5.5 Identification of pET32-E by double digestion

The expression vector pET- E was verified by restriction enzyme *Bam*HI and *Sal*I, the results suggested there were two DNA on the gel with the size of 5 900 bp and 1 500 bp, all together was a 7.4kb, the results were consistent with expectations, showing that the gene fragment cloned in pET-32a (+) vector is positive (Figure.6).

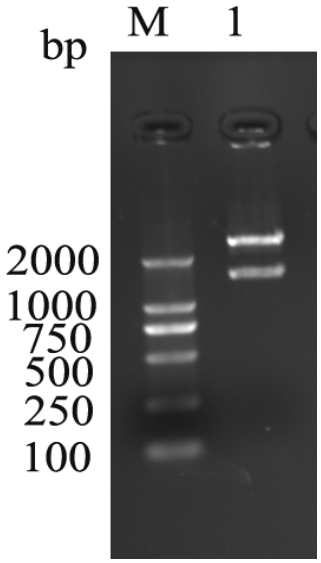

Figure6: Identification of the PET-32(a) plasmid by restriction enzymes (BamHI and SalI) digestion.
M: DNA marker-DL 2000; lane 1: PET-32(a) 5900 bp
Analysis and sequence of E gene from West Nile Virus

The DNA fragment was separated by electrophoresis on a 1% agarose gel, excised, and purified. The amplified fragment was digested with the BamH1 and SAL1 enzymes, cloned into pET-32a (+) vector and confirmed by sequencing. The results of sequencing the recombinant plasmid amino acid analysis and sequence homology, and using DNASTAR software with the sequencing results of original genes have been analyzed and compared.

5.6 SDS-PAGE analysis of the recombinant protein pET-E

The plasmid pET-E was transformed into E. coli BL21 (DE3), and the E protein was expressed inducing by IPTG at 28 ℃ for 4 hours. The samples were analyzed by SDS-PAGE and the proteins were visually by staining with Coomassie blue. The molecular weight of expressed protein was about 73 kDa. (Fig .7)

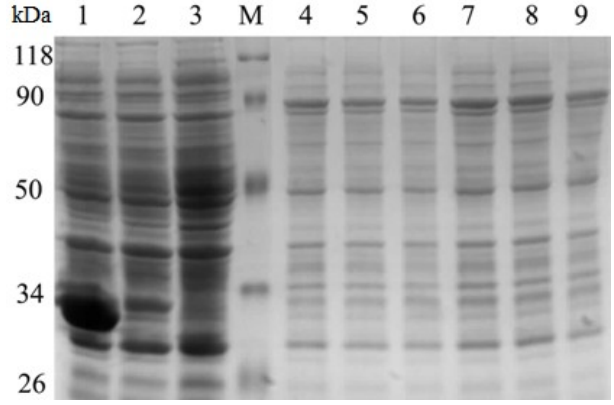

Figure 7: Analysis of the expression of E protein by SDS–PAGE

M: molecular mass protein markers, Lane 1: PET32 (a), Lane 2: pET32a-E without IPTG induction (control), Lane 3: cell lysates from *Escherichia coli* BL21, Lane 4-9: pET32a-E with IPTG-induced (Full-length (73 kDa).

5.7 Western-blotting analysis of the recombinant protein pET-E

Expression of the product obtained after transformation of strain supernatant, the preparation of the primary antibody and anti-mouse as secondary antibody **(a)** and the preparation of the primary antibody and specific WNV antibody as secondary antibody **(b)** for Western blot analysis. The results showed a protein band estimated at 73 kDa (Fig .8)

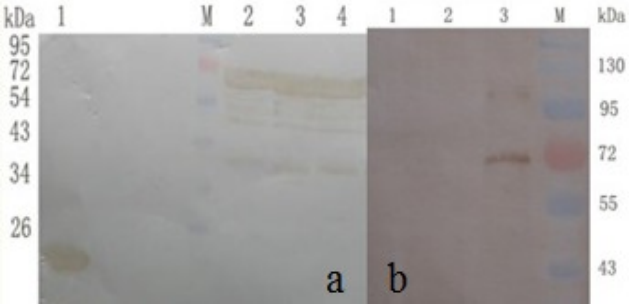

Figure 8: Analysis of the expression of pET-E by western blot
M: Marker
a: Lane 1: PET32 (a), Lane 2-3-4: pET32a-E with IPTG-induced
b: Lane 1: PET32 (a), Lane 2:BL21,Lane 3: pET32a-E with IPTG-induced

6. Discussion

The current epidemic of WNV is expected to continue over the coming years as the virus develops its geographical distribution. This impacts significantly both human and veterinary concerns. There is a lack of effective and specific antiviral treatment. The high production costs and limited scalability associated with mammalian-cell culture production may restrict the use of therapeutic mAbs against WNV and other flaviviruses in resource-poor settings [11, 12]. Moreover, Plasmid DNA is an emerging experimental vaccine, based on the plasmid ability to express a protein antigen which, in turn, promotes antibody production and

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confers host immunity. A recent review presents the various strategies followed to develop vaccines against WN virus and other flaviviruses [13] .It was reported that highly expressed level of the fusion pET32a-E protein was transformed into E.coli BL21 (DE3), and optimized the condition for induction [14] .Several groups are developing vaccines against WN virus. Two veterinary vaccines are now approved for use in the United States. One, manufactured by Fort Dodge Animal Health, consists of a killed virus preparation [15]. The other, a recombinant canarypox virus, is marketed by Merial [16] .Other vaccine development strategies are based on naked plasmid DNA, attenuated WN strains, non-infectious RNA vaccines and chimeric viruses [17,18,19,20,21;23] and [13] .A recent review presents the various strategies followed to develop vaccines against WN virus and other flaviviruses [13] .It has been shown that truncated envelope protein from dengue virus, when expressed in Drosophila S2 cells, folds into a conformation resembling that of native envelope protein [22]. Here we show the results of experiments with a truncated form of WN virus envelope protein produced in Drosophila S2 cells. Mice vaccinated with this antigen were protected against a lethal challenge with WN virus. Vaccination of horses elicited neutralizing antibodies. The antigen was immunogenic when administered with aluminum hydroxide, the only adjuvant currently approved for human use. We propose that this form of truncated E protein is a candidate antigen for further development as equine and human vaccines to protect against WN virus infection. However, the risk of immune enhancement of heterologous flavivirus infection with inactivated virus and the legitimate safety concerns with the chimeric flaviviruses are highly debatable. In this study, the glycoprotein E was used as antigen in rabbits. The serum was able to neutralize West Nile virus. Our present indicated that Glycoprotein (E) plays an important role in diagnostic tests, inhibition of hemagglutination and induces protective immunity.

7. Conclusions

The results indicated that WNV neutralizing antibodies alone are considered sufficient to impart protective immunity to vaccines.

8. Acknowledgements

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