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IMMUNE RESPONSE IN CATTLE FOLLOWING IMMUNIZATION WITH A HETEROLOGOUS LUMPY SKIN DISEASE VIRUS VACCINE: A LONGITUDINAL STUDY

Muhammad Awais Memon[1], Shahid Hussain Abro[1]*, Dildar Hussain Kalhoro[1], Nazeer Hussain Kalhoro[2], Rani Abro[3]

[1]**Department of Microbiology**, Faculty of Animal Husbandry & Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan

[2]**Sindh Institute of Animal Health**, Karachi, Pakistan

[3]**Department of Animal Nutrition,** Faculty of Animal Husbandry & Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan

***Corresponding Author:** Shahid Hassain Abro . E. mail: shahidabro9@yahoo.com

Abstract

Lumpy skin disease (LSD) is a highly contagious disease of cattle. The samples (n= 240) were collected from 30 cattle and buffalo across five farms in potential risk regions of Sindh, Pakistan, employing stratified random sampling. Both young (≤ 1 year) and adult (≥ 1 year) animals and a control group comprising of non-vaccinated animals to compare immunity with the LUMPYVAC® vaccine. The immune response of cattle to heterologous lumpy skin disease virus (LSDV) vaccination was assessed by evaluating physical health, antibody production, and interferon-gamma (INF-ɣ) levels. Blood samples were collected from two groups: one consisting of animals recovered from clinical LSDV infections and the other consisting of vaccinated animals. Throughout the experimental period, no clinical signs or abnormalities were observed, with slight variations in body temperature noted. Before vaccination, animals showed antibody titers below the seropositive threshold of 30%. Following vaccination, cattle began to develop antibodies against LSDV 30 days post-vaccination, with young animals showing high antibody levels (80%) after the first dose and adult animals achieving 83.31% seropositivity. After two doses, antibody levels ranged from 51.72% to 94.35%. However, six months post-vaccination, a decrease in antibody levels was observed, with only 6.7% of younger cattle and 43.3% of older cattle retaining detectable antibodies. After 11 months, no younger cattle had detectable antibodies, while only 6.7% of older cattle showed low levels just above the threshold. Significant differences ($p<0.05$) were observed in antibody levels before vaccination and post-vaccination six months of vaccination. However, no statistically significant difference ($p>0.05$) was detected in antibody levels from 1 month to 11 months post-vaccination. Interferon-gamma (INF-ɣ) production was also monitored, revealing that vaccinated cattle produced 100 pg/ml of INF-ɣ against LSDV 30 days post-vaccination. The first immunization resulted in low INF-ɣ concentrations (100 pg/ml), while the second dose six months later induced peak INF-ɣ levels of 1400 pg/ml in young animals and 1100 pg/ml in adults. Statistical difference ($p<0.05$) was recorded in increase Gamma- interferon (INF-ɣ) in both Groups following vaccination 1 month and 6 months. However, no statistically significant difference ($p>0.05$) was observed for the decrease in gamma-interferon (INF-ɣ) at 11 months post-vaccination. In summary, vaccination against LSDV triggered an immune response in cattle, marked by increased antibody production and interferon-gamma (INF-ɣ) levels. The research highlights the efficiency of the vaccine in the induction of a strong immune response in dairy animals, and the requisite for being used vaccination to maintain an immune response against LSD.

Keywords: *Antibodies, Cattle, Interferon-ɣ , LSDV, Vaccination*

INTRODUCTION

Lumpy skin disease (LSD) is a highly infectious disease caused by virus belongs to genus Capri pox and family, Poxviridae (1-3). In general, the disease produces severe infections in cattle and buffalo (4, 5). The disease is clinically manifested by elevated body temperature, skin nodules/lesions, frailty, nasal discharges, excessive salvation, enlarged nodes, lachrymation, edema in skin, pox lesions/ multiple nodules on skin and mucous membrane, of respiratory and digestive tract of the affected animals (6, 7).

Herd immunity, also known as community immunity, is a fundamental concept in epidemiology. It acts as a powerful defense mechanism against the spread of infectious diseases within a population (8). The

immunity may be achieved by recovery from a natural infection that results from the development of antibodies against the organism (9). When a sufficient proportion of individuals in a community become immune to a specific pathogen (either through vaccination or previous infection), the overall transmission of the disease is significantly reduced (10). As a result, even those who are not immune (such as individuals who cannot be vaccinated due to medical reasons) are indirectly protected because the disease has fewer opportunities to spread. There is influence of herd immunity in protection and prevention of the infections in dairy animals (11). It has been estimated that for high contagious diseases, herd immunity may need immunity levels ranging from (70% - 90%). This threshold frequency might be low in case severe infections in the herd (12). However, this immunity may in result of developed protection against the particular disease before the infection or vaccination (13). Indirect protection plays a crucial role in safeguarding livestock populations, particularly in preventing the transmission of diseases like lumpy skin disease virus (LSDV) among cattle. Herd immunity is a critical goal in managing infectious diseases. It necessitates a significant proportion of the population to be immune. Herd immunity is associated to vaccination against the specific disease. The vaccines provide protection against the infection. It also involved in protection of vaccinated animals and non-vaccinated animals by disruption of chain of infection transmission within the population (14). Vaccine-induced herd immunity hinges on the proportion of vaccinated individuals within a population. Achieving herd immunity involves a large percentage of the population becoming immune to a specific disease through vaccination or prior infection. This indirect immunity provides protection to those herds that were not vaccinated and decreasing the risk of infection spread. However, may show variations in different regions due to vaccine coverage and efficacy (15). LSDV outbreaks may pose a threat of recurring infection in dairy animals; therefore, knowledge of immunity development may help in effective prevention and control of the infection (3). The present research is designed to evaluate the immune response following the LSDV infection before and after vaccination in dairy cattle.

MATERIALS AND METHODS

The samples were taken from LSD affected cattle and buffalo of various areas of province Sindh, Pakistan. Stratified random sampling was approached, to obtain a representative sample of the cattle population, comprising of young animals (less than 12 months old) and adult cattle with no prior vaccinations. This method is valuable for the evaluation of development of natural immunity response and the effectiveness of vaccination campaigns in achieving herd immunity. The history regarding LSDV infections and/or immunization were obtained from farmers. The selection of the samples was performed based on areas at high risk of LSD infections. The representative herd samples were obtained using stratified random sampling. A total of 240 samples were collected from thirty (n=30) animals from five (05) different livestock farms. The sampled animals' age comprising of young ≤ 1year and adult ≥ 1 year old were chosen for the experimental trials. Each animal used in this study was identified and tagged individually. The cattle were vaccinated by commercially available LUMPYVAC@ (Lumpy skin disease) vaccine subcutaneously at neck region according to manufacturer's prescription. The vaccination injection site was monitored for any local reaction, redness and swelling. A control group consisting of non-vaccinated animals was kept for natural infection to compare development of immunity to the vaccinated group. any interference, which provide comparison of immunity between non-vaccinated and vaccinated groups. The size of all the animals in experimental groups was kept similar to maintain the balance. The vaccinated animals were monitored for clinical signs/symptoms, discomfort, adverse reactions, behavioral changes, feed and water intake. Post-vaccination, the animals were monitored for signs of LSDV infection or other infectious diseases. To retain potency and efficacy, vaccine (vials) was transported and maintained in cold chain conditions (4-8°C). Aseptically, blood samples were collected from the cattle before vaccination and at 1, 6, and 11 months following the administration of vaccine and transported to the laboratory for further analysis.

The immunity development in the cattle was determined and compared using antibody titers before vaccination and/or infection. The Serum samples were analyzed for the presence of antibodies against LSDV

using a validated ELISA kit (ID Screen® Capripox Double Antigen Multi-Species) and enzyme-linked immunosorbent assay (ELISA) (IDVet, Grabels, France) according to the manufacturer instructions The vaccine effective response was assessed by significant production of antibody titers by the vaccinated animals. Comparing antibody levels in naturally infected animals to vaccinated animals can help determine if natural infection also leads to robust immunity. The threshold of herd immunity was measured by the percentage of animals exhibited an antibody response above the established cut-off value. In addition, the findings were interpreted based on the following formula:

$$\text{Sample/Positive} = \frac{\text{Test sample OD} - \text{Negative control OD}}{\text{Positive sample OD} - \text{Negative control OD}} \times 100$$

The induction of antigenic cellular immunity was measured after vaccine challenged Group I and Group 2 animals by the production of Gama-interferon in the blood samples. Blood samples were incubated in 24-well cell-cultured plates and incubated at 37°C with 5% CO2 containing phosphate buffer saline. Following the centrifugation of plates, plasma were collected for determination of production of gamma-interferon in each well-plate. The gamma-interferon was measured ELISA kit for determination of bovine, caprine and ovine Gamma interferon (ID Screen ® Ruminant IFN-g Vet, Grabels, France). The antibody levels were measured using ELISA (ELISA) (IDVet, Grabels, France), in young animals (1-12 months old) and adult animals before vaccination and again at 01, 06 and 12 months post-vaccination. The t-tests were performed for this comparison of antibody levels. Any p-value less than 0.05 were considered as significant for analyzed tests. The statistical analysis was performed using software SAS 9.4 (SAS Institute in Cary, North Carolina, USA).

The statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, North Carolina, USA). The statistical analyses were carried-out employing t-tests for comparison of antibody levels and Gamma-Interferon (INF-ɣ) production at various durations (pre- vaccination, 1 month, 6 months, and 11 months after vaccination). Significant difference was calculated using p-values, where ($p<0.05$) represented statistical significance for comparisons at different time intervals before and after vaccination. Statistically, p-value ($p>0.05$) was considered as non-significant.

The study was approved by Ethics committee of Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University TandoJam and Sindh Institute of Animal Health, Karachi No.MICR/39/2023/25.03.2023; No. DAS/98/2024/: 11.01.2024. The experiments were performed according to the international standards and guidelines of animal research.

RESULTS

Immune response was determined by immunization to cattle with heterologous LSDV vaccine. The blood samples were obtained from two groups of animals; First group of the animals recovered from the clinical infections and second group comprised of animals received vaccination. During the experimental period the animals did not show any symptoms and signs of the LSD or any physical or physiological abnormality. However, slight variation was observed in body temperature.

The data indicated that before vaccination, the sampled animals exhibited titers below the sero-positive cut-off value of 30% (Table I). The results revealed that vaccinated cattle started developing antibodies against lumpy skin disease virus (LSDV) post-vaccination 30 days. The first dose of immunization in young animals produced high level of antibodies approximately 80% (Sero-positive 45.82-83.34 %) in the samples. Following, twice immunization adult animals generated the level of antibodies (83.31%) against LSDV. The serum positivity ranged (51.72 - 94.35%) after twice immunization.

Six months after the vaccine, we noticed a big drop in the number of cattle with antibodies against LSDV. In the first group of younger cattle, only 6.7% (that's 2 out of 30) still had a noticeable level of antibodies, with readings of 43.2% and 47.8%. In the second group of older cattle, 43.3% still had antibodies, with levels ranging from 31.8% to 65.7% (Table II). After 11 months, none of the younger cattle had any detectable antibodies, while only 6.7% of the older cattle (again, 2 out of 30) had very low antibody levels, just above the threshold, with readings of 30.9% and 31.2%. Significant differences ($p<0.05$) were observed in

Table I. Measurement of antibody titers in cattle at different interval with pre-vaccination and post-vaccination of sheep poxvirus vaccine

Group	Pre-vaccination and recovered				PV (1 Month)				PV (6 Month)				PV (11 Month)			
	SP		SP		SN		SP		SN		SP		SN		SP	
	N	%	n	%	N	%	n	%	n	%	N	%	n	%	n	%
Group I: Young animals 1 to 12 months old	30	100	Nil	-	6.5	20	24	80	28	93	2	7.6	30	100	Nil	-
Group II: Adult animals >12 months old	30	100	Nil	-	5.5	17	25	83	17	57	13	43	28	93	2	6.7

*Post Infection =PI Post vaccination = PV, SN = % SP < 30%. * n = number of animals

Table II. Antibody levels before and after vaccination of the sampled animals

No	Group I				Group II			
	Pre-vaccination	PV (1 month)	PV (6 month)	PV (11month)	Pre-vaccination	PV (1 month)	PV (6 month)	PV (11month)
1	8.9	44.9	20.7	14.0	20.8	78.1	27.8	11.3
2	7.8	74.9	27.0	17.3	27.7	92.2	57.2	27.8
3	-0.1	24.7	18.5	7.6	18.3	74.8	25.6	9.7
4	3.9	57.8	28.2	14.8	9.9	68.2	10.4	2.1
5	5.5	46.7	16.7	5.6	8.7	86.4	38.3	18.7
6	23.9	82.7	46.7	28.7	24.8	70.0	26.0	16.0
7	-1.9	22	14.3	4.0	10.5	27.8	20.0	18.8
8	4.5	58.4	20.7	3.6	25.7	88.2	39.0	19.6
9	20.1	67.5	23.0	19.2	7.8	28.3	24.9	19.2
10	23.9	46.6	27.7	19.5	17.8	68.8	34.3	23.8
11	18.01	62.5	24.6	24.0	25.6	28.7	20.0	4.4
12	16.9	68.2	28.0	17.2	18.8	69.7	38.8	9.8
13	1	27.1	25.01	2.3	20.4	27.7	24.8	2.8
14	2.9	57.3	10.2	2.8	16.7	58.2	23.5	5.5
15	22.8	66.8	26.2	21.9	28.3	93.2	64.8	30.1
16	10.9	80.8	28.0	17.2	27.8	80.2	48.6	16.2
17	0.6	53.9	16.8	4.5	8.8	67.0	31.5	12.8
18	22.7	78.01	42.3	21.8	12.3	58.8	30.7	6.2
19	8.7	48.7	20.5	16.4	18.0	68.6	28.6	10.2
20	0.7	28.4	17.3	4.2	17.5	26.4	13.0	8.2
21	1.2	74.7	25.0	11.7	8.7	67.0	23.7	7.3
22	-0.3	18.3	26.1	2.2	28.0	58.5	24.5	1.7
23	8.7	65.3	10.7	9.8	27.8	83.3	59.7	29.8
24	24.5	78.1	28.8	17.4	13.4	50.6	19.5	8.8
25	1.2	25.3	10.7	6.5	24.2	88.0	24.8	5.7
26	1.8	81.3	28.7	12.0	8.8	76.8	27.8	13.0
27	16.3	78.3	17.7	17.0	17.5	74.7	32.0	18.8
28	9.4	68.8	20.4	16.7	9.8	63.6	27.2	16.3
29	5.5	58.6	13.4	7.7	23.5	89.2	55.6	19.2
30	4.7	60.0	9.8	5.5	16.2	83.0	48.1	10.8

the levels of antibodies before the vaccine and six months after the vaccine (Table III). However, at one month and 11 months after the vaccine, the differences weren't significant. This suggests we need to test more cattle to be sure of these findings.

Table III. Statistical Analysis of ELISA SP titers (%) in pre-vaccination and post-vaccination in dairy cattle

Group	Pre-vaccination		PV(1 Month)		PV(6 Month)		PV(11 Month)	
	Mean (SD)	*p*-Value	Mean (SD)	*p*-Value	Mean (SD)	*p*-Value	Mean (SD)	*p*-Value
I	9.59	<0.0001	57.75 (19.59)	0.064	23.26 (8.65)	0.001	13.50 (7.41)	0.0630
II	18.96 (7.18)		67.55 (20.65)		33.35 (13.76)		14.47 (8.14)	

* Post-vaccination (PV)

The data indicated that before vaccination, the sampled animals exhibited interferon gamma (INF-ɣ) average production in the samples before and after vaccinated animals (Figure 1). The results revealed that vaccinated cattle started producing Gamma-Interferon (INF-ɣ) 100 pg/ml against lumpy skin disease virus (LSDV) post-vaccination 30 days. The first dose of immunization in young and adult animals produced low concentration of INF-ɣ 100 pg/ml in the sero-positive samples. The second dose immunization at six months young animals generated the peak level of INF-ɣ 1400 pg/ml and 1100 pg/ml in adult animals (Fig. 1). Statistical difference ($p<0.05$) was recorded in increase Gamma- interferon (INF-ɣ) in both Groups following vaccination 1 month and 6 months. However, no statistically significant difference ($p>0.05$) was observed for decrease in Gamma- interferon (INF-ɣ) at 11 months post-vaccination.

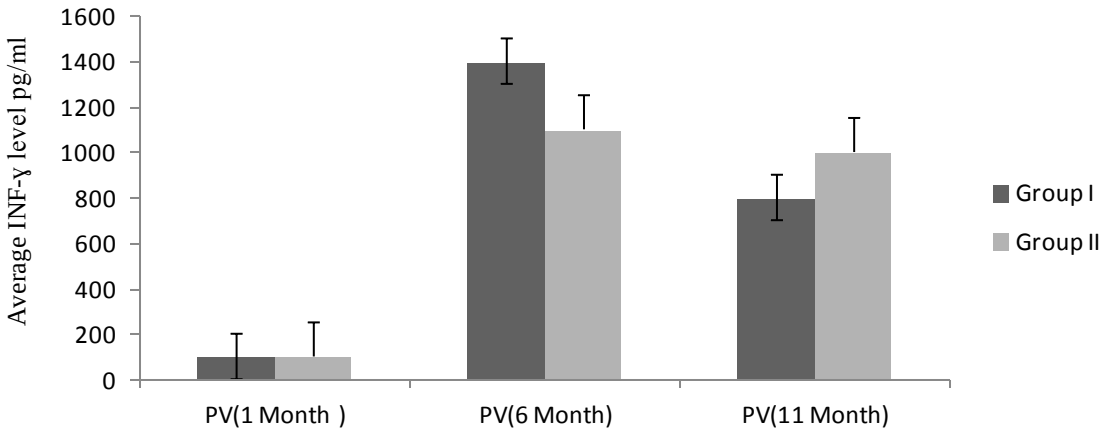

Fig. 1. Gamma-Interferon (INF-ɣ) production in before and post-vaccination in dairy cattle
* Post-vaccination (PV). Concentration of interferon (INF-ɣ) measured in pg/ml

DISCUSSION

Initial reports of outbreak of LSD infections in dairy cattle and buffalo were identified in various regions of Pakistan during 2022 (16). Due to increase in cases and severity of the disease, vaccination strategies were adopted for the prevention and control of LSDV infections. Considerable challenges associated to vaccination including protective efficacy, quality and costs were encountered for selection of suitable vaccine (17, 18). Animals were immunized to heterologous sheep pox vaccine for the prevention and control of LSDV infections (19). However, duration of immunity after vaccination with the heterologous vaccine was not satisfactory. It has been reported that vaccines have been used for the prevention of LSD in dairy cattle (20). The presence of specific immune response pre-vaccination and post-vaccination against the LSD infection was observed. It was found that the population immunity was high 86.1% with no adverse effects in vaccinated dairy animals. In this study, similar immune response (antibody titer 80.0 to 83.3%), was determined pre-vaccination and post-vaccination at different intervals. Different classes of attenuated/inactivated vaccines have been developed to control and eradicate poxvirus diseases. Many vectored vaccines prepared from alternative strains of poxviruses have been licensed to be administered in humans and animals (21).

The study aimed to assess the development of immunity post-LSDV infection and following vaccination against the virus in cattle herds within the Sindh province. The results of the study provide valuable insights into the dynamics of herd immunity in the context of LSDV. It has been reported that the Neethling vaccine for Lumpy Skin Disease Virus (LSDV) starts to work 10 days after it's given to the animals, and it works best after 21 days. The protection it offers can last for one year (22). Though, scientists have different findings about how well they can detect antibodies, which are the body's defense against the virus, after vaccination. There's also not much information on how the level of antibodies relates to how well the vaccine protects the animals. This is crucial because sometimes animals are safe from the disease even if tests show very few or no antibodies.

Past research has found that the Neethling vaccine for lumpy skin disease can make antibodies in cows that last for a long time. Even after 30 weeks, a test called the virus neutralization assay (VNT) could find these antibodies. Mostly research showed that when cows were given another dose of the vaccine, about 35% of them still had antibodies nearly a year later (46–47 weeks). This was true whether the antibodies were measured with the VNT, a test called immunofluorescence test (IFAT), or a commercial ELISA test. All three tests gave similar results, which mean they all seems to work well in finding antibodies in cows that have been vaccinated. In simpler terms, the ELISA test, which is used to detect antibodies against lumpy skin disease in cows, is quite accurate (23). It matches up with another test called the VNT 91% of the time when it says antibodies are present, and 87% of the time when it doesn't find antibodies. Also, a study using a vaccine made from a local virus strain named Ismailia showed that all the young cows had enough antibodies to protect them for at least 30 weeks. Even after 40 weeks, the ELISA test showed that the young cows still had protective antibodies (24). To put it simply, there's a vaccine developed from a weakened version of the lumpy skin disease virus called the Neethling strain. The neethling strain vaccine has been reported to provide good protections in dairy cattle up to three years (25). However, vaccine developed from sheep pox virus provided protection against the disease in cattle up to two years (26).

Live vaccine (LSDV LAV) and inactivated vaccine were employed and compared in the animals. The LSDV LAV live exhibited good immune response and the animals stayed protected for up to 18 months. Upon diagnosis there was no presence of the virus in the dairy cows. The inactivated vaccine showed good response up to12 months. However, inactivated vaccine did not provide sufficient protection to the cows when exposed to the infection after 12 months (27). To effectively manage lumpy skin disease, it's important to know how well vaccines work and how long they keep cattle immune. Since there's not much information about how long the immunity lasts with different lumpy skin disease vaccines. It was investigated by using a sheep pox vaccine in cattle, which is a different type of vaccine than the usual ones for this disease. In this study, the ELISA results showed that before getting vaccinated, young cattle had much lower levels of antibodies (with an average score of 9.59 and a standard deviation of 8.74) compared to adult cattle (average score of 18.96 and a standard deviation of 7.18). However, both groups were still below the threshold for a positive test result for antibodies. One month after vaccination, the difference in antibody levels wasn't significant. But at six months, the increase in antibody levels was significant (with a p-value less than 0.001), and then at 11 months, the difference was not significant again. From these findings, we conclude that the immunity lasts for six months for young cattle after getting their first vaccine and 11 months for cattle that are revaccinated every year. This duration aligns with the results from other similar research (24).

Investigation into how these different vaccines trigger immune cells to provide long-term immunity. It has been demonstrated in past research on a deactivated capripoxvirus vaccine has shown that immunity against poxviruses involves antibodies and immune cells (27). It has been shown that not every animal will show a detectable level of antibodies after vaccination, but they still may be fully protected from lumpy skin disease virus (LSDV) when tested (24). This means that just looking for antibodies might not be enough to guarantee an animal is protected. However, testing for these antibodies is still an important initial measure in assessing the success of vaccinations in real-world conditions (17).

The measurement of production of INF-ɣ in blood of the cattle is recommended to detect the infection and vaccine efficiency in dairy cattle (28). Determination of interferon gamma (INF-ɣ) in blood of animals is easy and swift replicates in vivo conditions, providing accurate information of immune response development. This in relation to preserved cellular interactions and the occurrence of plasma proteins in the blood, that may have inhibitory or stimulatory effects on immune response (29, 30). In this research, the data regarding interferon gamma (INF-ɣ) production suggested sampled animals showed low concentration that before and after vaccination. Following the vaccination, the animals started to generate INF-ɣ in response to 30 days after vaccination with initial level INF-ɣ 100 pg/ml against lumpy skin disease virus (LSDV). However, immunization 2nd dose of interferon INF-ɣ, production increased significantly at peak concentration INF-ɣ 1400 pg/ml in young animals and 1100 pg/ml in adult animals. These findings demonstrated profound immune influence, particularly after second dose of the vaccine. In contrast, Bassiouny *et al.,* (2016) (31) indicated that INF-ɣ production was detected at peak level after 35 days post-vaccination of sheep pox against the LSD in dairy cattle.

The findings of this research heterologous LSDV vaccine have proven efficiency for production of immune response in vaccinated animals by generation high concentration of antibodies and interferon-gamma (INF-ɣ) against LSDV, particularly following the second dose at six months. In fact, sufficient decrease in INF-ɣ production and antibody levels certain period, the vaccine's efficiency highlights its potential for robust production of immune response for short duration.

CONCLUSION

In conclusion, the vaccination against LSDV induced an immune response in cattle, including significant antibody production and interferon-gamma (INF-ɣ) levels at different periods. However, antibody levels declined six months post-vaccination, with no detectable antibodies in younger cattle after 11 months. The research highlights the efficiency of the vaccine in induction of strong immune response in dairy animals, and the requisite for being used vaccination to maintain immune response against LSD. However, further research is needed for sustaining long-term immune response for prevention and control of the infection.

Conflict of Interest:

The authors have no conflict of interest.

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