0,05$)). The grey line separates the LSDV negative (below) and positive (above) antibody titres.

A27L and L1R were selected for vaccine development based on their critical role in the viral entry. This study demonstrated the immunogenicity of LSDV A27L, L1R and combination of these two proteins as vaccine candidates. Furthermore, the diagnostic potential of the proteins was evaluated as diagnostics reagents.

Several factors challenge subunit vaccine development, these include but are not limited to the choice of an expression system, yield of antigens and the choice of adjuvants used in the vaccine formulations [42,43]. The *E. coli* expression system is widely used to produce recombinant proteins for pharmaceutical purposes [44]. One of the common challenges with the *E. coli* system is the formation of inclusion bodies with about 70% of expressed proteins reported to be insoluble [45]. The L1R protein was expressed in inclusion bodies and solubilised using SDS and sarkosyl prior purification [33], whereas the A27L was expressed in the soluble fraction. Both proteins were successfully purified using affinity chromatography with total pure protein yields of 6 mg/mL and 8.5 mg/mL for A27L and L1R, respectively.

A27L, L1R and A27L + L1R were formulated with the Montanide™ Gel 01 PR adjuvant and used to inject rabbits. The Montanide™ Gel 01 PR adjuvant was previously shown to be safe and compatible with a wide range of animal and antigenic models [46]. Moreover, Matsiela *et al.* [18] reported the use of Montanide™ Gel 01 PR adjuvant in the inactivated LSDV vaccine formulation and suggested that this adjuvant is compatible and safe for LSDV formulations [18]. The current study

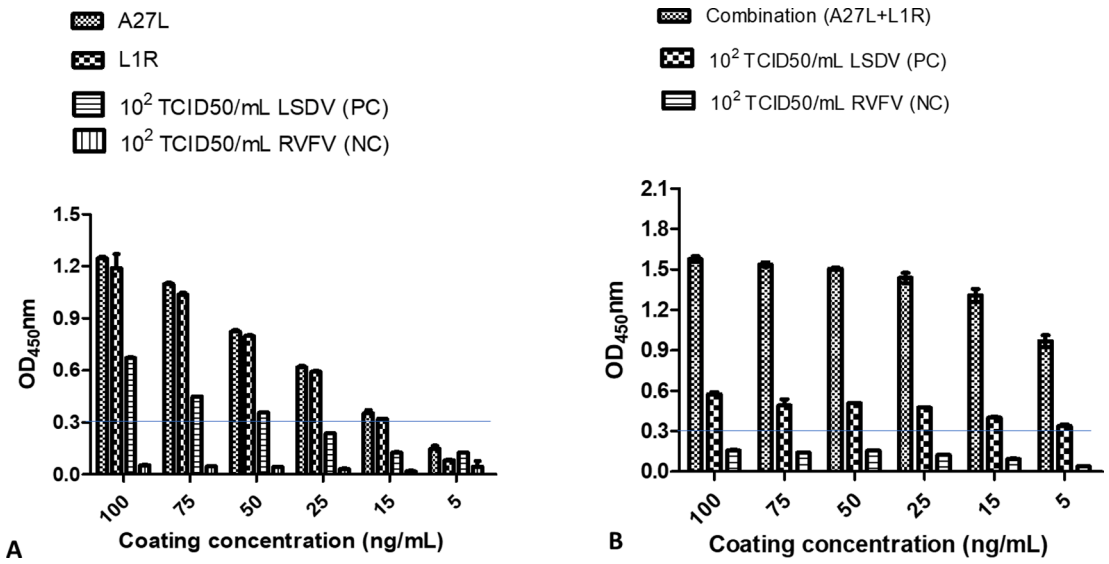

Fig. 5. Evaluation of diagnostics potential of A27L and L1R and a combination of both proteins using an ELISA. A. Reactivity of LSDV live attenuated vaccine-positive serum (titre of 8,75 log_2) with different coating concentrations of A27L and L1R. **B**. Reactivity of LSDV live attenuated vaccine-positive serum (titre of 8,75 log_2) with different coating concentrations of the combination of A27L + L1R proteins each present at the indicated coating concentration. The grey lines separate the negative (below) and positive (above) antibody detection. RVFV- rift valley fever virus.

demonstrated that A27L, L1R and A27L + L1R formulated with the Montanide™ Gel 01 PR adjuvant were able to elicit neutralizing antibodies from as early as day 14 post-vaccination. The formulation with combined A27L and L1R presented higher antibody titres and neutralizing activity compared to individual proteins. Maximum antibody titres were detected on day 42 post-primary vaccination for all products. These results were similar to immune response profile induced with A27L and L1R from goat poxvirus [38].

In parallel to the immunogenicity studies, the recombinant A27L and L1R proteins were evaluated for application as ELISA diagnostic reagents. The diagnosis of LSDV with high sensitivity and specificity antigens is essential in the management of the disease. Our data demonstrate that A27L and L1R can be developed as diagnostic reagents for LSDV detection, since these proteins indicated high specificity and were highly reactive with anti-LSDV sera. In addition, reactivity and specificity is improved when A27L is used in combination with L1R. No response was detected for non-related disease (RVF). The high specificity is critical for development of effective diagnostic tools to assist in the serosurveillance and the management of LSDV.

Despite previous reports of high immunogenicity, results from this study showed that A27L alone was not the best candidate for LSD vaccine development but can be efficiently used in diagnostics. This could be due to several factors which include the choice of adjuvant, or the antigen concentration used. Thus, we can conclude from the results of this study that other LSD antigen targets other than A27L can be investigated alone or in combination with L1R for vaccine development.

Conclusions

Our research demonstrated that recombinant LSDV A27L and L1R can be developed as potential subunit vaccines for effective control of LSD disease since they were able to elicit neutralizing antibodies. The vaccine efficacy will still need to be evaluated in a virulent virus challenge. The research further proved specificity and high reactivity of these proteins in diagnostic application as a proof of concept.

Availability of data and materials

The datasets and relevant reagents used during the current study are available from the corresponding author following the legal process of the University of KwaZulu Natal and Onderstepoort Biological Products policies on material distribution.

Funding

The Research was financially supported by the Council for Scientific and Industrial Research through a student bursary of N Ntombela. The Research was also financially supported by National Research Foundation (NRF), grant 115969 and internally funded by University of KwaZulu-Natal and Onderstepoort Biological Products Research budget.

Author contributions

Nobalanda Mokoena and Thandeka Khoza contributed to the conception of the work. Nomfundo Ntombela performed experiments and analysed the results. Sbahle Zuma contributed to the production of antigens. Suhavna Hiralal contributed to the cloning of A27L. Nobalanda Mokoena and Leeann Naicker contributed antigen production and interpretation of the results. Matome Selina Matsiela contributed in the formulation of the vaccines. Nomfundo Ntombela, Nobalanda Mokoena and Thandeka Khoza took the lead in writing and revising the manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr L Naicker reports financial support was provided by National Research Foundation. Dr N Mokoena reports financial support was provided by Onderstepoort Biological Products. Dr T Khoza reports financial support was provided by University of KwaZulu-Natal. N Ntombela reports financial support was provided by Council for Scientific and Industrial Research.

Data availability

Data will shared following the material transfer policies of the organization

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