A field study evaluating the humoral immune response in Mongolian sheep vaccinated against sheeppox virus

A field study evaluating the humoral immune response in Mongolian sheep vaccinated against sheeppox virus

Petra Fay[1*], Georgina Limon[1*], Gerelmaa Ulziibat[3*], Buyantogtokh Khanui[3], Odonchimeg Myagmarsuren[3], Gessica Tore[1], Bodisaikhan Khishgee[3], John Flannery[1], Batkhuyag Sandag[4], Batchuluun Damdinjav[3], Philippa M. Beard[1,2,†]

[1] The Pirbright Institute, Ash Road, Pirbright, GU24 0NF, UK

[2] The Roslin Institute, Easter Bush, University of Edinburgh, EH25 9RG, UK

[3] State Central Veterinary Laboratory, Zaisan, Khan-Uul District, Ulaanbaatar 210153, Mongolia

[4] The Mongolian General Authority for Veterinary Services (GAVS), Ulaanbaatar 210349, Mongolia

*Joint first authors

†Corresponding author, Pip.Beard@pirbright.ac.uk

Abstract (250 words)

Sheeppox is a transboundary disease of small ruminants caused by infection with the capripoxvirus sheeppox virus (SPPV). Sheeppox is found in Africa, the Middle East and Asia and is characterised by fever, multifocal cutaneous raised lesions, and death. Vaccination with live attenuated capripoxvirus (CPPV) strains is an effective and widely used strategy to contol sheeppox outbreaks, however there are few reports of post-vaccination field surveillance studies. This study used a commercially available ELISA to examine quantitative and temporal features of the humoral response of sheep vaccinated with a live attenuated CPPV strain in Mongolia. 400 samples were tested using the ELISA

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/tbed.14163.

commercial kit, and a subset of 45 samples were also tested with a virus neutralisation test (VNT). There was substantial agreement between the VNT and ELISA tests. Antibodies to CPPV were detected between 40 and 262 days post vaccination. There was no significant difference between serological status (positive / negative) and sex or age, however an inverse correlation was found between the length of time since vaccination and serological status. Animals between 90 and 180 days post-vaccination were more likely to be positive than animals greater than 180 days post vaccination. Our results show that a commercial CPPV ELISA kit is a robust and reliable assay for post CPPV vaccination surveillance in resource-restricted settings and provide temporal parameters to be considered when planning sheeppox post-vaccination monitoring programmes.

Keywords

Sheeppox, sheeppox virus, poxvirus, Mongolia, capripoxvirus, post-vaccination monitoring, humoral immunity, virus neutralisation assay.

Background

Sheeppox (SP), goatpox (GP) and lumpy skin disease (LSD) are transboundary diseases caused by infection with viruses of the genus capripoxvirus (CPPV), namely sheeppox virus (SPPV), goatpox virus (GTPV) and lumpy skin disease virus (LSDV). All three viruses cause systemic disease in ruminants characterised by fever, multifocal cutaneous raised lesions, and death. LSDV causes disease only in cattle, while SPPV and GTPV cause disease in sheep and goats. The host preference of SPPV and GTPV varies with some isolates of SPPV causing disease only in sheep, some isolates of GTPV causing disease only in goats, and some isolates of SPPV and GTPV causing disease in both sheep and goats [1]. SP and GP are high consequence diseases. They reduce production of meat,

milk, wool and cashmere and decrease the value of affected animals, therefore having a substantial negative effect on farmers' livelihoods [2-5]. Furthermore, countries with endemic SP or GP face restrictions on trade of live animals and animal products [6].

Mongolia is a landlocked country located in central Asia bordered by Russia to the north and China to the south, east and west. Mongolia has one of the highest livestock *per capita* ratios in the world, with a human population of 3.2 million compared to 4.3 million cattle, 30.1 million sheep and 27.3 million goats [7]. Since 1977 there have been three SP outbreaks in Mongolia: the 2006-2007 outbreak affecting five provinces [8], the 2013 outbreak affecting two provinces, and the extensive 2015-2017 outbreak affecting eight provinces. During this latest, the Mongolian General Authority for Veterinary Services (GAVS) started a risk-based vaccination campaign as a control strategy. In 2016 a targeted post-vaccination surveillance programme was employed.

Vaccination is considered one of the most effective methods for control of CPPVs [6]. Live-attenuated strains of SPPV and GTPV are the most common type of vaccine used against SP and GP, however the duration of the humoral immune response following vaccination with live attenuated CPPV vaccines is poorly understood. Manufacturers often recommend annual vaccination regimes to maintain herd immunity, however these recommendations are often based on research conducted for LSD in cattle and/or under controlled conditions [9-12]. There is very little published research describing the humoral immune response following regional or national vaccination programmes to protect against SP or GP.

In this study we used a subset of serum samples collected from sheep during post-vaccination surveillance in Mongolia to investigate the humoral immune response of vaccinated sheep following

a risk-based SP vaccination campaign. We used the results to identify potential factors that might play a role in the detection of seroconversion and to assess the suitability of a commercial ELISA test for post-vaccination monitoring in a non-endemic LMIC.

Methods

Vaccination

All animals were vaccinated with a live attenuated capripoxvirus vaccine produced by Biocombinat SOI, in Mongolia. The sheeppox vaccine was the live-attenuated SPPV Perego strain (original date 1978), grown in primary lamb testis cell cultures. The vaccine was administered to sheep subcutaneously into a hairless skin area (fore-flank or tail patch) in compliance with Mongolian hygiene regulations. Mongolia does not carry out sheeppox vaccination programmes routinely, therefore to the best of the authors' knowledge this was the first sheeppox vaccine administered to the animals.

Sample collection

Blood samples from sheep were collected as part of the post-vaccination surveillance programme for SP implemented by the Mongolian General Authority for Veterinary Services (GAVS) in 2016. All provinces that were part of the vaccination programme in the country (n=8; Figure 1) were part of the post-vaccination monitoring evaluation. Multistage sampling was used to select animals for testing. Briefly, in each province between one and three soums (or districts) were randomly selected. Within each soum herds were randomly selected and 20 sheep in each herd were randomly selected for sampling. A total of 2000 samples were collected.

For each sample collected, herder location (province and soum name), name of the herder/owner, age and sex of the animal, date when the animal was last vaccinated for SP (according to the vaccination records) and date of sampling was recorded.

Sample processing

Blood samples were stored at 4°C to clot after which the separated serum was collected. Samples were stored at 4°C and transported to the State Central Veterinary Laboratory (SCVL) of Mongolia, in Ulaanbaatar, within two days post-collection. On receipt, serum samples were centrifuged at 3000 rpm for 10 min, serum supernatant collected, aliquoted, and stored at -20°C.

ELISA

From the 2000 samples collected as part of the post-vaccination monitoring, 400 samples were randomly selected for comparative serological testing by both SCVL in Mongolia and The Pirbright Institute in the UK using a commercially available ELISA (ID Screen® Capripox Double Antigen Multi-species ELISA kit; IDvet, Grabels, France) following manufacturer instructions. Aliquots of the same serum samples were tested singly at both the SCVL and Pirbright. The optical density readings were used to calculate the percentage of seropositivity (%SP) for each sample. A sample with a %SP value of ≥ 30% was considered positive.

Fluorescent Virus Neutralisation Test (FVNT)

The recombinant EGFP-095-LSDV Neethling virus was generated through insertion of the EGFP marker at the N terminus of the LSDV ORF 095, encoding for a putative core protein of the LSDV Neethling strain (GenBank: AF409138.1). BS-C-1 (ATCC® CCL-26™) cells were infected with the LSDV Neethling strain at a MOI of 0.1. Two hours after infection, cells were transfected with a recombinant transfer plasmid carrying the EGFP sequence fused to the LSDV095 gene and flanked by

an upstream and a downstream LSDV homology region of, respectively, 427 bp and 338 bp. Infected/transfected cells were incubated for about five days during which homologous recombination occurred between the LSDV DNA genome and the recombinant construct. After the incubation period, single EGFP positive BS-C-1 cells were sorted into pre-seeded 96-well plates twice using a BD FACSAria™ III sorter (BD Biosciences). The recombinant fluorescent virus was recovered from supernatants of infected cell lysates and separated from the parental virus through 5-fold limiting dilutions of the viral suspension. The isolated recombinant EGFP-095-LSDV Neethling virus was then purified through a sucrose cushion to obtain a working stock to be used in the development of the FVNT. Full genome sequencing of EGFP-095-LSDV Neethling was carried out to confirm the genetic modification (data not shown).

Forty-five serum samples were selected and tested for neutralising antibodies by the FVNT. Samples were randomly selected based on the ELISA test results spread over time. Time between vaccination and sampling was re-categorised into (i) short (<98 days), (ii) medium (99 to 132 days) and (iii) long (>133 days), and %SP values were re-categorised into (i) low (%S/P ≤ 0.50), (ii) medium (%SP 0.51 to 74.38) and (iii) high (%S/P ≥ 74.39), using the first and the third quartile as cut-offs in both cases. Samples were then grouped into the nine categories and five samples selected from each category for testing by FVNT.

Briefly, Madin-Darby bovine kidney (MDBK) cells were seeded in 96-well tissue culture plates (Corning) at a cell suspension of 3 x 10^5 cells/ml and incubated overnight at 37°C in a 5 % CO_2 incubator. All sample and control sera were heat-inactivated at 56°C for 30 min and diluted 1:10 in culture medium (DMEM supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin and 2.5 % heat-inactivated fetal bovine serum; Life Technologies). Twofold serial dilutions (1:10 to 1:1280) were then prepared from the test sera. To all serum samples, an equal volume of EGFP-095-LSDV

Neethling virus (7 x 10^2 PFU/ml) was added, and samples incubated at 37°C for 1 h in a 5 % CO_2 incubator. In addition to the serum controls, a cell and virus only control were included. All sample and control sera were tested in duplicate.

To corresponding wells, 150 µl of the test and control serum samples were added to prepared MDBK cells and plates incubated at 37°C in a 5 % CO_2 incubator for 4 days. Fluorescent foci (indicative of cytopathic effect) were determined using a fluorescent UV light microscope (Olympus CKX53). The neutralising antibody titre for each sample was determined as the highest dilution at which no foci were identified, indicative of complete neutralisation.

Statistical analysis

Considering FVNT test as the gold standard, diagnostic sensitivity (Dse) and specificity (Dsp) of the ELISA was calculated.

McNemar's chi-squared test for paired data was used to assess whether there was a statistically significant difference in the proportion positive between (i) FVNT and ELISA test results from SCVL in Mongolia (n=45), (ii) FVNT and ELISA test results from Pirbright in the UK (n=45) and (iii) ELISA test results from SCVL in Mongolia and Pirbright in the UK (n=400).

Descriptive statistics were obtained for all sheep tested in both laboratories (n=400). Frequency distribution of values were explored for time between vaccination and sampling, and %SP values for the ELISA test considering the results from each lab separately. For samples in which the exact date of vaccination was not available and a range of potential time was given (e.g. May 2016 or between 15th of May and 15th of June), the midpoint date within this range was considered. The extent to which sex, age, time (in days) since vaccination and Province were associated with seroconversion (positive / negative) was determined using univariable mixed effects binomial models including herder as random effect. Age was re-categorised as ≤1year and >1 year to consider that sheep aged more than 1 year might have been vaccinated more than once. Time since vaccination was re-

categorised in to 3 categories (<90 days, 91 to 180 days and >180 days) based on the expected dynamics of the immune response [10, 12, 13]. Collinearity was assessed between all predictor variables for which $P < 0.2$ in the univariate analysis and, when present, only one of the variables was kept in the model. Multivariable analysis was used to assess the relationship between the individual predictor variables and the outcome, accounting for the potential confounding effect of other variables. The %SP values were used to classify animals as positive / negative. The analysis was conducted using the results from each laboratory separately.

Statistical analysis was performed in R.3.3.2 (R Development Core Team 2017) using packages EpiR, car and lme4.

Results

Blood samples were collected between 23rd and 30th of August 2016 from 400 sheep that had all been vaccinated with a live attenuated capripoxvirus vaccine. The 400 samples came from sheep herds belonging to 77 herders from seven provinces. Two thirds of the sheep (266; 66.5%) were female and 134 (33.5%) were male. Age ranged between 1 and 9 years (median 4 years) with similar age distribution between male and females. Time between vaccination and sampling ranged from 40 to 243 days (median 116 days). Day of vaccination was not recorded in four animals.

The 400 serum samples were tested in both the SCVL (Mongolia) and Pirbright (UK) using the ID Screen® Capripox Double Antigen Multi-species ELISA kit (IDvet) following the manufacturer's instructions, and the results from the two laboratories compared. A higher number of samples were classified as positive when tested in SCVL (n=188; 47%) compared to Pirbright (n=165; 41.3%), this difference was statistically significant (p=0.02) and the agreement was moderate (Table 1).

The Virus Neutralisation Test (VNT) is considered the gold standard for assessing the level of protective immunity (neutralising antibodies) to CPPV with high specificity [14]. Therefore 45 serum samples, representing a sample spread across time post vaccination and ELISA test result, were tested for the presence of neutralising antibodies using a fluorescent virus neutralisation test (FVNT). Out of the 45 samples selected to be tested by FVNT, 20 were deemed positive. Using the ELISA performed in SCVL (Mongolia), 22 of these 45 samples tested positive. Using the ELISA performed at Pirbright (UK), 18 tested positive (Tables 2 and 3). The range of values for those samples that were deemed positive are presented in Figure 2. There was a substantial agreement between the FVNT and ELISA tests regardless of the lab where the samples were tested (Kappa 0.82 and 0.73;

Days post vaccination	ELISA Mongolia lab (%S/P)	ELISA Mongolia lab	ELISA UK lab (%S/P)	ELISA UK lab	FVNT Titre	FVNT
40	41.93	Positive	83	Positive	80	Positive
40	0	Negative	2.09	Negative	0	Negative
40	3.01	Negative	0	Negative	0	Negative
40	5.32	Negative	2.58	Negative	0	Negative
74	45.7	Positive	0.48	Negative	0	Negative
75	17.2	Negative	0	Negative	0	Negative
76	1.61	Negative	0	Negative	0	Negative
76	9.07	Negative	1.25	Negative	0	Negative
76	29.23	Negative	0	Negative	0	Negative
77	232.86	Positive	210.9	Positive	480	Positive
77	160.18	Positive	32.81	Positive	120	Positive
80	7.81	Negative	0.55	Negative	0	Negative
80	117.84	Positive	325.5	Positive	320	Positive
80	156.96	Positive	49.47	Positive	80	Positive
81	83.47	Positive	82.25	Positive	120	Positive
92	26.77	Negative	1.42	Negative	0	Negative
92	72.25	Positive	143.3	Positive	30	Positive
92	62.25	Positive	0.32	Negative	0	Negative
102	1.91	Negative	3.7	Negative	0	Negative
107	46.07	Positive	0	Negative	0	Negative
110	105.24	Positive	54.56	Positive	30	Positive
114	3.63	Negative	0.26	Negative	0	Negative
114	24.4	Negative	0	Negative	0	Negative
119	33.47	Positive	0	Negative	10	Positive
121	14.76	Negative	22.32	Negative	20	Positive

122	0.11	Negative	139.5	**Positive**	60	**Positive**
122	34.83	**Positive**	181.6	**Positive**	160	**Positive**
122	173.3	**Positive**	400.1	**Positive**	1280	**Positive**
130	88.57	**Positive**	130.7	**Positive**	60	**Positive**
131	0	Negative	3.76	Negative	0	Negative
131	0	Negative	0	Negative	0	Negative
131	158.88	**Positive**	252.2	**Positive**	30	**Positive**
132	0	Negative	6.6	Negative	0	Negative
133	50.27	**Positive**	101.2	**Positive**	160	**Positive**
135	40.82	**Positive**	46.3	**Positive**	0	Negative
135	0	Negative	0	Negative	0	Negative
193	43.25	**Positive**	3.49	Negative	20	**Positive**
229	132.41	**Positive**	169.2	**Positive**	80	**Positive**
229	132.19	**Positive**	185.3	**Positive**	60	**Positive**
229	0	Negative	2.07	Negative	0	Negative
229	0	Negative	0.03	Negative	0	Negative
234	0	Negative	0.19	Negative	0	Negative
234	0	Negative	1.53	Negative	0	Negative
236	121.73	**Positive**	255	**Positive**	640	**Positive**
243	0	Negative	0.3	Negative	0	Negative

Table 3). Considering FVNT as the gold standard, the Dse of the ELISA test was slightly better when used in Mongolia (0.90; 95% CI 0.66 – 0.98) than when used in the UK (0.85; 95% CI 0.61 – 0.96); while the Dsp was better in the UK (0.96; 95% CI 0.78 – 0.99) than in Mongolia (0.84; 95% CI 0.63 – 0.95). In other words, there were less false positives when the samples were tested in the UK and less false negatives when the samples were tested in Mongolia.

The 188 positive samples from the SCVL dataset are represented in Figure 3. Positive ELISA results were detected from day 40 to 243 post-vaccination. The ELISA dataset was used to identify factors which might influence seroconversion. An inverse correlation was found between the length of time since vaccination and serological status (Figure 3) with a statistically significant difference between short (up to 90 days) and long periods of time (>180 days) (Table 4; Supplementary material tables S1 and S2). There was no significant difference between serological status (positive / negative) and sex or age (Table 4). Differences were found between serological status and province (Table 4),

however, length of time since vaccination and province exhibited strong collinearity (Supplementary material table S3) and therefore the univariate models were kept. The same patterns were found using ELISA results from both labs (Table 4).

Discussion

This study examined the humoral immune response of sheep to vaccination with a live-attenuated capripoxvirus vaccine. Most importantly, the study evaluates sheeppox vaccination under field condition, therefore addressing a key gap in literature.

The inter-assay repeatability of the ELISA was examined by testing the same 400 samples using the same kit and protocol but in two different laboratories. The results from the two datasets differed slightly when compared, with a higher number of samples classified as positive when tested in SCVL compared to Pirbright. The differences between the ELISA results reported from the SCVL and Pirbright laboratories may be due to differences in equipment, users, environment (such as storage conditions of samples) or the quality control regimes in the two laboratories. Unfortunately, the limited volume of sera precluded the re-analysis of samples which may have helped resolve the differences in results.

The FVNT was used as a gold standard to determine the Dse and Dsp of the ELISA. The two sets of ELISA results were in substantive agreement with the FNVT across the 45 sera tested. The values calculated using data from SCVL and Pirbright were Dsp 84% and 96%, and Dse 90% and 85%, respectively. These are similar to parameters previously reported for ELISA conducted in cattle with LSD (Dsp 87% and Dse 91%) [12] but lower than the Dsp data reported by IDvet (Dsp 99.7%) [15]. This difference in Dsp estimate may be attributed to the origin of the samples used (field versus

experimental), the level of antibodies present in the sample and the sample quality (e.g. haemolysis) [13, 16].

Occasional disagreements between the ELISA and FVNT were not unexpected as they are fundamentally different tests that detect different subsets of antibodies. The ELISA detects antibodies against immunogenic CPPV antigens, whereas the FVNT detects antibodies with the ability to neutralise CPPV. Sporadic disagreement between CPPV serological tests, particularly at early times post-infection or post-vaccination, has been reported previously [12, 15]. This should be a factor to consider when designing the timing of a CPPV ELISA-based post-vaccination testing programme.

ELISAs, IPMA, virus / serum neutralisation tests and immunofluorescent antibody tests have all been published for detecting humoral response to CPPV and recently comprehensively reviewed [15]. The ELISA is the easiest and cheapest of the four techniques and the most suitable for inter-laboratory standardisation. The IDvet ELISA has been validated in a number of peer-reviewed publications, showing good concordance with the VNT [12, 13, 17, 18]. Most of these studies use sera from cattle vaccinated or infected with LSDV. This study reports the use of the IDvet ELISA with CPPV vaccine field sera, demonstrating that it provided a high throughput means of assessing the overall CPPV antibody status of a large cohort of animals, therefore broadening the potential utility of the test.

Examination of factors which might influence seroconversion in the sheep identified no significant difference between serological status (positive / negative) and sex or age but did find an inverse correlation between serological status and the length of time since vaccination. Differences were also found between serological status and province, with the data suggesting this is due to timing of vaccination in different provinces. There were no important different geographic, environmental, or

cultural features or husbandry practices identified between the provinces, which are all in the eastern and central region of Mongolia.

A temporal response similar to that reported previously [9, 10, 12, 19] was seen in our study, with a correlation found between length of time since vaccination and serological status as measured by commercial ELISA. The ELISA detected seroconversion between 40 and 76 days post-vaccination albeit with borderline positive %SP values at these time points. Animals categorised into medium time period post-vaccination (between 90 and 180 days) were more likely to be positive and animals categorised into the group with long time period (>180days) were less likely to be positive. Antibodies against CPPV were still detected in this study in four samples at 236 days post vaccination. A limitation of this study is that sheep were only tested at one point in time. In the future, longitudinal studies with fewer animals tested but at frequent and uniform intervals post vaccination, particularly at early timepoints, would be beneficial and should be implemented in future post vaccination monitoring programmes.

Correlating antibody levels with protection against viral challenge requires further study. Antibodies alone are known to provide protection against poxviral disease including SP [20-22], and levels of poxvirus antibodies, measured either by ELISA or neutralisation assay, are often used as a correlate of protective immunity in people and animals. However, the level of antibodies that confers protection against poxviruses is unknown. One study found people with pre-existing neutralizing titres <1:32 against vaccinia virus were more susceptible to smallpox infection than those with antibody titers ≥1:32 [23]. A neutralisation index (calculated as the log titre difference between the titre of the virus in the negative serum and in the test serum) of ≥1.5 is considered positive for LSDV, SPPV and GTPV [14] although there is no data to link this index with protection from challenge. Importantly, a low antibody response by ELISA or neutralisation tests post-vaccination may not

necessarily mean absence of protection as (i) there may be sufficient memory B cells present to provide a rapid anamnestic antibody response and therefore protection post challenge [24], (ii) it is likely only a low amount of neutralising antibodies is required for protection [25, 26], and (iii) cell mediated immunity or non-neutralising antibodies may provide protection. The levels of antibodies detected at later time points in this study (>180 days) were lower than earlier time points. While this indicates that >180 days would not be the ideal time to carry out a post-vaccination monitoring survey, we would caution against extrapolating this information to estimating protection against challenge. Further studies to understand the protective immune response (humoral and cell-mediated) to SPPV are required.

Conclusions

Our results show that the use of a commercial CPPV ELISA kit provides a robust and reliable assay for post-vaccination surveillance on a regional or national level for SP in low resource settings. Our work builds on previous studies investigating the humoral immune response to CPPV vaccination and addresses particularly the limited number of studies assessing SP vaccination under field conditions. Our results have indicated that the timing of a post-vaccination SP testing survey is an important factor to consider when planning post vaccination monitoring.

Ethical approval

The animal samples used in this study were archived sera that had been collected in 2016 as part of a national Mongolian surveillance programme. Ethical approval for the national surveillance programme was granted by Mongolian authorities at the State Central Veterinary Laboratory and the Mongolian General Authority for Veterinary Services (GAVS).

Consent for publication

Not applicable

Availability of data and materials

The dataset supporting the conclusions of this article are included within this article, its additional files, and available in a public repository.

Competing interests

None

Funding.

This project was funded by the Biotechnology and Biological Science Research Council of the United Kingdom (BBSRC) Impact Acceleration Account and project BB/R008833. The Pirbright Institute receives strategic funding support from BBSRC (BBS/E/I/00007031, BB/E/I/00007036, BB/E/I/00007037, and BBS/E/I/00007039). PMB was partially funded by BBSRC-funded Institute Strategic Programmes BB/J004324/1 and BBS/E/D/20002173 awarded to The Roslin Institute. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773701. Targeted surveillance in Mongolia was financed by FAO Mongolia (FAO Project TCP/MON/3603 Emergency assistance for the control of sheep and goat pox). The ID Screen® Capripox Double Antigen Multi-species ELISA kits were supplied by IDvet.

None of the funding bodies played a role in the design of the study, collection, analysis or interpretation of the data, or in writing the manuscript.

Author's contributions.

P.M.B, G.L., G.U., B.D., and O.M. contributed to the conception of the study, P.M.B, G.L., G.T., G.U., Bo.K., B.S., B.D., and P.F. designed the study, G.T. generated the fluorescently-labelled LSDV strain, P.F. developed and carried out the FVNTs, G.U., Bu.K., O.M., and J.F. carried out the ELISAs, G.L., P.F., P.M.B., and G.U. analysed and interpreted the data, P.F., G.L., and P.M.B drafted the manuscript. All authors have approved the submitted version of the manuscript.

Table 1 Number of positive and negative results using ELISA in two labs (Mongolia and UK) (n=400)

	Num. negatives samples	**Num. positive samples**	**P value***	**Kappa test**
	ELISA Mongolia			
ELISA UK				
Num. negatives samples	178	57		
Num. positive samples	34	131	0.02	0.54

*McNemar's chi squared test for paired data

Table 2 Results from samples tested by ELISA and FVNT (n=45)

Days post vaccination	ELISA Mongolia lab (%S/P)	ELISA Mongolia lab	ELISA UK lab (%S/P)	ELISA UK lab	FVNT Titre	FVNT
40	41.93	Positive	83	Positive	80	Positive
40	0	Negative	2.09	Negative	0	Negative
40	3.01	Negative	0	Negative	0	Negative
40	5.32	Negative	2.58	Negative	0	Negative
74	45.7	Positive	0.48	Negative	0	Negative
75	17.2	Negative	0	Negative	0	Negative
76	1.61	Negative	0	Negative	0	Negative
76	9.07	Negative	1.25	Negative	0	Negative
76	29.23	Negative	0	Negative	0	Negative
77	232.86	Positive	210.9	Positive	480	Positive
77	160.18	Positive	32.81	Positive	120	Positive
80	7.81	Negative	0.55	Negative	0	Negative
80	117.84	Positive	325.5	Positive	320	Positive
80	156.96	Positive	49.47	Positive	80	Positive
81	83.47	Positive	82.25	Positive	120	Positive
92	26.77	Negative	1.42	Negative	0	Negative
92	72.25	Positive	143.3	Positive	30	Positive

92	62.25	Positive	0.32	Negative	0	Negative
102	1.91	Negative	3.7	Negative	0	Negative
107	46.07	Positive	0	Negative	0	Negative
110	105.24	Positive	54.56	Positive	30	Positive
114	3.63	Negative	0.26	Negative	0	Negative
114	24.4	Negative	0	Negative	0	Negative
119	33.47	Positive	0	Negative	10	Positive
121	14.76	Negative	22.32	Negative	20	Positive
122	0.11	Negative	139.5	Positive	60	Positive
122	34.83	Positive	181.6	Positive	160	Positive
122	173.3	Positive	400.1	Positive	1280	Positive
130	88.57	Positive	130.7	Positive	60	Positive
131	0	Negative	3.76	Negative	0	Negative
131	0	Negative	0	Negative	0	Negative
131	158.88	Positive	252.2	Positive	30	Positive
132	0	Negative	6.6	Negative	0	Negative
133	50.27	Positive	101.2	Positive	160	Positive
135	40.82	Positive	46.3	Positive	0	Negative
135	0	Negative	0	Negative	0	Negative
193	43.25	Positive	3.49	Negative	20	Positive
229	132.41	Positive	169.2	Positive	80	Positive
229	132.19	Positive	185.3	Positive	60	Positive
229	0	Negative	2.07	Negative	0	Negative
229	0	Negative	0.03	Negative	0	Negative
234	0	Negative	0.19	Negative	0	Negative
234	0	Negative	1.53	Negative	0	Negative
236	121.73	Positive	255	Positive	640	Positive
243	0	Negative	0.3	Negative	0	Negative

Table 3 Number of positive and negative results using FVNT and ELISA in two labs (Mongolia and UK) (n=45)

	Num. negatives samples	**Num. positive samples**	**P value***	**Kappa test**
	ELISA Mongolia			
FVNT				
Num. negatives samples	21	4		
Num. positive samples	2	18	0.68	0.73
	ELISA lab UK			

FVNT					
Num. negatives samples	24	1			
Num. positive samples	3	17	0.61	0.82	

*McNemar's chi squared test for paired data

Table 4 Distribution of factors considered for sheep pox serological status after vaccination following univariable analysis. All models include herder as random effect

Variable	Mongolia laboratory					UK laboratory				
	Number of negative (%)	**Number of positive (%)**	**Estimate**	**Std. error**	**P value**	**Number of negative (%)**	**Number of positive (%)**	**Estimate**	**Std. error**	**P value**
Sex										
Female	142 (67.0)	124 (66.0)	*Ref.*			155 (66.0)	111 (67.3)	*Ref.*		
Male	70 (33.0)	64 (34.0)	-0.15	0.30	0.61	80 (34.0)	54 (32.7)	-0.09	0.33	0.78
Age										
Up to 1 year	4 (1.9)	7 (3.7)	*Ref.*			10 (5.3)	1 (0.6)	*	*	*
>1 year	208 (98.1)	181 (96.3)	-0.31	0.93	0.74	225 (95.7)	164 (99.4)			
Days since vaccination										
Up to 90 days	43 (20.7)	52 (27.8)	*Ref.*			58 (25.1)	37 (22.5)	*Ref.*		
91 to 180 days	123 (59.1)	122 (65.2)	-0.52	0.53	0.33	127 55.0)	118 (72.0)	0.35	0.67	0.60
>180 days	42 (20.2)	13 (6.9)	-2.23	0.80	**0.006**	46 (19.9)	9 (5.5)	-2.16	1.03	**0.04**
Province										
Dornod	30 (14.2)	49 (26.1)	*Ref.*			50 (21.3)	29 (17.6)	*Ref.*		
Dornogovi	52	9 (4.8)	-3.00	0.73	**<0.001**	44	17	-0.86	0.87	0.32

Dundgovi	(24.5)	18 (9.6)	-1.72	0.69	**0.01**	(18.7)	(10.3)	-0.34	0.92	0.71
Govisumber	36 (17.0)	10 (5.3)	-1.49	0.84	**0.08**	33 (14.0)	21 (12.7)	-0.50	1.15	0.66
Khentii	17 (8.0)	39 (20.7)	-1.16	0.59	**0.04**	18 (7.6)	9 (5.4)	1.06	0.78	0.18
Sukbaatar	48 (22.6)	46 (24.5)	-0.18	0.60	0.77	38 (16.2)	49 (29.7)	-0.47	0.82	0.56
Tuv	27 (12.7)	17 (9.0)	1.86	1.10	**0.09**	50 (21.3)	23 (17.0)	3.58	1.40	**0.01**
	2 (0.9)					2 (0.8)	17 (10.3)			

*Model does not converge

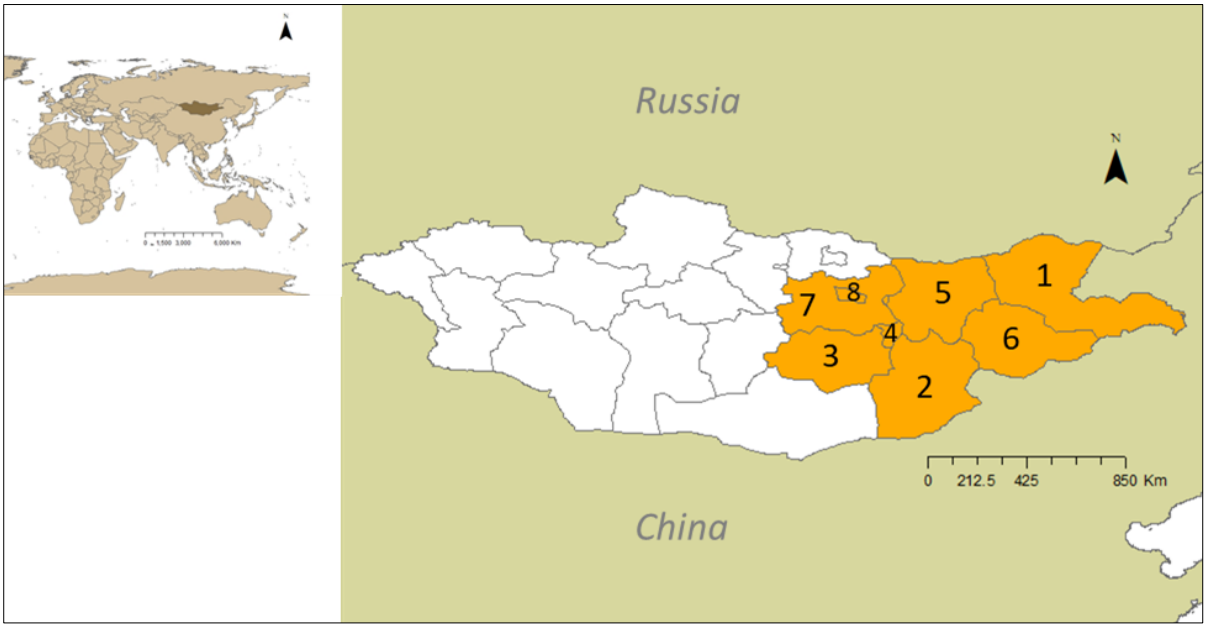

Figure 1 Geographical location of Mongolia (dark brown – top left map) and provinces where vaccination was conducted (in alphabetical order) (1)Dornold, (2)Dornogovi, (3)Dungovi, (4)Govisumber, (5)Khenthi, (6)Sukhbaatar, (7)Tuv and (8) Ulaanbaatar

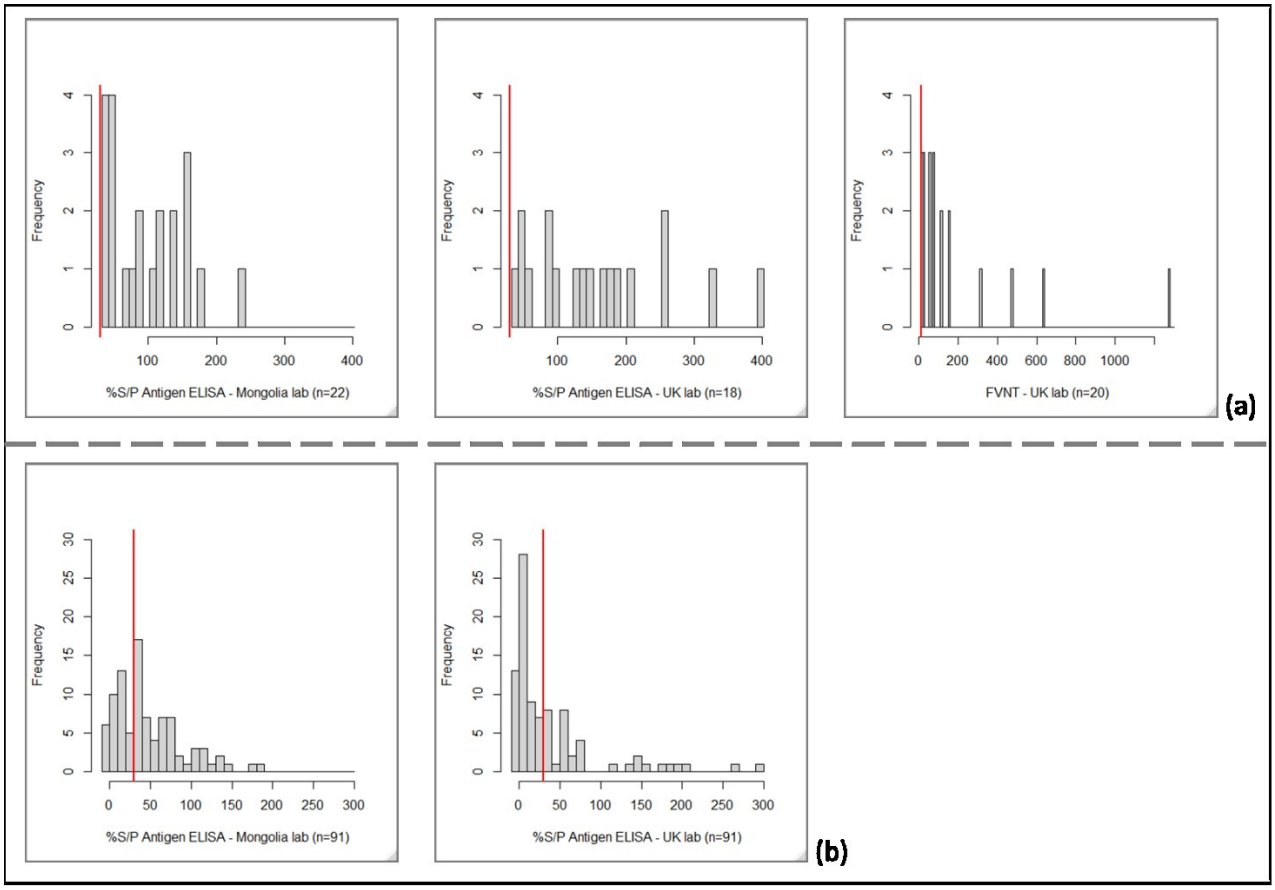

Figure 2 Frequency distributions of values among samples, belonging to the 45-sample subset, that were deemed positive by each test a); and values among those samples with contradictory results between the two labs considering all samples tested (b). Red vertical lines show cut off value used to classify samples as positive/ negative (ELISA S/P30% and FVNT 1:10).

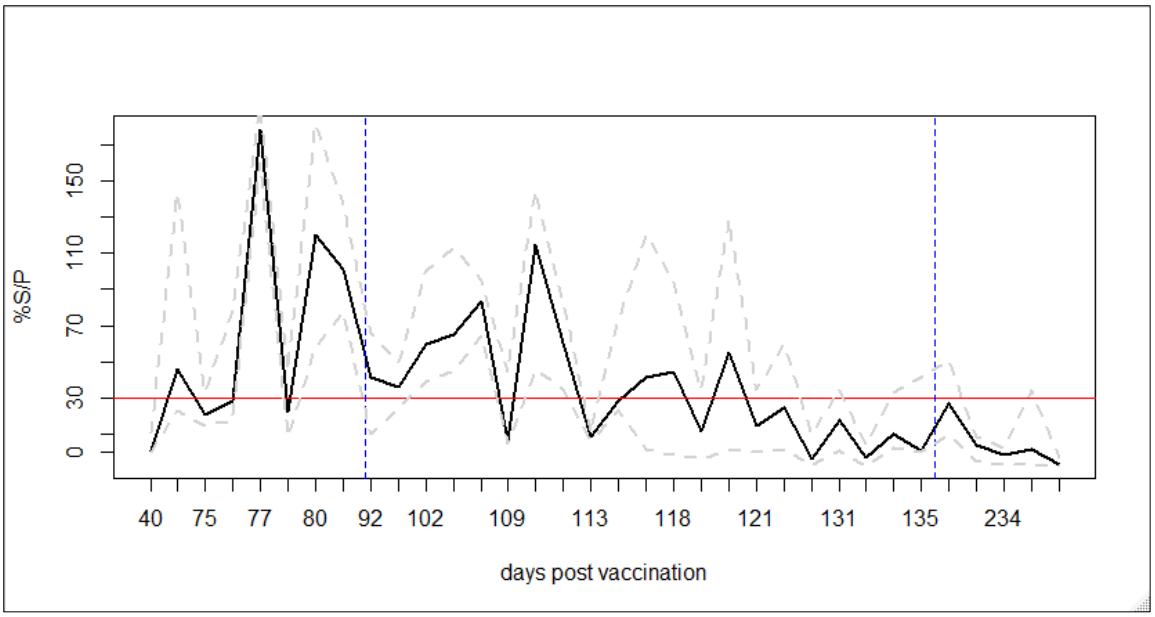

Figure 3 Median %SP value using results from SCVL (black line), 1st and 3rd quartile (grey dashed lines) and days post vaccination. Red horizontal line represent %S/P cut-off used to classify samples as positive/negative. Blue vertical dashed lines are cut-off used to classified days post vaccination in categories (<90 days, 91 to 180 days and >180 days) based on the expected dynamics of the immune response.

Supplementary material

Table S1 Median and interquartile range for %S/P values stratified by days since vaccination

Days between vaccination and sampling	ELISA S/P SCVL, Mongolia			ELISA S/P Pirbright, UK		
	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile
Up to 90 days	9.08	34.62	117.84	-0.05	2.10	73.14
Between 91 and 180 days	1.6	28.83	64.31	1.58	24.77	79.77
More than 180 days	-6.37	2.89	23.69	-0.15	0.29	1.80

Table S2 Median and interquartile range of %SP value, stratified by day of sampling, using results from SCVL.

Number of animals	Days post vaccination	Median %SP (1st and 3rd quartile)	Number of animals	Days post vaccination	Median %SP (1st and 3rd quartile)
25	40	0.7 (-0.2 – 11.1)	25	40	0.70 (-0.20 – 11.13)
9	74	45.7 (23.3 – 143.0)	42	70-79	29.7 (17.5 – 90.9)
11	75	20.3 (14.3 – 33.1)			
13	76	28.1 (17.4 – 77.8)			
5	77	178.2 (160.2 – 191.4)			
4	79	22.5 (8.3 – 45.5)			
24	80	120.4 (57.9 – 181.3)	28	80-89	117.8 (63.7 – 179.4)
4	81	100.7 (77.0 – 136.6)			

15	92	41.5 (9.9 – 65.7)	33	90-99	36.8 (17.9 – 58.7)
18	98	34.0 (24.3 – 48.7)			
19	102	59.8 (38.8 – 100.2)	36	100-109	60.9 (39.4 – 109.0)
10	105	65.3 (45.4 – 113.3)			
3	107	83.3 (64.7 – 95.1)			
4	109	5.9 (4.8 – 44.4)			
9	110	114.7 (46.1 – 144.5)	79	110-119	28.1 (2.9 – 85.1)
5	112	60.3 (34.8 – 83.9)			
4	113	8.2 (6.3 – 10.9)			
11	114	28.1 (23.3 – 72.0)			
13	116	41.4 (1.8 – 120.0)			
10	118	44.1 (-1.7 – 94.0)			
27	119	11.3 (-4.0 – 34.2)			
6	120	55.3 (1.9 – 128.5)	23	120-129	14.8 (1.1 – 84.4)
5	121	14.8 (0.3 – 34.6)			
12	122	24.8 (1.6 – 59.6)			
11	130	-4.1 (-6.9 – 10.0)	74	130-139	4.4 (-0.2 – 35.1)
24	131	17.6 (0.6 – 34.4)			
6	132	-2.9 (-7.7 – 4.2)			
6	133	10.2 (2.3 – 33.3)			
27	135	0.8 (-0.25 – 41.4)			
12	193	27.2 (10.0 – 50.7)	55	≥140	2.9 (-6.4 – 23.7)
16	229	3.7 (-5.3 – 8.3)			

4	234	-1.7 (-6.7 – 2.6)	
13	236	1.9 (-6.9 – 34.7)	
10	243	-6.7 (-7.9 - -3.3)	

Table S3 Time between vaccination and sampling stratified by Province

Province	**Time between vaccination and sampling (days)** **Median (min- max)**
Dornod (n=79)	80 (74 – 193)
Dornogovi (n=61)	229 (130 – 243)
Dundgovi (n=54)	121 (40 – 131)
Govisumber (n=27)	135 (135 – 135)
Khentii (n=87)	119 (98 – 131)
Sukbaatar (n=73)	105 (76 – 114)
Tuv (n=19)	102 (102 – 102)

References

[1] Babiuk S., Bowden T.R., Parkyn G., Dalman B., Hoa D.M., Long N.T., Vu P.P., Bieu do X., Copps J., Boyle D.B., Yemen and Vietnam capripoxviruses demonstrate a distinct host preference for goats compared with sheep, The Journal of general virology. (2009) 90:105-114.

[2] Bolajoko M.B., Adedeji A.J., Dashe G.D., Òsemeke O.H., Luka P.D., Molecular epidemiology and economic impact of goat pox on small holder sheep and goats farmers in north central Nigeria, Small Ruminant Research. (2019) 179:75-78.

[3] Garner M.G., Sawarkar S.D., Brett E.K., Edwards J.R., Kulkarni V.B., Boyle D.B., Singh S.N., The extent and impact of SGP in the state of Maharashtra India, Trop Anim Health Prod. (2000) 32:205-223.

[4] Limon G., Gamawa A.A., Ahmed A.I., Lyons N.A., Beard P.M., Epidemiological characteristics and economic impact of lumpy skin disease, sheeppox and goatpox among subsistence farmers in northeast Nigeria, Front Vet Sci. (2020).
[5] Babiuk S., Bowden T.R., Boyle D.B., Wallace D.B., Kitching R.P., Capripoxviruses: an emerging worldwide threat to sheep, goats and cattle, Transbound Emerg Dis. (2008) 55:263-272.
[6] Tuppurainen E.S.M., Venter E.H., Shisler J.L., Gari G., Mekonnen G.A., Juleff N., Lyons N.A., De Clercq K., Upton C., Bowden T.R., Babiuk S., Babiuk L.A., Review: Capripoxvirus Diseases: Current Status and Opportunities for Control, Transbound Emerg Dis. (2017) 64:729-745.
[7] Mongolian Statistical Information Service 2017, http://1212.mn/ (Accessed: September 2018) [on line]
[8] Beard P.M., Sugar S., Bazarragchaa E., Gerelmaa U., Tserendorj S., Tuppurainen E., Sodnomdarjaa R., A description of two outbreaks of capripoxvirus disease in Mongolia, Veterinary microbiology. (2010) 142:427-431.
[9] Boumart Z., Daouam S., Belkourati I., Rafi L., Tuppurainen E., Omari Tadlaoui K., El Harrak M., Comparative innocuity and efficacy of live and inactivated sheeppox vaccines, BMC Vet Res. (2016) 12.
[10] Kali K., Kardjadj M., Touaghit N., Yahiaoui F., Hind Ben-Mahadi M., Understanding the epidemiology of sheep-pox outbreaks among vaccinated Algerian sheep and post vaccination evaluation of the antibodies kinetics of the commercially used vaccine, Comparative Immunology, Microbiology and Infectious Diseases. (2019) 65:128-131.
[11] Klement E., Broglia A., Antoniou S.E., Tsiamadis V., Plevraki E., Petrović T., Polaček V., Debeljak Z., Miteva A., Alexandrov T., Marojevic D., Pite L., Kondratenko V., Atanasov Z., Gubbins S., Stegeman A., Abrahantes J.C., Neethling vaccine proved highly effective in controlling lumpy skin disease epidemics in the Balkans, Prev Vet Med. (2018) 18:30537-30533.
[12] Milovanovic M., Dietze K., Milicevic V., Radojicic S., Valcic M., Moritz T., Hoffmann B., Humoral immune response to repeated lumpy skin disease virus vaccination and performance of serological tests, BMC veterinary research. (2019) 15:80.
[13] Haegeman A., De Leeuw I., Mostin L., Van Campe W., Aerts L., Vastag M., De Clercq K., An Immunoperoxidase Monolayer Assay (IPMA) for the detection of lumpy skin disease antibodies, Journal of virological methods. (2020) 277:113800.
[14] OIE, Lumpy skin disease, Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019, 2019.
[15] Haegeman A., De Vleeschauwer A., De Leeuw I., Vidanovic D., Sekler M., Petrovic T., Demarez C., Lefebvre D., De Clercq K., Overview of diagnostic tools for Capripox virus infections, Preventive veterinary medicine. (2019):104704.
[16] Bowden T.R., Coupar B.E., Babiuk S.L., White J.R., Boyd V., Duch C.J., Shiell B.J., Ueda N., Parkyn G.R., Copps J.S., Boyle D.B., Detection of antibodies specific for sheeppox and goatpox viruses using recombinant capripoxvirus antigens in an indirect enzyme-linked immunosorbent assay, Journal of Virological Methods. (2009) 161:19-29.
[17] Milena S., Vladimir P., Vladimir G., Diana L., Gospava L., Tamaš P., Sava L., Detection of antibodies against lumpy skin disease virus by virus neutralisation test and ELISA methods, Acta Veterinaria-Beograd. (2019) 69:47-60.
[18] Moller J., Moritz T., Schlottau K., Krstevski K., Hoffmann D., Beer M., Hoffmann B., Experimental lumpy skin disease virus infection of cattle: comparison of a field strain and a vaccine strain, Archives of virology. (2019) 164:2931-2941.
[19] Abdelwahab M.G., Khafagy H.A., Moustafa A.M., Saad M.A., Evaluation of humoral and cell-mediated immunity of lumpy skin disease vaccine prepared from local strain in calves and its related to maternal immunity, Journal of American Science. (2016) 12 (10):38-45.

[20] Kitching R.P., Passive protection of sheep against capripoxvirus, Research in veterinary science. (1986) 41:247-250.
[21] Law M., Putz M.M., Smith G.L., An investigation of the therapeutic value of vaccinia-immune IgG in a mouse pneumonia model, The Journal of general virology. (2005) 86:991-1000.
[22] Kempe C.H., Bowles C., Meiklejohn G., Berge T.O., St Vincent L., Babu B.V., Govindarajan S., Ratnakannan N.R., Downie A.W., Murthy V.R., The use of vaccinia hyperimmune gamma-globulin in the prophylaxis of smallpox, Bull World Health Organ. (1961) 25:41-48.
[23] Mack T.M., Noble J., Jr., Thomas D.B., A prospective study of serum antibody and protection against smallpox, Am J Trop Med Hyg. (1972) 21:214-218.
[24] Crotty S., Felgner P., Davies H., Glidewell J., Villarreal L., Ahmed R., Cutting edge: long-term B cell memory in humans after smallpox vaccination, Journal of immunology. (2003) 171:4969-4973.
[25] Hammarlund E., Lewis M.W., Hansen S.G., Strelow L.I., Nelson J.A., Sexton G.J., Hanifin J.M., Slifka M.K., Duration of antiviral immunity after smallpox vaccination, Nature medicine. (2003) 9:1131-1137.
[26] Boshra H., Truong T., Nfon C., Bowden T.R., Gerdts V., Tikoo S., Babiuk L.A., Kara P., Mather A., Wallace D.B., Babiuk S., A lumpy skin disease virus deficient of an IL-10 gene homologue provides protective immunity against virulent capripoxvirus challenge in sheep and goats, Antiviral research. (2015) 123:39-49.