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Recombinant N protein of Rift Valley Fever expression in baculovirus system and its use in immunoenzymatic assay: preliminary data

Serroni A., Armillotta G., Laguardia C., Ulisse S., Iorio M., Testa L., Parolini F., Capista S., Traini S., Fanì S., Palucci C., Ronchi G.F., Di Ventura M., Mercante M.T.
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale"

ISTITUTO
ZOOPROFILATTICO
SPERIMENTALE
DELL'ABRUZZO
E DEL MOLISE
"G. CAPORALE"

Introduction

Rift Valley fever (RVF) is an arthropod-borne, zoonotic viral disease caused by the RVF virus, which belongs to *Bunyavirales* order, *Phenuiviridae* family, and *Phlebovirus* genus. The virus primarily affects domestic animals and can affect humans, in which the disease mostly manifests with flu-like symptoms and can rarely have a fatal outcome.

The viral particles are enveloped, and the single strand RNA genome is composed by three segments: Large (L), Medium (M) and Small (S), which encode four structural proteins (nucleocapsid protein N, glycoproteins Gn and Gc, and polymerase L), two non-structural proteins (NSs and NSm) and another 78 kDa protein of unknown function [1].

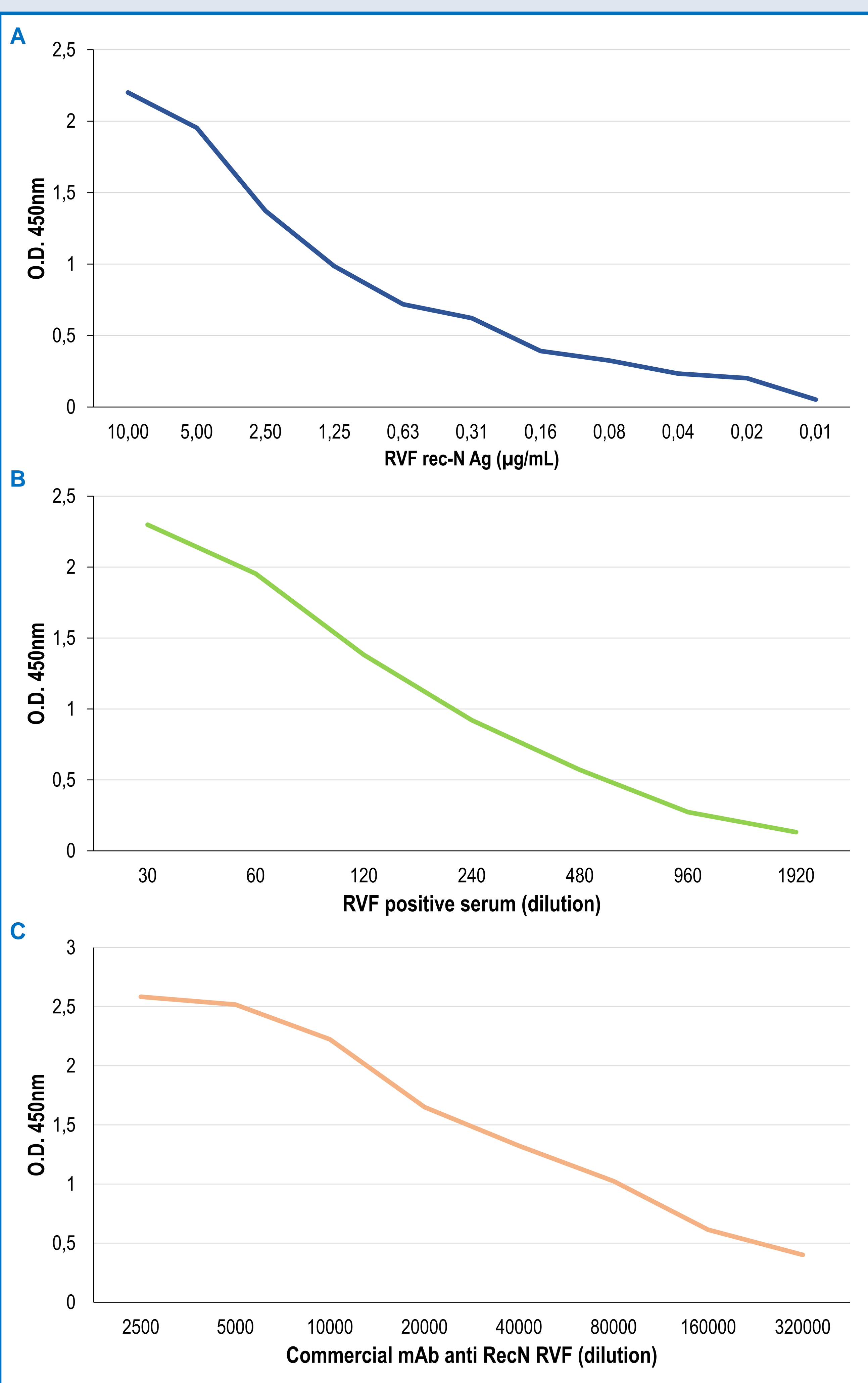
Nowadays, the virus is present in the African continent and in some islands of the Indian Ocean. Recent outbreaks in a French overseas department of Mayotte and the finding of seropositive animals in North Africa suggest the potential risk of RVF incursion into European territory [2]. For this reason, the availability of effective diagnostic tools is essential to implement adequate national and international surveillance. The aim of this study is the production, purification, and characterization of RVFV N protein, which will be useful for the development of a diagnostic kit.

Results

Approximately, 10 mg of RVF recN protein with a high grade of purity was obtained infecting 2 $\times$ 10^9 Sf9 cells. SDS-PAGE (**Figure 1A**) and Western blot analyses (**Figure 1B**) were performed to assess the quality and integrity of the recombinant protein. As shown in Figure 1, a clear band corresponding to the expected molecular weight of about 30 kDa was observed.

To verify the potential application of the obtained protein as reagent in a diagnostic tool, a series of indirect ELISAs using a positive sheep serum, and a commercial anti-RVFV N protein were performed. As reported in **Figure 2**, the O.D. measured at 450 nm progressively decreases, in correlation with decreasing concentrations of both antigen and antibody. This behavior strongly suggests the specificity of the antigen-antibody interaction, further confirming that the antigenic structure of the obtained recN protein was preserved during the expression and purification processes.

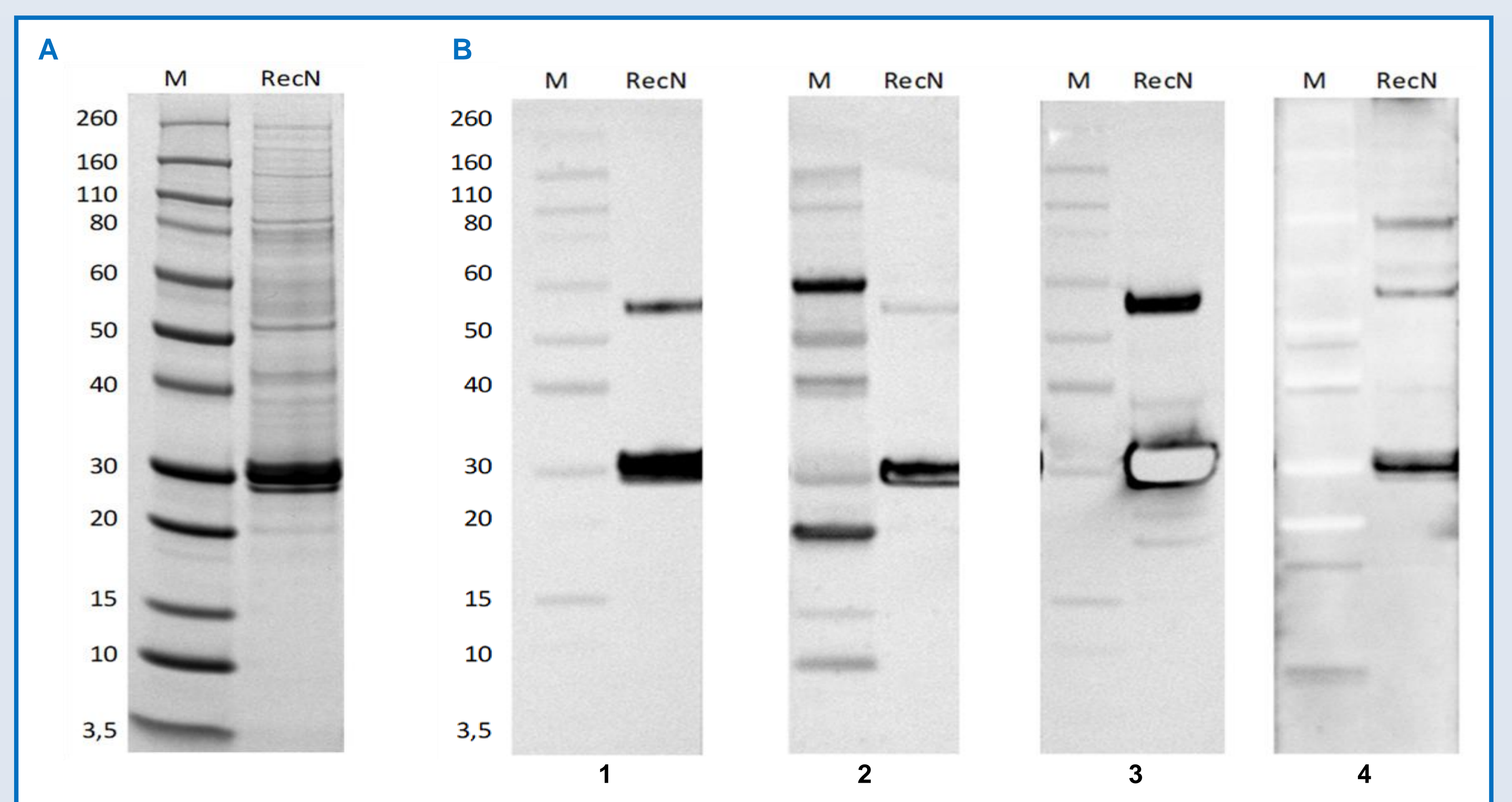
Figure 2. Graphical representation of i-ELISA results for obtained recN protein. Different recN concentrations, as coating antigen, were tested using the reference positive sheep serum diluted 1:60 (**A**). Different positive serum concentrations were used with 5 µg/mL recN antigen coating (**B**). Different commercial mAb anti RecN dilutions were tested using 3,125 µg/mL recN antigen coating (**C**).



Materials and methods

The his-tagged recN protein was expressed exploiting the baculovirus system. For this purpose, the baculovirus vector was specifically engineered for the expression of RVFV recN protein, and the optimal protocol for protein production was developed testing different infection conditions, such as varying multiplicity of infection (MOI) and harvest times. The production was performed infecting 1.5 L of Sf9 (*Spodoptera frugiperda*) cells (1.5 $\times$ 10^6 cells/mL) at a MOI of 0,001. The cell supernatant was collected at 120 hours post infection, supplemented with 0.2 M L-arginine hydrochloride, and incubated at 4°C overnight until the purification, based on IMAC (immobilized metal affinity chromatography) principle, using the AKTAPurifier 100 FPLC system. The eluted fractions containing the recN protein were pooled and concentrated using an Amicon® Ultra, MWCO 10 kDa filter device in a swinging bucket rotor. A buffer exchange was also performed using the same filter, in order to remove the excess of imidazole. The purified protein was quantified by Bradford assay. The quality and integrity of the purified recN protein were then assessed through several analytical techniques, including SDS-PAGE, Western blot and indirect ELISA using different antibodies and a positive serum, obtained from a sheep vaccinated with an inactivated RVF vaccine.

Figure 1. SDS-PAGE (Panel A) and Western blot analyses (Panel B) of recN protein purified from supernatant of Sf9 infected cells. For the immunoblot analyses different antibodies were used: anti-Rift Valley Fever NP Monoclonal antibody (1); His-tag antibody anti 6xHis C-term (2); V5-tag antibody (3). Immunoreactivity of membrane was also tested using a reference RVFV positive sheep serum (4). All the samples were analysed under denaturing conditions.



Discussion and Conclusions

The current context of increased risk of Rift Valley Fever incursion into European countries requires national and international health authorities to increase surveillance. For this reason, it becomes essential to implement surveillance with appropriate diagnostic and preventive tools.

The immunogenic properties of the surface glycoproteins Gn and Gc and the nucleocapsid protein N make them good candidates for both diagnostic tests and vaccine development [3,4].

In this study, RVFV recN protein was expressed in baculovirus system and procedures for its large-scale production and purification were developed. The purification of recombinant protein was performed from the supernatant using IMAC. This method offered several advantages, such as the automatization of the process, which allows to speed the process and improve its standardization.

The results from the assays performed (Western blot and ELISA) provide evidence supporting the hypothesis that the recN protein retains its native antigenic properties, making it a viable candidate for use in diagnostic applications. The observed correlation between O.D. values and antigen/antibody concentrations highlights the protein suitability for sensitive detection of RVFV antibodies in serological tests, indicating its potential utility in developing reliable diagnostic tools for Rift Valley fever. The preservation of the correct antigenic structure, as suggested by the ELISAs, reinforces the protein stability and functionality, which are both critical factors for its successful incorporation into diagnostic tools.

These results lay the foundation for further studies to develop a diagnostic method, to support the disease monitoring in case of incursion into Europe.

References

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