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Towards Effective Vaccine Production: A Controlled Field Trial on the Immunological Response of Three Lumpy Skin Disease Vaccine Strains in Dairy Farms

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Abstract: Longitudinal study design was devised for field trial of lumpy skin disease virus (LSDV) vaccine strains in two dairy farms of Bishoftu, Ethiopia from October 2012 to May 2013 to give an insight for the comparative serum neutralization test (SNT) antibody responses and dynamics in cattle population. Three LSD vaccines were administered to dairy cows of two farms and followed up to 63 days post vaccination. The vaccines were Capripox vaccine (n= 44), Neethling vaccine (n= 39) and Romanian vaccine (n= 40) strains. A total of 425 sera samples (including both before and after vaccinations) were tested for LSDV antibody. Among the study animals, only 13.3% had protective antibody at cut-off value of $\geq \log_5^2$ before vaccination. Before vaccination, 50% (31/62) of the study animals from Debre Zeit Agriculture Research Centre (DZARC) and 22.6% (14/62) from Ethiopian Meat and Dairy Technology Institute (EMDTI) showed no detectable antibody. Antibodies production were started before day 7 of post vaccination and the mean antibodies of all the 3 vaccines were increased across each day of the followed up. The peak antibody titres were observed in the monitored cattle of EMDTI at day 35 of post vaccination. After vaccination all animals had antibody titres of $\geq \log_5^2$ starting from day 21 and remain within protective range at day 63 too. Age and parity did not show significant effect on sero-conversion rate (antibody titre) of the vaccinated animals. The three vaccines appeared equally immunogenic. It should be proven through challenge to recommend for a wider animal population.

Key words: Lumpy Skin Disease Virus · Serum Neutralization Test · Three Strains of LSD Vaccines

INTRODUCTION

Lumpy skin disease (LSD) is an infectious, eruptive, occasionally fatal disease of cattle caused by a double stranded DNA virus of the family *Poxviridea* and genus *Capripox* which is also termed as Neethling virus [1, 2]. The disease was first described in Northern Rhodesia (currently Zambia) in 1929 and then rapidly spread in cattle over most of the African countries [3]. LSD was first reported outside Africa in Middle East [4, 5] in 1991 and possibly introduced in the region through import of live animals from endemic countries of African continent [6]. Further spread of the disease to Eastern Asia and Northern Europe through Middle East and Turkey was reported [7].

Lumpy skin disease is an OIE list A disease, which shows its serious socio-economic status. It has significant economic importance to cattle industry due to chronic debility in infected cattle, reduction in milk production, abortion, temporary or permanent sterility, damaged hides and deaths [7]. The disease presents itself as an acute, sub-acute or inapparent disease with variable severity depending upon *Capripox virus* strain and the host breed. *Bos indicus* is known to be less susceptible to clinical disease than *Bos Taurus* [8] and cows in lactation are more at risk [9]. It is less contagious with low mortality (less than 10%) but has been as high as (20-75%) in some outbreaks and varying (1-90%) morbidity rate, even sometimes reach as high as 100% during some outbreaks [3,10, 11, 2]. Transmission is

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thought to be primarily by biting insects. The incidence of LSD occurrence is high during wet seasons when biting insect populations are abundant and decreases during the dry season [12].

Four live attenuated strains of *Capripox virus* have been used as vaccines for the control of LSD [4, 9, 13]. These are a strain of Kenyan sheep and goat pox virus, Yugoslavian sheep pox strain, Romanian sheep pox strain and lumpy skin disease virus strain from South Africa. It is thought that strains of *Capripox virus* share a major neutralizing site, so that animals recovered from infection with one strain are resistant to infection with any other strain. Consequently, it is possible to protect cattle against LSD using strains of *Capripox virus* derived from sheep and goat [14, 13].

In Ethiopia lumpy skin disease was first observed in the North-western part of the country (Southwest of Lake Tana) in 1983 [15]. It has spread to almost all the regions and agro-ecological zones of the country. Because of the wide distribution of the disease and the size of the cattle population in Ethiopia it is likely that LSD is one of the most economically important livestock diseases in the country [12]. Ethiopia has been striving to control the disease using mass vaccination. The currently used LSD vaccine is a homologous LSD vaccine produced from attenuated Kenyan sheep and goat pox strain virus (KSGPV). National Veterinary Institute (NVI) is the only institute producing and providing this vaccine to all regions of the country. However, there are compliances from animal owners and farm managers about occurrence of the disease after animals have been vaccinated. Scientific studies done on these compliances of the vaccine strains are very scarce in Ethiopia. Therefore, this field trial is important to evaluate the status of the existing vaccine strains and to select the best vaccine strain that effectively protects cattle population against LSD virus. Therefore the objectives of this study were:

- To measure, compare and rank antibody responses of cattle to three LSD vaccine types using Serum Neutralization Test
- To monitor the persistence of the induced antibody in the vaccinated cattle population and
- To evaluate whether the quantity of antibody induced is dependent on age and parity level of the vaccinated animals

MATERIALS AND METHODS

Study Area and Study Population: The study was conducted in Ethiopian Meat and Dairy Technology Institute (EMDTI) and Debre Zeit Agricultural Research Centre Dairy Farms (DZARC) located in Bishoftu town. Bishoftu is located in East Shewa Zone, Oromia regional state, in the central highlands of Ethiopia and is about 47 km distance from Addis Ababa. Its altitude ranges from 1,500-2,200 meter above sea level. It has a short rainy season from March to April and a long rainy season from June to September. The average annual rainfall is 1,150 mm, while the maximum and minimum temperature is 28.6°C and 12.9°C, respectively [16]. The study population comprised of apparently healthy Holstein Friesian (crossbred) dairy cows above six months of age from the two farms.

Study Design and Sampling Methods: A longitudinal experimental study was carried out in the two government dairy farms. These farms were purposively selected based on their LSD outbreak occurrence reports after the animals have been vaccinated and easiness for monitoring. Random sampling method was used to select the study animals from each farm and to vaccinate the animals with the three vaccine strains. Animals were assigned randomly to the three groups of LSD vaccine strains in each farm. The immunological statuses of the study animals were evaluated before and after vaccinations via sera sampling to check their antibody titres.

Types of Vaccine and Vaccination of Study Animals: The three types of vaccine strains used were Kenyan sheep and goat pox (KSGP), South African Neethling (SAN) and the Romanian sheep pox (RSP) produced by NVI. All these vaccines were live, freeze dried and in a bottle of 100 doses of each containing 10^3 $TCID_{50}$ (50% tissue culture infective dose) viruses/ml. The safety, potency and the sterility of the vaccines were checked before and after freeze drying as per the protocol of NVI vaccine production protocols. A total of 425 sera were tested using SNT for lumpy skin disease virus (LSDV) before and after vaccinations with KSGP (n= 44), SAN (n= 39) and RSP (n= 40) strains. A 100ml of sterile saline water solution was transferred to the bottle containing the freeze-dried vaccine and then mixed well till the powder is completely dissolved. From EMDTI 22, 21 and

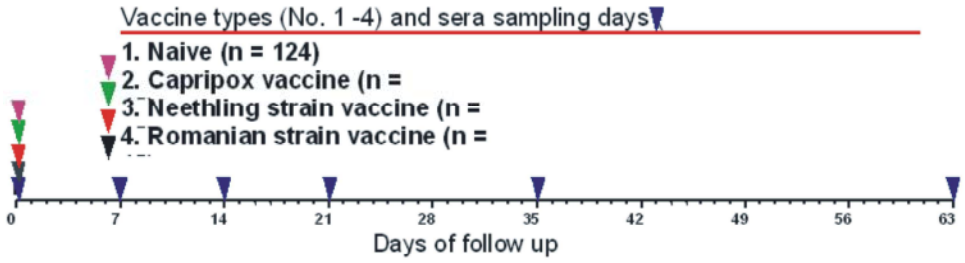

Fig 1: Summary of the study design.

Key: *The n in naive group indicated the number of cattle sampled before vaccination from both EMDTI and DZARC farms whereas the rest n showed the number of regularly monitored animals in EMDTI farm but day 35 is exceptional*

19 of the study animals were vaccinated by KSGP, SAN and RSP vaccine strains respectively. In the same manner from DZARC, 22, 18 and 21 animals were vaccinated with the three vaccines at a dose of 1 ml per animal.

Sample Collection: A total of 124 animals (62 from DZARC and 62 from EMDTI) were sampled at day 0 (before vaccinations). The animals were restrained by the attendants during every blood sample collections. About 5-7ml blood samples were collected from the jugular vein using plain vacutainer tubes from each animal. Each sample was labelled appropriately for identification. Before vaccination blood sample was collected from all study animals if there are antibodies of LSD before vaccination from natural infections. This is also important data to compare the antibody levels before and after vaccinations. Blood sample after vaccination was collected regularly at days 7, 14, 21 and 63 from 45 animals (i.e. only 15 animals per vaccine type) from EMDTI farm only due to the budget constraints. However, specifically at day 35 blood samples were collected from 61 cattle from DZARC (one animal is excluded because the animal is culled out due to sever physical injury) and 62 from EDMTI. The collected samples were transported to NVI virology laboratory and allowed to clot for 16-24 hrs at room temperature and then the sera samples were transferred to cryovials under laminar air flow hood in ordered to avoid contamination and the serum was kept at -20°C temperature until processed.

Serum Neutralization Test (SNT): The serum neutralization test is the most specific serological test and gold standard to detect antibodies against LSDV with sensitivity and specificity of 78% and 97% respectively though time consuming [1, 11, 17]. The procedure was carried out using 96-well flat-bottomed cell culture micro-titre plates, according to the standard protocol of the virology section of NVI, Debre Zeit, Ethiopia following OIE [1] manual.

The sera stored at -20°C were thawed at room temperature for subsequent SNTs. A 1:5 dilution of the test sera serially diluted starting at 1/5, 1/25, 1/125, 1/625 and 1/3125 dilutions in a Glasgow's minimum essential cell culture medium (GMEM). A volume of 75µl of GMEM was added to columns 1 and 7 of each well; whereas from columns 2 up to 6 and 8 up to 12 a volume of 100µl GMEM was added in each well. A volume of 25 µl tested sera was added in columns of 1, 2, 7 and 8 in each well. Using multichannel pipette 5-fold serial dilutions was performed (from columns 2 to 6 and 8 to 12) with initial dilution of 1/5 and 25 µl of suspension was discarded from the end point dilutions 1/3125 (that is from column 6 and 12).

A volume of 100µl of 1000$TCID_{50}$/ml (50% tissue culture infective dose) viral suspension of each vaccine strain was added in each well from columns 2 to 6 and 8 to 12; for virus control, different dilutions of the antigen was added into 5 wells in each row; then the plates were sealed with plate sealer, mixed with plate mixer and incubated at 37°C, containing 5% carbon dioxide (CO_2) for 1hr. A cell control was prepared into five wells of the last row. After an hour of incubation 50 µl of Vero cells preparations at the concentrations of $4x10^5$cells/ml was added in each well and checked for cell concentration using microscope. The plates were again sealed and further incubated at 37°C, containing 5% carbon dioxide (CO_2) for 4-9 days.

The plates of monolayer cells were examined under inverted microscope for the presence of cytopathic effect (CPE) starting from day 4. The final reading was taken on day 9 and the results were recorded from the highest dilution which inhibited CPE in both or either of the

duplicate wells and recorded as the reciprocal of the log titration. Interpretations of the results were made in such a way that wells with no CPE in 1/25 and more dilutions were considered as positive. This signifies that the antibody against the LSD virus has reacted with the vaccine strains and inhibited the growth of the virus [1, 3, 12].

Data management and analysis: The collected data was entered and stored into Microsoft office Excel spread sheet 2007 and thoroughly screened before subjecting to statistical analysis. Descriptive statistics was used to quantify the proportion (percentage) of cattle with different levels of SNT antibody titres across each sampling day. Prism 5 software was used to compare the mean and confidence intervals of SNT antibody titers across time for the three vaccine strains. Based on the explanation by Gari *et al*. [12] referred from OIE [1] SNT antibody titers with $\geq \log_5^2$ were considered as a cut-off value. This is an effectively protective antibody concentration for LSD. Vaccinated animals were classified either protected or at risk group using this cut-off value based on their SNT antibody titers at each sampling day. The number of days' taken-to-protective SNT antibody production for each vaccinated animal was calculated using Kaplan-Meier survival analysis. A log-rank was used to compare whether the before and the after vaccination animal proportions categorized either as protected or as at risk group. It was also used to test whether or not the three vaccines induced similar proportion of animals either protected or at risk group after vaccination at each time point of follow up. This leads to the conclusion to rank and select the best protective vaccine among the three vaccine strains we have experimented on. Cox-proportional hazard ratio analysis was used to test the ratio of sero-conversion among the three vaccines and the underlying risk factors (age and parity). In all the analyses, confidence interval was at 95% and $p < 0.05$ was set for significance.

RESULTS

Serum Neutralization Test (SNT): A total of 425 sera samples were tested for neutralizing antibodies using the SNT for lumpy skin disease virus (LSDV) before and after vaccinations with Capripox (n= 44), Neethling (n= 39) and Romanian vaccine (n= 40) strains from the two farms and the results are presented in the following sections.

Assessments on Snt Antibody Titres of Cattle Before (Day 0) and after Vaccination at (Day 35): The mean of the SNT antibody titres of the DZARC and EMDTI farms were similar at day 0 (before vaccination) though appeared slightly higher in EMDTI farm. After vaccination, the SNT antibody remarkably increased at day 35 compared to day 0 but it was without statistically significant variation between both farms and three vaccine types as shown in Figure 2.

At day 35 all the vaccinated animals produced detectable SNT antibody as high as 5 times dilution indicating induction of high concentration of antibody after vaccination as shown in Table 1.

Seroconversion Status of the Monitored Cattle after Vaccination: As days went on post vaccination, the SNT antibody titres increased with different percentages of animals for each seroconversion level among the three vaccine types as shown in Table 2 and Figure 3.

The three vaccines appeared to induce insignificantly ($p > 0.05$) different levels of SNT antibody levels indicating the three vaccines were equally immunogenic and usable as shown in Figure 3.

Comparison of the Proportion of Sero-Converters Using Cut-off Value $\geq \log_5^2$ for Snt Antibody Titres Before and after Vaccinations: After vaccination the number of animals with antibody titres of $\geq \log_5^2$ were increased across each time point of follow up. The comparative

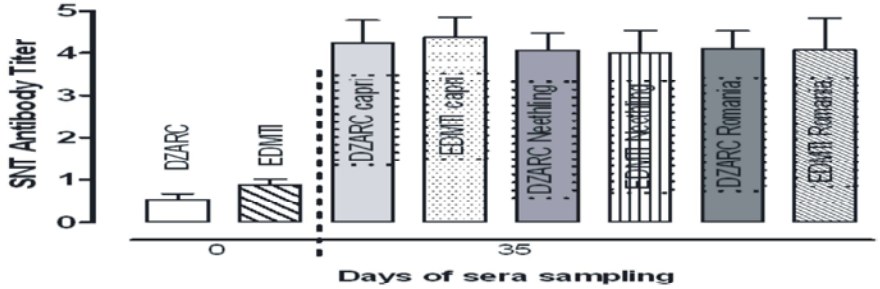

Fig 2: Comparisons on the antibody responses (mean and confidence intervals of SNT antibody titres) of cattle before vaccination (at day 0) and after vaccination (at day 35) with the three types of LSDV vaccine strains in DZARC and EMDTI dairy farms

Table 1: Comparative SNT antibody titres at day 0 (before vaccination) and the after vaccination seroconversion rates of the cattle at day 35 in DZARC and EMDTI dairy farms

		DZARC	EMDTI
Vaccine type	SNT antibody titre via serial dilution	Day 0 (n= 62)	Day 0 (n=62)
Non-vaccinated	0	50% (31/62)	22.6% (14/62)
	1	46.8% (29/62)	67.7% (42/62)
	2	3.2% (2/62)	9.7% (6/62)
		Day 35 (n = 61)	Day 35 (n= 62)
Capripox strain vaccinated	0	-	-
n= 22= DZARC	1	4.5% (1/22)	-
n= 22= EMDTI	2	13.6% (3/22)	-
	3	-	13.6% (3/22)
	4	18.2% (4/22)	40.9% (9/22)
	5	63.6% (14/22)	45.5% (10/22)
Neethling strain vaccinated	0	-	-
n= 18= DZARC	1	-	-
n= 21= EMDTI	2	-	4.8% (1/21)
	3	33.3% (6/18)	19% (4/21)
	4	27.8% (5/18)	33.3% (7/21)
	5	38.9% (7/18)	42.9% (9/21)
Romanian strain vaccinated	0	-	-
n= 21= DZARC	1	-	10.5% (2/19)
n= 19= EMDTI	2	4.8% (1/21)	-
	3	28.6% (6/21)	5.3% (1/19)
	4	19% (4/21)	31.6% (6/19)
	5	47.6% (10/21)	52.6% (10/19)

n= total animals vaccinated with the three types of LSDV vaccine strains across each farm

Table 2: Sero-conversion profile of the monitored cattle after vaccination with the three types of LSDV vaccine strains in EMDTI across each days of follow up

		Days of follow up					
Vaccine type	SNT titre	0	7	14	21	35	63
Capripox (n=15)	0	22.2% (10/45)	6.7% (1/15)	-	-	-	*
	1	64.4%(29/45)	33.3% (5/15)	13.3% (2/15)	-	-	-
	2	13.3% (6/45)	60% (9/15)	80% (12/15)	20% (3/15)	-	14.3% (2/14)
	3	-	-	-	53.3% (8/15)	20% (3/15)	35.7% (5/14)
	4	-	-	6.7% (1/15)	20% (3/15)	20% (3/15)	35.7% (5/14)
	5	-	-	-	6.7% (1/15)	60% (9/15)	14.3% (2/14)
Neethling (n=15)	0	22.2% (10/45)	6.7% (1/15)	-	-	-	-
	1	64.4% (29/45)	46.7% (7/15)	6.7% (1/15)	-	-	-
	2	13.3% (6/45)	46.7% (7/15)	73.3% (11/15)	13.3% (2/15)	6.7% (1/15)	6.7% (1/15)
	3	-	-	13.3% (2/15)	66.7% (10/15)	26.7% (4/15)	26.7% (4/15)
	4	-	-	6.7% (1/15)	20% (3/15)	26.7% (4/15)	20% (3/15)
	5	-	-	-	-	40% (6/15)	46.7% (7/15)
Romanian (n=15)	0	22.2% (10/45)	13.3% (2/15)	-	-	-	*
	1	64.4% (29/45)	33.3% (5/15)	40%(6/15)	-	13.3% (2/15)	-
	2	13.3% (6/45)	53.3% (8/15)	53.3% (8/15)	40% (6/15)	-	-
	3	-	-	6.7% (1/15)	53.3% (8/15)	6.7% (1/15)	57.1% (8/14)
	4	-	-	-	-	26.7% (4/15)	8.6% (4/14)
	5	-	-	-	6.7% (1/15)	53.3% (8/15)	14.3% (2/14)

* At day 63 samples did not taken from two animals which were vaccinated by Capripox and Romanian vaccine strains because the animals were slaughtered due to physical injury

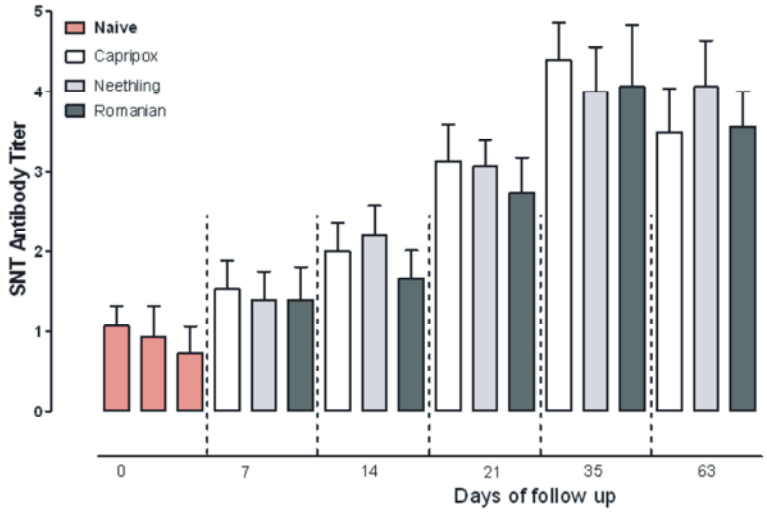

Fig 3: Comparisons of the SNT antibody responses (mean and confidence intervals) among the monitored cattle from EMDTI vaccinated with the three types of LSDV vaccine strains across each day of follow up for each vaccine types

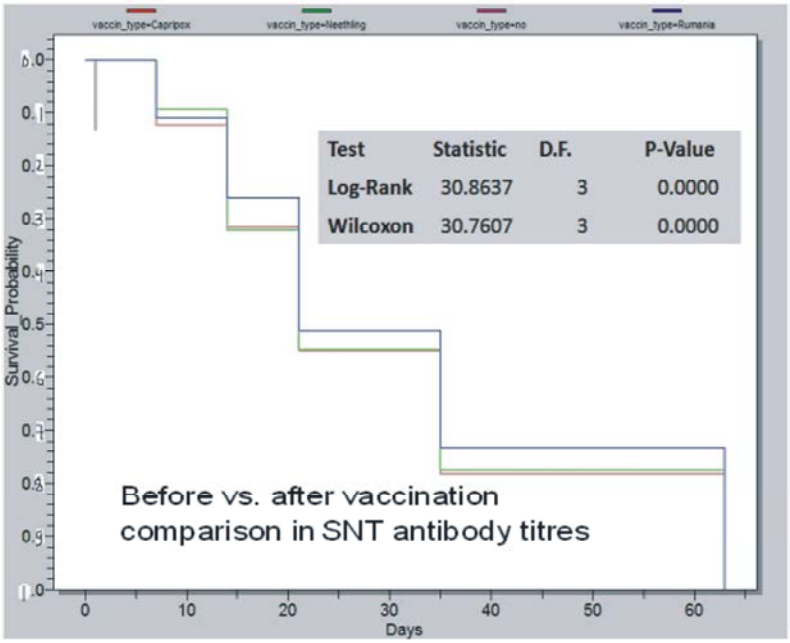

Fig 4: Proportions of monitored cattle with SNT antibody titres $\geq \log_5^2$ before and after vaccination at each time point as assessed by Kaplan-Meyer survival analysis among the 3 vaccines

SNT antibody increment between the before vs. after vaccination were statistically significant as shown in Figure 4.

Comparison of the Seroconverters after Vaccination Using a Cut-off Value ≥ Log_5^2 for Snt Antibody Titres among the 3 Vaccines: The proportions of cattle whose SNT antibody titres $\geq \log_5^2$ were increased after vaccination with the three types of vaccines in each days of follow up but no significant difference noticed statistically among the three vaccine types as shown in Figure 5.

Seroconversion Levels among Vaccine Types, Cattle Age Groups and Parity: The likelihood of seroconversion appeared to be higher in Capripox vaccinated cattle compared to the rest vaccine types by Cox proportional hazard ratio model. However, the seroconversion levels

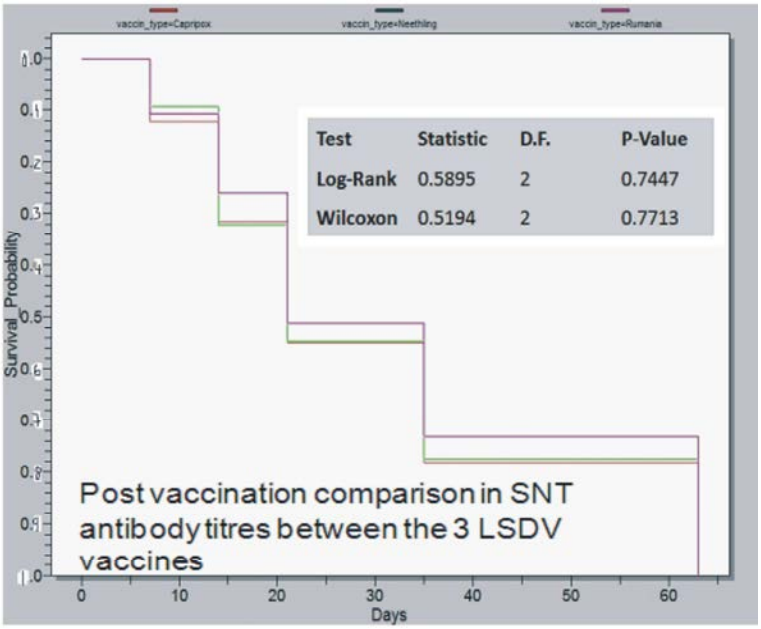

Fig 5: Proportions of monitored cattle with SNT antibody titres > log52 after vaccination at each time point as assessed by Kaplan-Meyer survival analysis among the 3 LSD vaccines

Table 3: Cox proportional hazards ratio for comparison of seroconversion levels among vaccine types, cattle age groups and parity levels of the vaccinated animals

Term	Hazard Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
vaccine(Neethling/Capripox)	0.9824	0.6834	1.4123	-0.0177	0.1852	-0.0957	0.9238
vaccine(Rumania/Capripox)	0.9217	0.6341	1.3399	-0.0815	0.1909	-0.427	0.6694
age group(1/0)*	0.9821	0.6254	1.5422	-0.018	0.2302	-0.0783	0.9376
Age group(2/0)	0.9463	0.4172	2.1463	-0.0552	0.4178	-0.1321	0.8949
parity(1/0)	0.9016	0.5835	1.3931	-0.1036	0.222	-0.4668	0.6407
parity(2/0)	1.0285	0.5133	2.0608	0.0281	0.3546	0.0793	0.9368
parity(3/0)	1.0061	0.4319	2.3436	0.0061	0.4314	0.0142	0.9887
parity(4/0)	0.8986	0.3277	2.4638	-0.1069	0.5146	-0.2077	0.8354
parity(5/0)	0.9663	0.3413	2.7361	-0.0342	0.531	-0.0645	0.9486

*Age group = 0 means < 2 years; 1 means 2 - < 5 years and 2 means > 5 years.

among the three vaccine types did not show significant difference statistically ($p > 0.05$). Age and parity level of cattle were assessed to know if in case they influence the seroconversion rate in vaccinated cattle. However, neither age nor parity could have significant ($p > 0.05$) effect on seroconversion rate of the vaccinated animals as indicated in Table 3.

DISCUSSION

Vaccination is widely prescribed as an effective control measure for LSD [9, 13, 4, 1]. However, little is known on the immunological response and immune dynamics against this disease. Despite regular cattle vaccination with Capripox vaccine in Ethiopia, there is a continuous outbreak in vaccinated animals. Therefore, the objectives of this field trial on the three LSD vaccine strains were to give an insight to the comparative SNT antibody response and its dynamics in cattle population of two dairy farms of Bishoftu.

The SNT results indicated that before vaccination very low levels of antibodies were detected in both farms as well as very few animals had this antibody. However, in our study after immunizations (vaccinations) a higher fold increase in SNT antibody titres were detected in DZARC and in EMDTI dairy farms. The vaccinated animals were able to produce antibodies before day 7 of post vaccination. Similarly, in the literature it was indicated that vaccinated animals produce neutralizing antibodies before day 7 of post vaccination [18]. However, in newly infected cattle the antibodies appear after 10 days [19]. Both the percentages of

seroconversion rates of the animals and mean SNT antibody titres of all the three vaccine strains were increased at each days of followed up after day 7. The SNT antibody reached peak at day 35 and remained peak until the follow up ends at day 63. Although similar work on LSD in cattle is scarce in the body of literature, works on similar virus in goats (goat poxvirus) showed that the antibodies are increased above 1: 16 titres at day 21 and reached peak (1:32) at 3 months and remain peak for 1 year post vaccination [20].

Currently the humoral wing of immune response post vaccination is evaluated in this study. In the body of literature there exist unsettled hypothesis that both the humoral and the cellular immunity works in the protection of poxviruses including LSD infection. However, the effective protective immune system wing for LSD is said to be cell mediated although the antibody wing also relevant [18]. However, the work of Zheng *et al.* [21] and Barman *et al.* [20] in goats on goat pox virus revealed that it is not only the cellular immunity wing but also the antibody wing is actively protecting from the viral challenge. In the current study artificial challenge post vaccination was not conducted to evaluate the efficacy of the induced antibodies in protecting the animal from viral invasion. Furthermore, literature on immune response against LSD vaccination in cattle is non-existent to use as a contrast to the current findings. Meanwhile, there is an argument that neutralizing antibody with 1:25 titre is protective for LSD viral challenge [12, 1].

From the works of Barman *et al*. [20] who worked on similar virus in goats, the lesson is neutralizing antibody with 1:16 titre is protective against goat pox viral challenge. In this line, according to Kaplan-Meyer survival analysis, before vaccination around 13.3% of the monitored animal population in EMDTI had SNT antibody titres of $\geq \log_5^2$. There was higher statistical significant difference ($p = 0.0000$) before and after vaccinations of the cattle proportion whose SNT antibody titres were $\geq \log_5^2$. But after vaccination with the three types of LSDV vaccine strains, higher proportion of animals developed higher levels of antibody titres across the different days of follow up. After day 21 post vaccination, majority of the monitored animals developed SNT antibody titres which were $\geq \log_5^2$ with insignificant variation among the three vaccine types statistically ($p > 0.05$) implying all the 3 vaccines gave similar antibody responses based on Log-Rank test.

There is accumulated evidence that sheep pox, goat pox and LSD are related viruses as well as their induced antibodies cross-react one another [13, 1]. Accordingly, though our viral strains were derived from sheep pox (Rumanian strain), goat and sheep pox (capripox strain) and LSD (Neethling strain), remarkable difference was not observed in antibody induction among the three viral strains used for our experiment across the study time.

Between the farms the seroconversion rates were relatively higher in DZARC as compared to in EMDTI. The reasons might be due to differences in management or a difference in the percentages of exotic germplasm (gene) compositions (in DZARC on average 60% of the gene composition of the animals were exotic while in EMDTI around 85% gene composition of the animals were exotic).Whereas the slightly low level of seroconversion rates were recorded in animals vaccinated by Neethling vaccine strain in both farms but insignificantly compared to the rest two vaccine types. Furthermore, seroconversion (antibody titre) levels of all the three vaccine types were not affected by the age and parity of the cattle in this study. As age and numbers of parity of the animal increases, the seroconversion levels of the animals remained similar among the three vaccine types implying the seroconversion condition work independent of age and parity. There is no strong evidence in the literature as to the probable and known variables which influence the immune response to lumpy skin disease virus and vaccines in cattle to use as a template to compare with them.

Contrary to our findings, in other viral diseases of cattle (e.g. FMD) a number of variables are listed that influence the immune response of the cattle towards vaccine trial. These variables include hosts (species, breed, age, health status, physiological state), virus (dose, route of administration, virus strain) and response variables includes antibodies (specificity, half-lives, synergy or competition between different antibodies, titre and distribution), cells (density and number, distribution or tropism, types of cells, relative proportions of different cells) [22]. We never exactly know whether the LSD immune response behaves differently than FMD based on this preliminary study. Despite regular vaccination by farm owners every year, we didn't able to detect antibody at both farms in majority of the animals before we launched our study. It indicated that the half-life of the antibodies in blood circulation could be very short (less than 1 year) in Ethiopian cattle population and management context. However, in our experiment the antibodies persisted at higher level up to the 63 days of post vaccination during our follow up though we don't know thereafter.

CONCLUSION

In the present study, the three LSDV vaccine strains induced comparable SNT antibody responses after vaccination. SNT antibodies are induced before seven days, increased regularly thereafter. After day 21 post vaccination, majority of the monitored animals developed SNT antibody titres which were $\geq \log_5^2$. The SNT antibody reached peak at day 35 and remained peak until the follow up ends at day 63. The protectiveness of such peak titre of antibodies is not confirmed through viral challenge in this study though OIE suggests that such peak antibodies are protective.

From the obtained results of the present study, the following recommendations are forwarded:

- The protectiveness of the SNT antibody titres induced by the three vaccine types against LSDV should be evaluated via challenge.
- Further field or laboratory comparative studies on the three vaccine types should be undertaken to determine factors that affect vaccine efficacy as well as on the half-life, persistence and time taken for the disappearance of the induced antibodies after vaccination by allocating sufficient study period and budget.

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