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Comparative Studies of Nairobi-Sheep-Disease Virus Strains Infectivity, Immunogenicty and Cross-Protectivity in BALB/C Mice Model

Ngari P. Muriuki[1, 2 *]; Ithinji G. D[2]; Leonard O. Ateya[3]; Yatinder S. Binepal[3];
Caroline Wasonga[4]; Muthamia M. Kiraithe[5]; A. K Nyamache[1]

1. Department of Biochemistry, Microbiology & Biotechnology, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya.
2. Kenya Agricultural and Livestock Research Organization (KALRO), Veterinary Science Research Institute (VSRI)-Muguga), P.O Box 32-00902, Kikuyu, Kenya.
3. Kenya Agricultural and Livestock Research Organization (KALRO), Biotechnology Research Institute (BioRI)-Kabete, P.O. Box 57811-00100, Nairobi, Kenya.
4. Department of Biochemistry, University of Nairobi, P. O. Box 30197 - 00100. Nairobi, Kenya.
5. Department of Research& Development, Kenya Veterinary Vaccine Production Institute (KEVEVAPI), P.O. Box 53260-00200, Nairobi, Kenya.

*Correspondence; Ngari P. Muriuki
† Equally contributing Author

Abstract:- Nairobi sheep disease virus is a hemorrhagic virus that cause severe gastroenteritis in shoats resulting to significant morbidity and mortality in naïve small ruminants' populations. Vaccine platform to develop efficacious vaccine against the Nairobi sheep disease virus have been unsuccessful. This research detail the comparative infection, immunogenicity, and protection of three Nairobi sheep strains; I34, 1473 and Ansell. The three strains are marked with differences in their ability to cause disease in suckling mice model. Fatality rates range from 0-50% from the virulent pathogenic 1473 strain, I34 and the seemingly less virulent Ansell strain also shown by their relative time to death. Findings of this research demonstrate that protective efficacy mediated by inactivated Nairobi sheep disease virus strain I34 conferred a stronger cross protection against homologous and heterologous strains compared to 1473 and Ansell strains. Strain I34 sera neutralization against homologous I34 strain was similar to that against Entebe strain providing evidence of possible antigenic homology. Vaccine developed from I34 strain will protect against multiple strains of Nairobi sheep disease virus; 1473, Ansell and Entebe strain. Understanding immune response in mice elicited by different Nairobi sheep disease virus strains will facilitate development of a more efficacious vaccine. Using formalin inactivated NSDV vaccine, I34 strain showed complete protection from homologous and a partial protection heterologous strains in in vitro assay. Protection was associated by higher neutralizing antibodies against homologous and heterologous strains compared to that of 1473 and Ansell. Thus, this study deduce serum neutralizing antibody titers are associated with protection against homologous and heterologous challenge

I. INTRODUCTION

Nairobi sheep disease (NSD) is a tick-borne viral disease of sheep and goats (Olum & Muthamia, 2021). It causes high morbidity and mortality in sheep and goats with a fatality rate up to 90% in naive herd (IVVN, 2019). Nairobi sheep disease was first isolated in 1970' in Kenya (Bin Tarif et al., 2012; Davies et al., 1978; Krasteva et al., 2020). Since then, its impact and magnitude on small ruminants is yet not known. This orphaned economically significant disease have been accelerated by combination of factors revolving within humans-animals-ecosystem interface. Surveillance of the NSDV from various markets was based on the on the spatial and environmental model on the distribution of Nairobi sheep disease on small ruminant described by (Gong et al., 2015; Krasteva et al., 2020; Marczinke & Nichol, 2002). Surveillance models predict best strategies to respond to infectious diseases averting the risks involved decreasing the economic loses. Kenya is potentially at high risk of NSD shown by wide distribution of tick species and the diverse favorable environment. NSD is an OIE notifiable disease which can cause significant socioeconomic impacts with trade implications for endemic region. This disease impact small ruminants and pose a great threat to the populations and communities that directly and indirectly depend on them.

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Global climate change influence the distribution of vector borne diseases and changes in climate lead to devastating consequences resulting from corresponding increase of disease occurrences. (Marczinke & Nichol, 2002; Ramasamy & Surendran, 2011; Sudeep et al., 2009). The global span of NSDV vector distribution has increased significantly with the raising global temperatures. Nairobi sheep disease causes a febrile illness in man and have been classified as a biosafety level 3 agent as it can induce a fatal disease. (Hartlaub et al., 2021) It's not however known how the emerging pathway for its reemergence would impact the public health. The biology of the current circulating strain is not well known an important gap for research. Mice is a cheap model to investigate and compare the infection, immunogenicity and protection against homologous and heterologous challenge. Newly emerging and reemerging viral diseases have been occurring throughout an indefinite and long period of time in their environment. A combination of environmental factors revolving around environment, host and vector have increased their interaction over a time. This has increased their impact on public health due to unprecedented spillover to man and domestic animals leading to emergence of novel zoonotic diseases, particularly viral whose potential can lead to global pandemics. (Yi et al., 2021).

Circulating viruses exist as a heterogeneous swarm in their natural reservoirs prior spillover events that lead to emergence of these diseases in human and animals. Antigenic identities of these organisms remain unknown until their establishment in an outbreak situation which can result to huge global impacts in case of a pandemic as recently witnessed with severe acute respiratory syndrome coronavirus (SARSCoV-2). Heterogeneous swarm of a virus can be very notoriously difficult to control using already existing vaccines as a result of immune-senescence. (Hammerschmidt et al., 2021; Tawinprai et al., 2022). Cross reactive immunity is important consideration for vaccine development in order to sufficiently protect from both homologous and heterologous challenge.(Barros-Martins et al., 2021; Hillus et al., 2021) The main objective of this study is to explore and come up with a successful vaccine candidate that can produce a robust cross protective immunity to homologous and heterologous challenge strain. This will enhance the mechanism of a successful vaccination strategy for the circulating strains disproportionately affecting small ruminants in the region or other emerging NSDV strains in future. Theses study hypothesized that among the isolated circulating NSDV strains, I34, 1473, Entebe, Ansell, the lethal strain produced the superior immunity. Lethality of all the isolates was studied in suckling mice model. The most lethal isolate was titrated in the suckling mice to determine the IMLD50. Adult mice were immunized intraperitoneally with 100 IMLD50 with a booster dose given 14 days apart. Superior protection for the mice was observed following a lethal challenge using the virulent 1473 challenge strain.

II. METHODS

- *Handling of Mice and Recording of Clinical Parameters*

Mice handling procedures were carried out in accordance to the standard operating procedure published by (Weiss & Bürge, 2012). All mice were weighed and temperature before and throughout the challenge experiment. Mice were restrained by scuffling method and the temperature reading obtained using a thermo-gun from the region below the base of the sternum for consistent readings.

- *Studies on Infectivity and Lethality*

Sickling BALB/c n=4/group mice were inoculated via intracerebral (IP) with 20uL each group with I34, 1473 and Ansell respectively with serum free GMEM inoculated group served as the control. Mortality and morbidity were monitored during the 14 days post infection (d.p.i) and clinical scores recorded. End point titers for the brain, liver and spleen viremia was determined as described by (Zhao et al., 2021) with slight modification. Differences in end point titers for internal organs, brain, liver and spleen were determined through ANOVA at p=0.05 and mean differences compared using Dunnett's multiple comparison test.

- *Immunogenicity and Protection Protective Efficacy in Mice*

20 BALB/c mice (n=5/group) were giving two doses 14 days apart 100uL of inactivated Nairobi sheep disease virus in three matrices, I34, 1473 or Ansell containing 10^6 TCID50. Control animals received 100ul of serum free GMEM medium. Mice were bled at day 0, 7, 14, 21, and 28 to assess the neutralizing antibody immune responses. 28 days post immunization, mice were challenged with 100ul 10^6 TCID50 of virulent 1473 strain. Protective efficacy was assessed by determining the viremia in blood at day 4 post challenge by titrating the infected blood of confluent monolayer of BHK cell line.

- *Statistical Analyses*

Data was entered and organized in Microsoft excel and the means were exported to Graph Pad Prism 9.3.1. The statistical differences between the viral titers in blood tissues was determined by the Mann-Whitney U test. Differences between the weights, temperatures and log2 antibody levels were subjected to Mann-Whitney U test at $p<0.05$ level of significance.

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III. RESULTS

- *NSDV Strains Lethality and Infectivity in Suckling BALB/c Mice*

16 Suckling mice (n=4/group) were inoculated intracerebral with 20ul of 10^8 TCID50. Lethality and infectivity of the three viruses was measured by time to death (TTD) for suckling BALB/c mice inoculated Intracerebral (IP) with similar number of particles viral particles. All the strains, 1473, I34 and Ansell were observed to exhibit difference in lethality and infectivity shown by significant differences in time to die (TTD) relative to the comparison control group. Ansell strain inoculated group showed a mild illness with 50% mortality by 14 days of inoculation. 1473 strain and I34 strain inoculated mice showed 100% mortality with significant difference in their average time to death (TTD). Average time-to-death (TTD) shown above ***(Figure 2)*** for the three strains were; 1473- 7.75days, I34- 9days and Ansell 10.5days (50% mortality). All the Mock vaccinated showed 100% survival rates and survived until day 14. 1473 strain recorded the highest viral tropism and multiplicity in mice, followed by I34 and Ansell ***(Figure 3).*** Average viremia for intracerebral inoculated mice in brain, liver and spleen was highest in 1473 as shown in figure ***(Figure 3)***

- *Suckling Mice Lethal Dose (SMLD50)*

In-vivo titration of 1473 lethal virus was carried out mice using 10^7, 10^5, 10^3, 10^1 matrices and serum free medium mock. Suckling mice lethal dose (SMLD50) to was determined be 10^4 of 100TCID50 concentration at which there was 50% survival as shown in figure ***Figure 2).*** **(**Survival rates for the lethal 1473 inoculated mice at 10^7, 10^5 and 10^3 were 0%, 25%, 75% and 100% respectively. All serum free medium inoculated mock survived.

- *Comparative Immunogenicity of Inactivated NSDV Strains in BALB/c Mice*

Plaque Reduction Neutralization Test (PRNT) was used to determine neutralizing antibody titers for 1473, I34, and Ansell inoculated groups. I34 immunized group produced the highest neutralizing antibody titers against 1473, I34, Entebe and Ansell vruses. I34 strain produced the highest neutralizing titers against homologous and heterologous strains in mice. Similar results were observed from rabbit hyper-immune sera against homologous and heterologous strains. Entebe strain showed similar infectivity profile to that of I34 against I34, 1473 and Ansell homologous sera.

- *Comparative Protection of Homologous and Heterologous Vaccine against Lethal 1473 Strain*

All the mice in all the groups were challenged intraperitoneally (IP) with 200ul of 10^4 (SMLD50) lethal Mice 1473 challenge. All mice were characterized by insignificant weight loss and slight decrease in body temperature. Hypothermia was found to be more in mock vaccinated group and Mice lost less than 10% of their weight within 2-4 dpi, became less active, handled together and looked prostrated. Mock vaccinated exhibited a higher weight loss following challenge than the vaccinated groups which was not significant at p value of 0.05. At 4-5 d.p.i mice all the groups including the mock gradually increased in weight, normalized body temperature and became more active by 6 d.p.i.

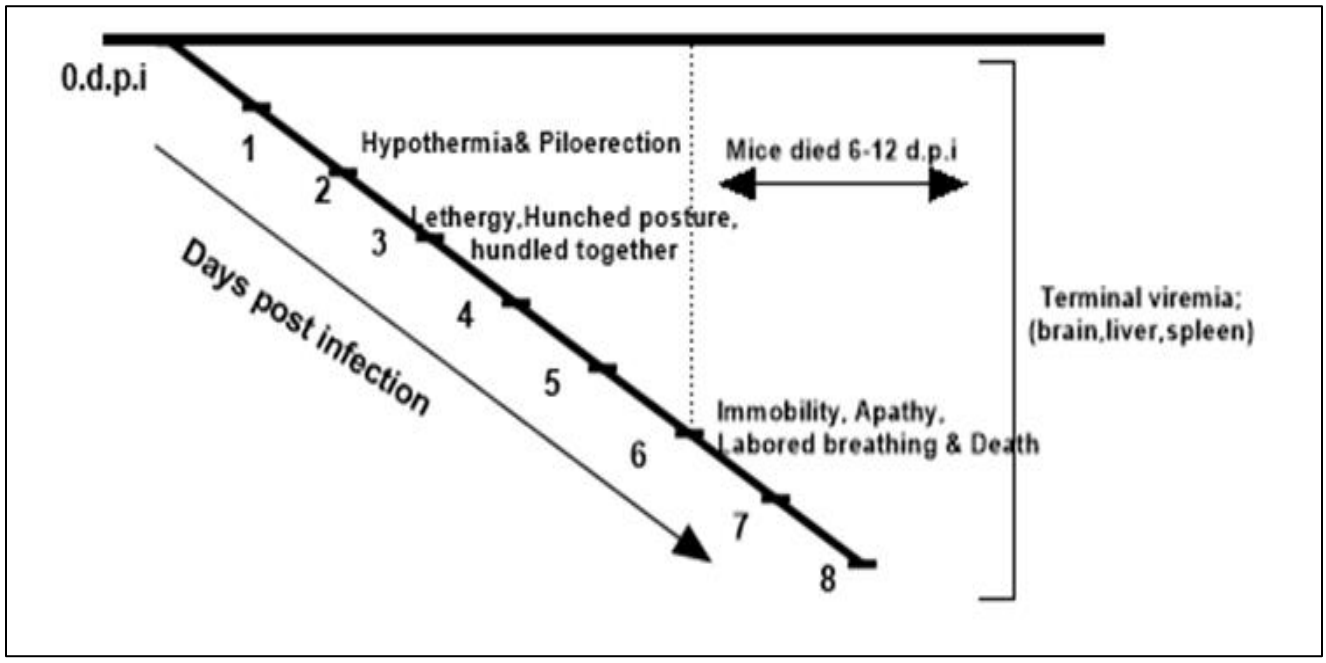

Fig 1 Experimental Illustration Highlighting Clinical Presentation in Suckling Mice Following Challenge with NSDV

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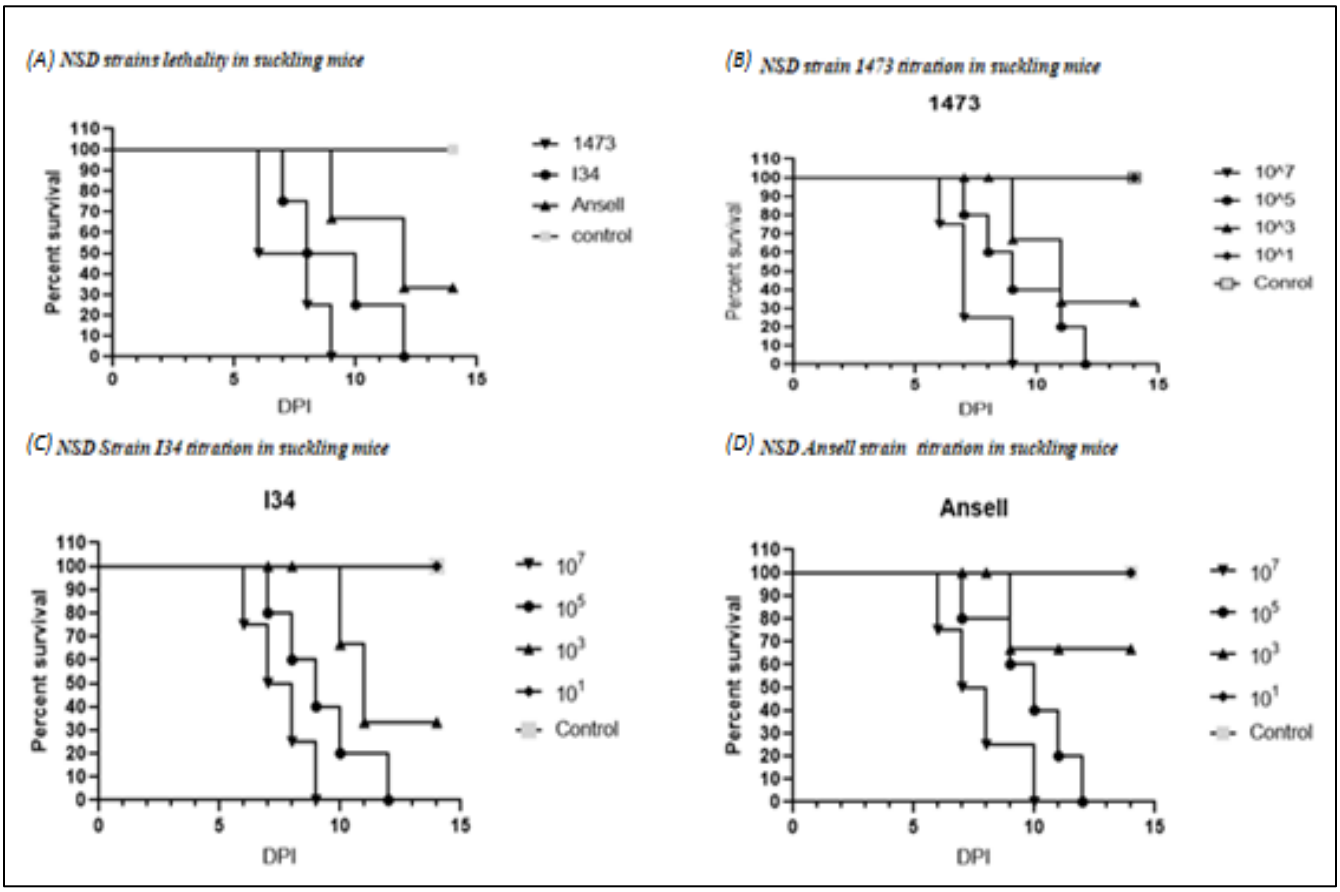

Fig 2; A: NSDV Strain Lethality. B: 1473 Challenge Virus SMLD50. C: I34 Challenge in SMLD50. D. Ansell Challenge in SMLD50

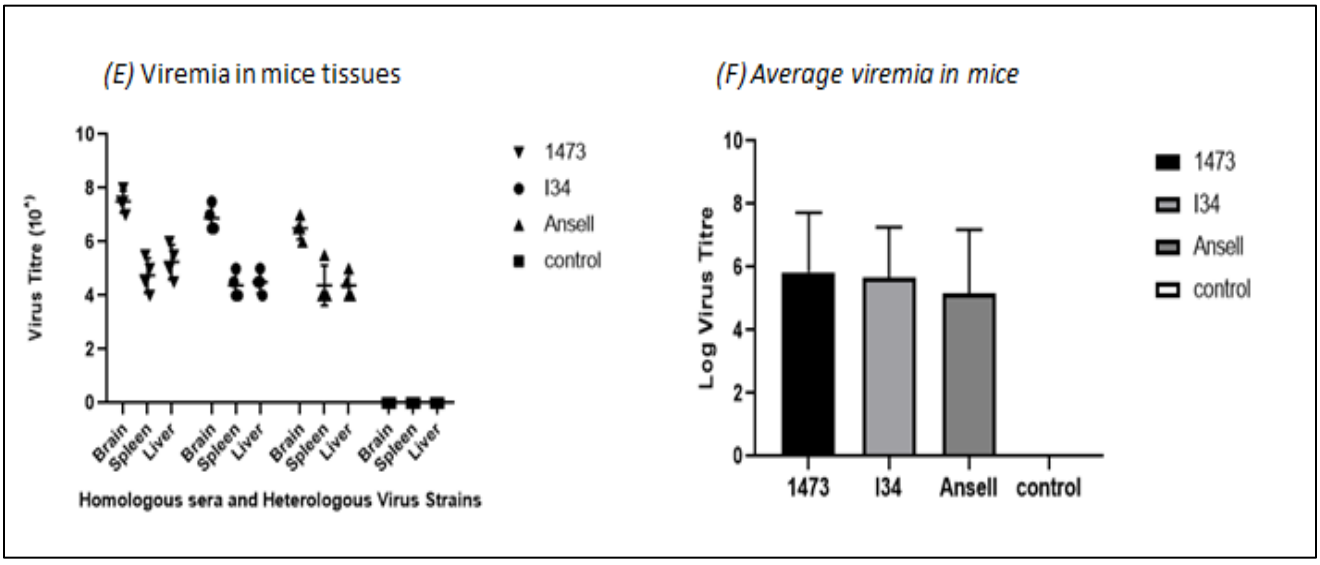

Fig 3. E. Average Viremia in Mice Organs. F Average Viremia in Mice

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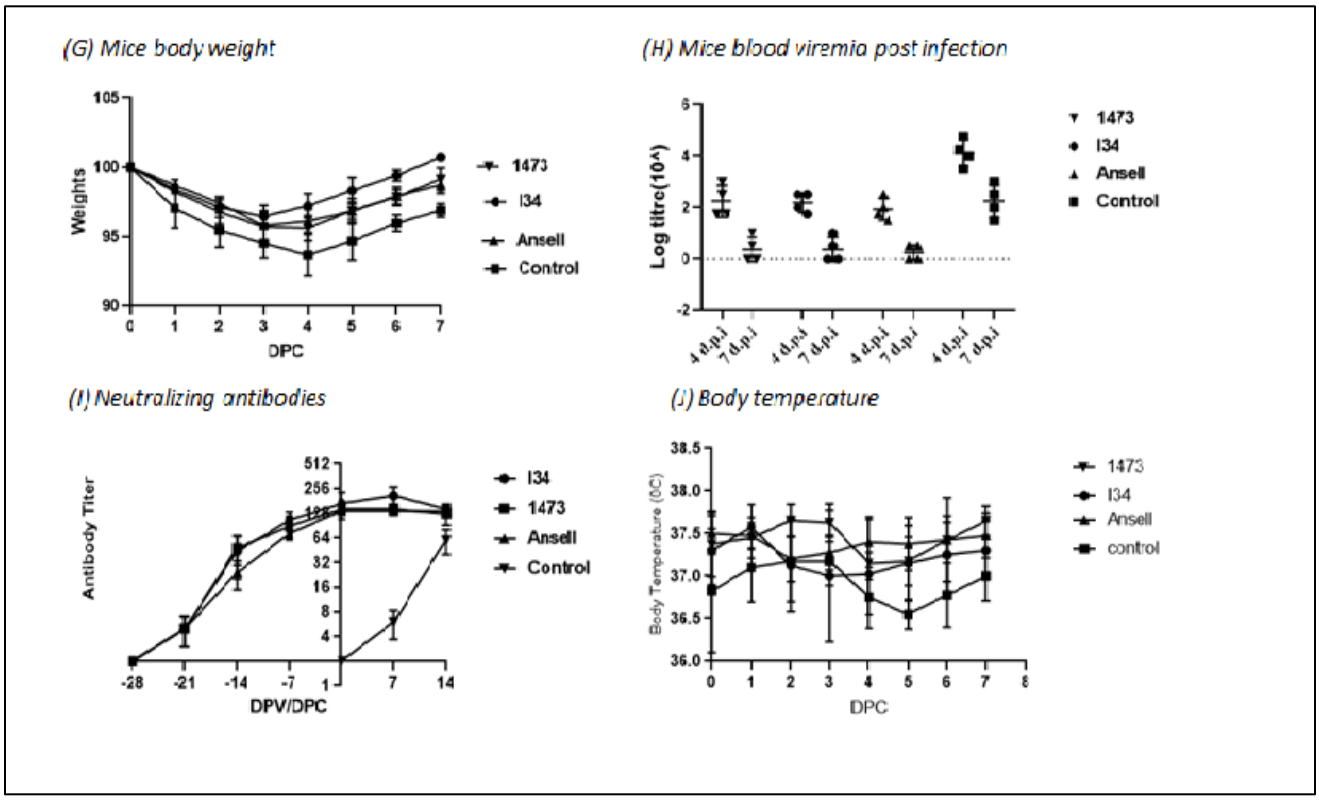

Fig 4. G: Change in Body Weigh Post Challenge. H: Viremia in Blood Post Challenge. I: Neutralizing Antibodies Against NSDV in Mice. J: Body Temperature Curve Post Challenge

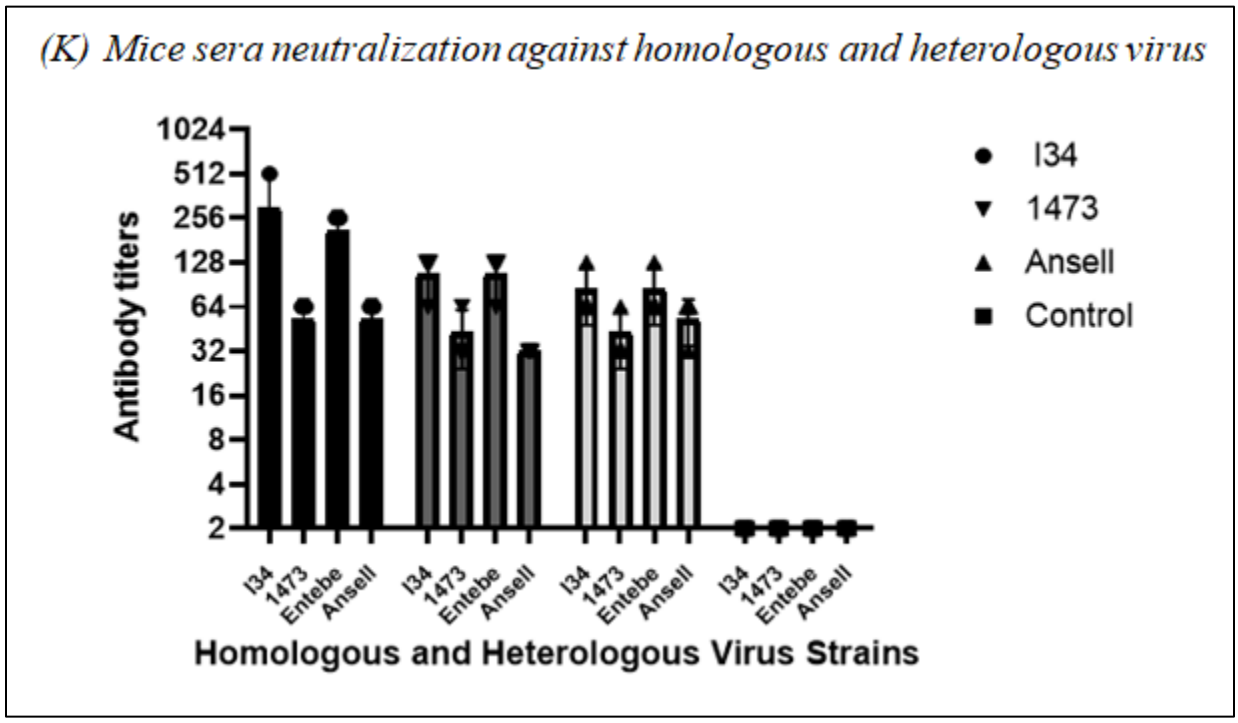

Fig 3 Homologous Sera Against Homologous & Heterologous Virus in Rabbit and Mice

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IV. DISCUSSION

Comparative studies on virulence, antigenicity and protective efficacy were carried to evaluate the differences between three Nairobi sheep disease virus (NSDV) strains I34, Ansell, and 1473. Three-day suckling mice (n=4/group) were inoculated through the intracranial (IC) route and the mice observed over a period of 14days. Nairobi sheep disease virus (NSDV) is a pantropic virus whose differences was evaluated following infection in day old mice. 1473 strains were observed to have grater neurotropic and tissue dissemination rates caused a fatal disease in mice and death. Relative indifference in disease and survival determined by the clinical score indicated the potentiality of the NSDV strains and their significance in disease and vaccine design.

Neutralizing antibodies development in mice following intraperitoneal (IP) inoculation in mice were evaluated by Plaque Reduction Neutralization Test (PRNT) and the serological differences in antibody levels over a period of 28days post vaccination determined. Serological relationships to homologous and heterologous strains by cross neutralizations test in-vitro was determined

- *Mice Infection Heterogeneity among NSD Virus Strains*

Day old BALB/c mice showed a much greater dissemination of the virus compared to adult mice due to a deficient adaptive immune response. The day old mice developed a lethal wasting disease with the virus substantially disseminated to the brain and kidney. (Yoshimatsu et al., 1997) Suckling mice developed a severe wasting disease characterized by ruffling of fur, labored breath, weight loss and death.

The choice of NSDV strain is of critical importance in vaccine development and the challenge to the NSDv animal models. NSDV isolates I34, Ansell, Entebe and 1473 were observed to cause different disease patterns in mice as was observed in arenavirus in guinea pig model. (*Joseph et al,.2015*). In addition, various NSDv strains exhibit a varying degree of lethality 0 -100% with 1473 strain being the most virulent and Entebe and I34 strains strain being least virulent with similar lethality. Ansell strain is least virulent in day old BALBC mice. Age is a factor to consider when testing the virus lethality and efficacy of the vaccine. Identification of biomarkers of virus virulence in different strains and host factors contributing to disease severity differences is important. Use of different strains provides greater insight as to the protective efficacy of each and their ability confer protective immunity against a heterologous virus.

This study could not differentiate the four strains as different serotypes as there are no significant differences in susceptibility to neutralizing antibodies raised against each strain. However, the study showed serum raised from I34 exhibited a higher neutralizing potential at neutralizing homologous versus heterologous two-fold higher compared to the other NSDv strains. This may suggest a unique antigenic difference and hence the small impact on the neutralizing titre. *(Jahrling et al,.1985)*. Antigen homology is an important consideration in selecting a vaccine seed strain to ensure protection against a heterologous strain for full protection.

Prevention and control of infectious diseases, emerging and reemerging viral diseases remains a high priority in endemic regions of the world. Development of safe and effective vaccine is the only effective method to avert associated mortalities due to lack of effective treatment options.

Nairobi sheep disease virus cause a severe disease in small ruminants in sheep associated with mortalities ranging from 70-90 % in a naïve herd. There different strains of Nairobi sheep disease virus that have been isolated in many endemic regions of the world, in Africa, Indian subcontinent and china. This study evaluated strains variations in infection, antibody response and protective efficacy in BALB/c mice. All the strains in this study showed differences in infection (lethality), immune response and protection to their homologous and heterologous strains tested in vitro. These differences suggest differences in the neutralizing epitopes within the viral glycoprotein located in the highly variable region. The three strains, I34, 1473 and Ansell have shown to differ significantly due to a differing corresponding difference in infections, immune response and protection. Significant differences in infection were shown in cell culture in BHK cells and lethality in 2-day old suckling BALB/c mice. Difference in immune response were evaluated by in vitro method plaque reduction neutralization test using sera raised from each strain against their homologous and heterologous strains. 6 weeks old BALB/c mice were intraperitoneally immunized with 10^6 100 TCID50 inactivated NSDV. This revealed that antibodies raised against the I34 strain was superior in neutralizing infection with homologous and heterologous strains. A booster dose 14days post first immunization significantly increased neutralization titers to both homologous and heterologous virus strains. This shows that a booster dose reduces the chances of the virus to escape the neutralization within the body conferring maximum protection to the virus. Sera raised against I34 strain demonstrated high cross protective response against the test strains showed by high baseline neutralizing antibody titers to homologous and heterologous. Limitations of this study is that it did not show cross protective response to the current circulating of Nairobi sheep disease virus due lack of current isolated virus strain.

To compare the protection of homologous and heterologous vaccine against the 1473 challenge virus, mice immunized with I34, 1473, and Ansell were challenged mice along the serum free control mice with 10^3 100ul TCID50. Body temperature and weight change in individual mice after challenge with virulent 1473 strain were monitored in all the groups. Viremia in blood for the four groups were determined

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and the differences in viral loads for each group compared relative to the control group. The control group showed a higher viremia than vaccinates. However, there was no significant difference in temperature and the clinical score index for all the groups. At day three post challenge, mice became less reactive and immobile, rolled up with piloerected hair and significantly lost weight. Day 7-10 post challenge, the mice started regaining their weights, symptoms cleared and all the mice survived. All vaccinates showed similar pattern of illness, weight loss and survival with slight differences in viremia. The control group showed higher viremia which was found significant to all the vaccinated groups.

V. CONCLUSION

The study demonstrated that 1473 strain was more lethal in mice and with a greater tissue tropism compared I34 and Ansell strain. Strain 1473 produced the highest viremia in brain, liver and spleen in suckling mice. 1473 strain showed the highest pathogenicity and tissue tropism I34 strain proved to be the most immunogenic antigenic identity of the three strains. This was demonstrated by high neutralizing titers against homologous and heterologous virus. 134 strain was the candidate strain choice for vaccine development.

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Data Availability

All data and material that support the findings of this study are included in this manuscript.

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Conflict of interest

The authors declare no conflict of interest

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