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Field trials for assessment of live attenuated Neethling and sheep pox virus vaccine in cattle

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ABSTRACT

Lumpy skin disease (LSDV) is of significant economic impact for the cattle industry in Africa, Middle East and Egypt. The disease is spreading aggressively posing threats to cattle industry. Due to cross-protection, Neethling and sheep pox virus (SPV) vaccines have been used for cattle against LSDV. In this work, living attenuated Neethling and sheep pox vaccines were used for vaccination of cattle and evaluated under field conditions.14susceptible calves were vaccinated with live attenuated Neethling vaccine and4 cattle were vaccinated with live attenuated sheep pox vaccine with 8cattleas non-vaccinated control (4cattle for each).The humeral immunity was measured by double antigen ELISA while, cellular immune response were evaluated by interferon gamma (IFN-γ) concentration real time PCR. Humeral immune response of cattle vaccinated with SPV vaccine evaluated by ELISA, showed that the immune response begins at 7thdayPV with absorbance of 1.4 ±0.075 then by the 14th& 28thDPV with mean ELISA 2.02 ±0.0015 and 2.5 ±0.085 then at 7thweek with a mean of 2.9 ±0.1755 then declined to 1.6 ±0.957 at 4m while, IFN-γ was expressed at 2 DPV with a fold change 1.75-3.31 and at 7 DPV with fold change 1.22-1.74. ELISA of Neethling vaccinated group showed response at 30th DPV with absorbance of 1.69 ±.07815 then by 40thDPV with titer of 1.96 ±.09141 then at 7weeks with a mean 2.45 ±.055 then1.82±.1066 at4m PV, Then IFN-γ wasexpressedat2 days PV withafoldchange6.52-11.16andat7th DPV withfoldchange1.22-1.78. According to the current study results, humeral and cellular immune response of two living attenuated Capripox viruses' vaccines were evaluated at the field conditions by ELISA and IFN-γ expression that proved to be reliable and sensitive techniques for assessment of vaccine potency.

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INTRODUCTION

CaPVs (Capripox viruses) is a genus comprised of LSDV, SPV, and GPV belongs to the Chordopoxvirinae (ChPV) subfamily of the Poxviridae **(Tulman et al. 2001)**; which are responsible for the most economically significant diseases of domestic ruminants in Africa and Asia **(Fields et al. 1996).**CaPVs generally are host specific with a wide geographic distribution **(Coetzer et al. 1994).**CaPVs virus members are immunologically indistinguishable from each other, having the ability of induction of heterologous cross-protection **(Carn, 1993).** LSD is a viral disease of cattle has two clinical forms including subacute and acute, which clinically characterized by extensive cutaneous lesions and other complications include hide scare, skin myiasis, severe emaciation, and death **(Salib and Osman, 2011).**

Direct contact and/or through arthropod vectors are The most common recorded method of transmission of LSD between cattle **(Omnia et al. 2014).**The first time for LSD reported in Egypt was in 1988 **(Ali et al. 2004).** 17 years after the first appearance in Egypt, LSD outbreak was recorded again in 2006 by LSDV infected cattle importation from the African Horn countries **(El-Kholy et al. 2008).**In endemic countries the only effective way to control LSDV is by vaccination which is the most cheaply and sustainable means of disease control **(Kallesh et al. 2009).**Sheep pox virus vaccines have been widely used for cattle against lumpy skin disease virus due to cross-protection within the Capripoxvirus genus **(Tuppurainen et al. 2014)** in Africa recently, these vaccines have been associated with incomplete protection against LSD which has been reported in cattle vaccinated with live attenuated SPV vaccines **(Ayelet et al. 2013; Omyma, 2008).**The main causes for the distribution of SPV and LSD are mainly poorly conditioned animals, overcrowding, poor feeding, general mismanagement, and false using of vaccination **(Zangana and Abdullah, 2013).**

Few studies assessed the field evaluation of vaccine efficacy at the field conditions **(Abutarbush and Tuppurainen, 2018).** Due to the inability to distinguish clinically or serologically between the CaPVs infections and incomplete protection against heterologous vaccines, some researcher recommended the annual regular evaluation of the currently used vaccines at the field conditions in addition to the need for more reliable tests for monitoring the humoral and cellular immune response and strain identification based on molecular methods **(Hosamani et al. 2004).** The aim of this study was designed to field assessment of attenuated sheep pox and Neethling vaccine at field conditions. To achieve this aim, we selected two cattle farms using the most used LSDV strain to vaccinate cattle against their respective diseases. Humeral immune response was evaluated by serological monitoring of protective antibodies using ELISA test and the non-specific cellular immune response was evaluated by detection of IFN gamma.

MATERIALS and METHODS

Animals: 26 cattle participated in this research. 8 cattle (2-3 years age) were vaccinated with a live attenuated SPV vaccine. The other 18 calves (7 and 10 month age) were vaccinated with living attenuated Neethling vaccine for LSD virus, with 8 cattle as control negative group (4 for each group).

Vaccine: Two types of vaccines are used (sheep pox vaccine and Neethling vaccine of lumpy skin disease virus). **attenuated Sheep pox vaccine (Romanian strain**) supplied from Servac, Veterinary Serum and Vaccine Research Institute (VSVRI), Egypt (batch NO.2038) dosage (1ml I/D for cattle in tail fold) of prepared vaccine (log $10^{2\cdot5}$TCID50) , kept at -20°C, package (10 doses for cattle), the route by I/D injection. **attenuated Neethling vaccine of LSDV (BOVIVAX LSD-N**) is supplied by Moroccan animal health company, AL Maghrib, (batch NO.19BLSN020), dose 2ml/cattle, stored at 2°C to 8°C, route of administration by S/C. Neethling contains log $10^{3\cdot}$ TCID50 per dose.

Serum samples182 Blood samples (56 samples from SPV vaccinated cattle and 126 samples from Neethling vaccinated cattle). SPV vaccinated sera were collected before vaccina-

tion, 2-, 7-, 14-, 28- daysPV,7th week, and 4th month PV, meanwhile, the Neethling vaccinated sera were collected at the 2-, 7-, 14-, 30-, 40 - days PV ,7th week and 4th month of vaccination. 5 ml/ sample was collected from the jugular vein then allowed to clot without anticoagulant and then separated and kept frozen at -20˚c till humoral and cellular immunity evaluation.

ELISA detection of Capripoxvirus antibodies

Double antigen ELISA (IDvet Company, FRANCE) is designed to detect antibodies against SPV and GPV and LSDV, was conducted according to the instructions of the manufacturers. The kits contain microplates coated with CPV purified antigen, a CPV purified antigen labeled with horseradish peroxidase conjugate, positive control, negative control, dilution buffer, wash concentrate(20x), a substrate solution (TMB), and stop solution (0.5M).

SYBER Green real-time PCR for detection IFN Gamma

RNA extracted using RNA easy mini kit (Qiagen) Catalogue no.74104 according to manufacturer's instructions. Quantitect SYBR green PCR kit used for preparation of PCR master mix according to manufacturer's instructions. Samples were checked using RT-PCR for detection of IFN-γ using specific primers and probes **(Harrington et al. 2007)** table (1) using cycling conditions according to manufactures instructions (table 2).

Table 1. Primers and probes used in SYBER Green real-time PCR

Gene	Primer sequence (5-3)	Reference
β2M	AGACACCCACCAGAAGATGG, F	(Harrington et al., 2007)
	TCCCCATTCTTCAGCAAATC, R	
	FAM-TGGGTTCCATCCACCCCAGA-BHQ, P	
IFN-γ	GCGCAAAGCCATAAATGAAC, F	
	CTCAGAAAGCGGAAGAGAAG, R	
	HEX-CAAAGTGATGAATGACCTGTGCCA-BHQ, P	

Probes contained either the 6-carboxyfluorescein (FAM) or hexachloro-6- carboxyfluorescein (HEX) reporter dye covalently attached at the 5′ end and the BHQ molecule covalently attached at the 3′ end. F, forward primer; R, reverse primer; P, probe

Table 2. Cycling conditions of real time PCR

Reverse transcription	Primary denaturation	Amplification (40 cycles)		Dissociation curve (1 cycle)		
		Secondary denaturation	Annealing & Extension	Secondary denaturation	Annealing	Final denaturation
50˚c/30 min	94˚c/15 min	94˚c/ 15 sec	60˚c/45sec	94˚c/1 min	60˚c/1min	94˚c/1min

Amplification curves and CT values were determined by the stratagene MX3005P software. To estimate the variation of gene expression on the RNA of different sample, the CT of each sample was compared with that of the control group according to the"ΔΔCt" method stated by (Yuan et al., 2006).
Whereas ΔΔCt= ΔCt reference- ΔCt target
Ct target=Ct control – Ct treatment and ΔCt reference= Ct control -Ct treatment.

Statistical analysis

Data obtained from vaccinated subjects were compared with non-vaccinated groups by the student t test and SPSS programs in order to determine the mean and standard deviation and statistical significance of differences between the two groups as described by **Snedecor and Cochran (1982)**. P values below 0.05 were regarded as statistically significant.

RESULTS

Evaluation of the humoral immune response of cattle vaccinated with sheep pox vaccine by ELISA:

The immune response begins at 7th days PV with a mean absorbance of 1.4 ± 0.075 then increased gradually to be detected by the 14- &-٢٨ day PV with mean ELISA O.D of 2.02 ± 0.0015 and 2.5 ± 0.085 more than protective level (>1) then reached to the highest level at 7th week with a mean of 2.9 ± 0.1755 then declined to 1.6 ± 0.957 at 4 months PV (table 3). There is a significant difference in ELISA O.D between sheep pox vaccinated and non-vaccinated cattle at 7th -, 14th- , 28th- days,

Table 3. Mean ELISA absorbance of sheep pox vaccinated cattle:

Groups	No.of animals	Days post-vaccination				
		7 days	14 days	28 days	7 weeks	4 months
Non-vaccinated	4	0,09±0.004	0.05±0.004	0.05±0.004	0.45±0.004	0.05±0.004
Vaccinated	4	1.4 ±0.075	2.02±0.001	2.5 ± 0.08	2.9±0.175	1.65±0.095
P value		0.001	0.005	0.039	0.000	0.012

Evaluation of the humoral immune response of cattle vaccinated with attenuated Neethling vaccine by ELISA:

The immune response begins at 30 days PV with a mean absorbance of 1.69±.07815 then increased gradually to 40 day PV with mean ELISA O.D of 1.96±.09141 then reached the highest level at 7 weeks with a mean of 2.45±.055 then declined to 1.82±.1066 at 4 months PV(table 4). There is a significant difference in ELISA O.D between Neethling vaccinated and non-vaccinated cattle (P< 0.05).

Table 4. Mean ELISA absorbance of cattle vaccinated with attenuated Neethlingvaccine

Groups	No. of animals	Days post vaccination			
		30 days	40 days	7 weeks	4 months
Non-vaccinated	4	0.32±.00479	0.32±.00408	0.45±.0048	0.42±.00408
Vaccinated	14	1.69±.07815	1.96±.09141	2.45±.055	1.82±.1066
P value		0.05	0.030	0.014	0.034

Evaluation of interferon gamma expression in sera of cattle vaccinated with sheep pox vaccine:

IFN-γ were expressed at 2 days post-vaccination with a fold change ranged between 1.75 and 3.31 and decreased to an obvious low level at 7 days post-vaccination with fold change ranged between 1.22 and 1.74 compared to before vaccination (table 5).

Table 5. Interferon gamma expression in serum of cattle vaccinated with attenuated sheep pox vaccine

Animal	Before vaccination			2 days post vaccination		7 days post vaccination	
	Ct B2M	Ct samp	ΔΔ Ct	Ct samp	ΔΔ Ct	Ct samp	ΔΔCt
1	19.3	19.0	1.22	21.4	1.75	18.7	1.23
2	19.2	19.3	1.01	20.6	2.23	19.6	1.35
3	18.2	18.7	0.89	22.7	3.31	21.3	1.22
4	19.7	19.3	1.42	21.3	2.52	20.1	1.74

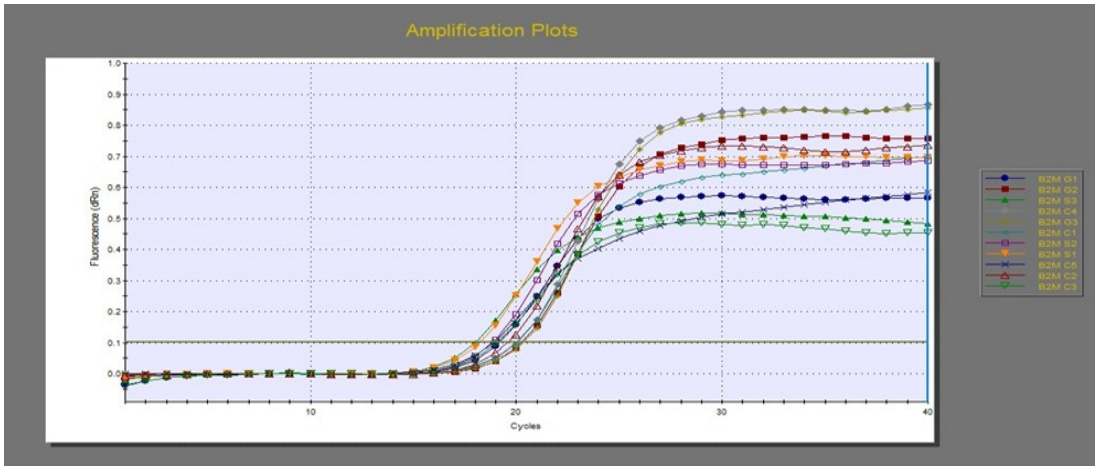

Fig 1. real-time PCR gene expression curve of IFN-γin cattle vaccinated with sheep pox vaccine

Expression of IFN-γ in cattle vaccinated with Neethling vaccine by real-time PCR: I

FN-γ were expressed at 2 days post-vaccination with a fold change ranged between 6.52 and 11.16 and decreased to an obvious low level at 7 days post-vaccination with fold change ranged between 1.22 and 1.78 compared to before vaccination (table 6).

Table 6. Interferon-gamma expression in serum of cattle vaccinated with Neethling vaccine.

Animal	Before vaccination			2 days post vaccination		7 days post vaccination	
	Ct B2M	Ct samp	ΔΔ Ct	Ct samp	ΔΔ Ct	Ct samp	ΔΔCt
1	19.3	19.3	1.22	21.4	11.16	18.7	1.78
2	19.2	19.2	1.01	22.0	10.23	19.6	1.55
3	18.7	18.2	1.81	20.1	9.25	20.1	1.75
4	18.2	18.4	0.89	21.3	9.31	21.3	1.22
5	19.7	19.3	1.42	20.1	6.52	20.1	1.74

DISCUSSION

LSD is one of the most important economic endemic animal diseases in developing countries **(Ben-Gera et al. 2015).** CaPVs genus diseases cause high mortalities and either dry form (fever and generalized skin eruptions) or the moist form (pox lesions in the lung, internal organs, and lymphadenopathy) **(Kitching and Carn, 2004).** LSD and sheep and goat poxviruses are antigenically related to each other's **OIE (2010)** and usually controlled by vaccination of cattle with heterologous SPV vaccine. **(Gari et al. 2015)** Vaccination has been considered to be the cheapest and sustainable means of disease control in the enzootic

situation like Egypt and the Middle East **(Kallesh et al. 2009).**The first LSD outbreak in Egypt was recorded between 1988 and 1989 and again in 2006, 2011, and 2014, and an attenuated sheep pox vaccine was used to vaccinate cattle **(Elhaig et al. 2017).** Enzyme-linked immunosorbent assay (ELISA) had already been proved to have great potentiality as a quantitative serological tool in the detection of antibodies against several viral infections including the poxviruses **(OIE, 2010; Tuppurainen et al. 2017).** Results documented in tables 3 revealed that heterologous sheep pox vaccine was used for vaccination of cattle against lumpy skin diseases, this is due to the cross-protection between CaPVs genus diseases as described by **Kitching and Taylor (1985).** Also, the results are parallel to that obtained by **Yogisharadhya et al. (2011)** who mentioned that live attenuated vaccines are safe and can be used in pregnant animals and have been used for decades in Capripox endemic countries. Humoral immune response against sheep pox vaccine in cattle presented in Table 2 and detected by ELISA were determined. It is showed that the immune response begins to appear at 7 days post-vaccination with a mean absorbance of 1.4 ±.07500 then increased gradually to be detected by the 14 and 28-day post-vaccination with mean ELISA O.D of 2.02±0.001and 2.5±0.08 more than protective level (> 1) then reached to the highest level at 7 weeks with a mean of 2.9±.175then declined to 1.65±0.095at 4 months post vaccination (PV). The same results were obtained by **A Mikhael et al. (2017)** who mentioned that ELISA antibody reached a peak at the 9th weak PV (mean O.D 1.8) and remained protective until the end at the week 20 PV (S/P = 1.38) and This is consistent with the results of humoral immune response against Neethling vaccine in cattle predicted in Table 4that detected by ELISA. It is showed that the immune response at 30 days post-vaccination with a mean absorbance of 1.69±.07815 then increased gradually to be detected by the 40day post vaccination with mean ELISA O.D of 1.96±.09141 more than protective level (> 1) then reached to the highest level at 7 weeks with a mean of 2.45±.055 then declined to 1.82±.1066 at 4 months post-vaccination (PV).

Cell-mediated immune response of cattle vaccinated with living attenuated sheep pox vaccine was evaluated through the expression of IFN-γ by real-time PCR (Table 4), showed that IFN-γ were expressed at 2 days PV with a fold change ranged between 1.75 and 3.31 and decreased to an obvious low level at 7 days PV with fold change ranged between 1.22 and 1.74 compared to fold change ranged between 0.89 and 1.42 in non-vaccinated animals. These results agree with that obtained by **Khafagy et al. (2016), and Norian and Azadmehr (2017)** who stated that IFN-γ can express in Iranian cattle 1 weak PV with either attenuated SPV or GPV vaccine and decreased in the week after, while the Cell-mediated immune response of cattle vaccinated with living attenuated Neethling lumpy skin disease vaccine was evaluated through the expression of interferon-gamma (IFN-γ) by real-time PCR (Table 6) showed that IFN-γ were expressed at 2 days PV with a fold change ranged between 6.52 and 11.16 and decreased to an obvious low level at 7 days PV with fold change ranged between 1.22 and 1.78 compared to 0.89- fold change in non-vaccinated animals. The results of the current study demonstrated that field evaluation of humoral and cellular immune response to SPV and Neethling vaccine is very important in designing control strategies to LSD in Egypt. ELISA had been proved to have great potentiality as a quantitative serological tool in the detection of antibodies. ELISA can be a reliable measure of the efficacy of sheep pox and Neethling vaccine and provide a good correlation between protective immunity and resistance and can be used as alternatives to challenge test. Also, gamma interferon proved to have superiority in the evaluation and early monitoring of cellular immune response against sheep pox and Neethling vaccine.

CONCLUSION

Based on the obtained results under field condition, the humoral and cellular immune response of two living attenuated Capri pox vaccines were evaluated at the field conditions. ELISA and gamma interferon expression tests proved to be reliable and sensitive techniques for the assessment of vaccine

potency. Significant differences between the two vaccines and the humeral and cellular immune tests were obtained. Furthermore, using of heterologous Capri pox vaccine is more efficient for the control of pox diseases in other animal species. Sheep pox vaccine is efficiently used for vaccination of cattle against lumpy skin disease.

Author Contributions:

Fawzy M., EL-Shahedy M.S. and Eman K. E., organized the whole process and drafted the manuscript. Fawzy M., EL-Shahedy M.S. and Eman K. E., designed the work. Rania H.E., Fawzy M., and Eman K. E., performed the work. Fawzy M and Eman K. E., performed the data analysis. Eman K.E. and Fawzy M., wrote the work. All authors read and approved the final manuscript.

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