Journal of Immunological Methods 502 (2022) 113226

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Evaluation of ELISA and VNT for sheeppox virus antibody detection and development of an immunoenzymatic quantitative method

Halima Rhazi [a,b,*], Karima Mikou [a], Youness Sadeqy [b], Meryeme Alhayane [b], Soufiane El Mejdoub [b], Najete Safini [b], Matthias Lenk [c], Khalid Omari Tadlaoui [b], Mehdi Elharrak [b]

[a] Laboratory of functional and environmental ecology, Faculty of Sciences and Techniques, Sidi Mohammed Ben Abdellah University, BP 2202 Imouzzer Road, Fez, Morocco
[b] Laboratory of Research and Development virology, MCI Animal Health, Lot. 157, Zone Industrielle Sud-Ouest (ERAC) B.P: 278, Mohammedia 28810, Morocco
[c] Departement of Experimental Animal Facilities and Biorisk Management, Collection of Cell Lines in Veterinary Medicine, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, D-17493 Greifswald - Insel Riems, Germany

ARTICLE INFO

Keywords:
Sheep poxvirus
Immunoenzymatic technics
Immunoperoxidase assay
Immunofluorescence assay
ELISA
Antibody detection

ABSTRACT

Vaccination against sheep pox (SPV) is the most efficient tool to control spread of the disease and virus neutralization test (VNT) is the gold standard for vaccination monitoring. In the presented study, we evaluated the use of ELISA and VNT for quantification of SPV humoral response post vaccination. Results confirmed that VNT is more sensitive since ELISA did not detect 22% of positive tested sera, and VNT weak positive sera were either negative or doubtful by ELISA. The most sensitive cells to perform VNT were ESH-L instead of Lamb primary cells. We also investigated immunoperoxidase IPMA and immunofluorescence IFA assays for detection of SPV specific antibodies and IPMA showed higher antibody titers comparatively to IFA. VNT using ESH-L cells with immune-enzymatic revelation provide specific quantitative SPV antibody titers, easier to read in shorter incubation time.

1. Background

Sheep pox is a devastating and contagious disease of sheep characterized by pyrexia, generalized skin and internal pox lesions, and lymphadenopathy (Beard and Kingdom, 2019). The diseases is caused by sheep poxvirus (SPPV), which are enveloped, double-stranded DNA viruses, classified in Capripoxvirus genus of the Poxviridae family (Bhanuprakash et al., 2006). SPPV occur in Africa, Middle East, and Asia including India and China, causing high morbidity (70–90%) and mortality up to 50% leading to important economical losses (Rao and Bandyopadhyay, 2000). For successful SPPV control, vaccination of all susceptible animals is considered the main pillar, supported by other control measures such as stamping out, animal movement restrictions, quarantine and disinfection (Tuppurainen et al., 2017). Vaccination is the most effective way to control the spread of SPPV, only live attenuated vaccines are currently used. Immunological studies for SPPV vaccination monitoring has shown that vaccination stimulates both humoral and cell-mediated immunity (Varshovi et al., 2021). Humoral immune response can be investigated by using virus neutralization test (VNT), indirect fluorescent antibody test (IFA) and ELISA (Gari et al., 2008; Office International des Epizooties, 2017) and other immunological tests such include CIE, and latex agglutination test, also agar gel immunodiffusion test and Western blot. However; they have shown low sensitivity (Chand et al., 1994; Shankar and Yadav, 1988; Jenson, 2014). So far, VNT is the only serological test validated by the OIE with high specificity for detecting capripoxvirus-specific antibodies (Edition, 2008).

In this study, we evaluated and compared the performances of the detection of SPPV specific antibody by different serological tests. Our work is divided into three parts:

- Comparative sensitivity study of ELISA commercial kit with VNT OIE reference method, investigation carried out on 220 sera.

* Corresponding author at: Laboratory of functional and environmental ecology, Faculty of Sciences and Techniques, Sidi Mohammed Ben Abdellah University, BP 2202 Imouzzer Road, Fez, Morocco.
E-mail address: h.rhazi@mci-santeanimale.com (H. Rhazi).

https://doi.org/10.1016/j.jim.2022.113226
Received 1 October 2021; Received in revised form 29 November 2021; Accepted 10 January 2022
Available online 13 January 2022

- Comparative study of VNT reference OIE method using testis cells (LT), Ovine Aries Testis (OA3.Ts) cell line and Embryonic Skin of sheep (ESH-L) cells, to determine cell sensitivity, investigation performed on 106 sera.
- Comparative investigation on the VNT revelation method, where 100 of weak and strong positive sera have been analyzed by VNT and sera titers by three methods; cytopathic effect presence or absence (OIE standard method), immunoperoxidase assay (IPMA) and immunofluorescence assay (IFA) both using specific antibodies.

The aim of the study is to propose a specific and sensitive method for the detection of antibody response to SPPV infection or vaccination.

2. Material and methods

2.1. Blood samples

For this study, 220 serum were collected from sheep in Morocco. Blood samples were taken randomly from local breeds, male and female of all ages, within the frame of post vaccination monitoring for SPPV. Sampling was performed in line with principles of good veterinary practices and in full respect of animal welfare, including unvaccinated control and vaccinated animals at different intervals of time after immunization. Blood were collected into dry tubes, allowed to clot for 3 h at room temperature, and then sera were extracted by centrifuging at 2000 rpm for 20 min, transferred in 2-ml tubes and stored at −20 °C until analysis.

2.2. Serological analysis by ELISA

Antibody detection by ELISA test was performed using ID Screen® Capripox double antigen Multi-species ELISA kit from ID vet® (Montpellier, France) according the manufacturer's instructions. Briefly, the samples and the controls are distributed in the wells, forming an antigen-antibody complex. The plates are then washed and the conjugate (purified CPV antigen labeled with peroxidase (HRP)) is added to wells. After elimination of the excess conjugate, the reaction is revealed by a tetramethylbenzidine (TMB) solution. In the presence of antibodies in the sample, a blue color appears which becomes yellow after blocking. However, in the absence of antibodies in the samples, no staining appears. The reading is performed at 450 nm.

2.3. Serological analysis by VNT

Sheep serum were analyzed using VNT to determine SPPV antibody titer. This method was based upon the OIE Terrestrial Manual Chapter 3.7.12. The test was performed in 96-microwell plates (Nunc, Thermo Fisher Scientific, USA):

- Serum preparation: Sera were heated at 56 °C for 30 min. A 3-fold dilution series was made in cell culture media (Dulbecco's modified Eagle's medium (Wisent Inc., cat n° 219–015-XK) supplemented with 1% Fetal Bovine Serum (FBS)) from the dilution 1/3 to 1/729 in 4 duplicates for each serum.
- Work virus: attenuated SPPV Romania strain (Precausta et al., 1979) (Causta and Kato, 1979) was used in VNT. A monolayer of LT cells in T75 flasks (Nunc, Thermo Fisher Scientific, USA) was infected with a MOI of 0,01, and incubated at 35 °C. After 80% CPE, the flask was frozen and thawed, aliquoted and stored at −80 °C until use. The viral suspension was submitted for titration 5 times to determine the infectious titer to be used in VNT. A suspension of 100 TCID50/well was prepared for the test. The suspension was added in the 96 well plates containing the serum serial dilution at equal volume (25 µl) and incubated 1 h at 37 °C, 5% CO2. Three 10-fold dilutions of the work virus were prepared and distributed in 10 replicates each in the virus control plate (25 µl of virus +25 µl medium).
- Cells: Primary lamb testis cells LT were obtained by castration of a healthy three-month-old male. The tissue was cut into small pieces, and submitted to enzymatic dissociation by trypsin to obtain single cells. Cells were diluted in Dulbecco's modified Eagle's medium (Wisent Inc., cat n° 219–015-XK) supplemented with 10% Fetal Bovine Serum (FBS), and incubated at 37 °C with 5% CO2. Two additional cell lines were used in VNT for sensibility evaluation: OA3. Ts cells (CRL-6546, ATCC) and ESH-L cells obtained from Federal Research Institute for Animal Health, the Friedrich-Loeffler Institut (FLI) (Cat N°: CCLVRIE 0175). The two cell lines were cultured in Dulbecco's modified Eagle's medium (Wisent Inc., cat n° 219–015-XK), supplemented with 10% FBS. For each type of cells, a suspension (120,000 cells/cm^2) was prepared and added to the incubated plates at a volume of 150 µl. Few wells in the plate were dedicated to cells growth control (150 µl of cells) without virus or serum.
- All plates were incubated 6 days at 37 °C for CPE detection. Wells were considered negative when a typical CPE was observed. In the absence of CPE, the virus is considered neutralized and the well positive. The antibody titer was calculated using the method of Reed & Muench method (1938) (Reed and Muench, 1938) (American, 1938) and expressed log neutralizing doses 50% (DN 50%).

2.4. Serological analysis by immunoperoxidase assay (IPMA)

50 sera with strong positive titers and 50 sera with weak titers by ELISA and VNT were used to compare VNT, IPMA and IFA methods. The IPMA were performed as described by Haegeman (Haegeman et al., 2020). The test was conducted similarly to the VNT described above. Briefly, after three days incubation of plates containing virus/serum/cells, the plates were fixed with 4% paraformaldehyde and methanol/30% H_2O_2, washed with PBS, and the home made anti-SPPV serum was added (sheep). After 1 h incubation, the anti-Sheep IgG-peroxidase (Sigma Aldrich) was added, and the plates were incubated 1 h at 37 °C. After a final wash, 50 µl per well of a substrate solution (3-amino-9-diethyl-carbazole in 50 mM Na-acetate buffer with 0.05% hydrogen peroxide) was added to reveal the reaction. The mixture was incubated at room temperature for 15 min. The staining was stopped by eliminating the substrate and adding 100 µl of the Na-acetate buffer. Observation with inverted contrast microscope characterize positive wells as uncolored monolayer and negative wells as red stained wells. The antibody titer is calculated as above.

2.5. Serological analysis by immunofluorescence assay (IFA)

The test is similar to VNT and IPMA, reading plate wells was carried out by using inverted IF microscope after addition of anti-SPPV antibody and fluorescent rabbit antimouse dye-light 594 (Bethyl Laboratories, Inc.). The fluorescence signal was analyzed using an Olympus fluorescence microscope. The wells that display fluorescent foci were designated negative. And the cells that shows no fluorescence were considered positive.

2.6. Statistical analysis

Differences between antibody titers obtained by different type of serological tests were evaluated for significance by the method of Fisher. Values of $p \leq 0.01$ were considered significant.

To determine the specificity and the sensitivity of ELISA, we used VNT as the gold standard. We determined true positives (TPs) (ELISA positive and VNT positive), false positives (FPs) (ELISA positive and VNT negative), false negatives (FNs) (ELISA negative and VNT positive), and true negatives (TNs) (VNT ELISA and VNT negative). The formulae used were TP/(TP + FN) * 100 for the sensitivity and TN/(FP + TN) * 100 for the specificity.

3. Results

3.1. Sensitivity study of ELISA and VNT for SPPV antibody detection

Investigations were conducted on 220 sera for detection of SPPV antibody in sheep serum. Table 1 demonstrate that the highest number of positives tested sera was detected by VNT (70%) vs ELISA (48%). All negative sera by VNT were also negative by ELISA and 15 sera tested negative by ELISA were doubtful (13 sera) or weak positive (two sera) by VNT. Results also showed that half of the VNT weak positive sera were found doubtful by ELISA, and among 220 tested samples, 22,2% were doubtful by ELISA and only 6,8% by VNT.

The sensitivity of ELISA compared to that of VNT was 87.60%, while specificity was 100% (Table 1).

Obtained results demonstrate a high correlation (R^2) between ELISA and VNT in strong positive samples (0,383), and low correlation for weak (0, 00343), doubtful (0.0128) and negative (0,0023). Which indicate a higher sensibility of VNT to detect SPPV antibody comparatively to ELISA (Fig. 1).

3.2. Cells sensitivity of VNT using LT, OA3.Ts and ESH-L cells

A total number of 106 sera were selected for antibody titration by VNT using three types of cells; LT, OA3.Ts and ESH-L cells. Results showed that 99 samples were detected positive on the three tested cells. Seven samples were negative on LT and OA3.Ts cells, while only three were negative on ESH-L cells (all weak positive, average titer 0,9). The global titer of the 106 tested sera obtained was 1,73 on LT cells, 1,63 on OA3.Ts and 1,97 on ESH-L. There is a significant difference between the three used cells to detect CPE in VNT (⟨0,001).

Results obtained on OA3.Ts cells compared to those on LT cells showed a correlation factor (R^2) of 0,76 while correlation between ESH-L and LT cells was significantly higher (0,81) which indicate that both LT and ESH-L cells provide almost similar results (Fig. 2).

3.3. Investigation on VNT revelation method: CPE, IPMA and IFA

One hundred positive sera were tested by VNT on ESH-L cells and revelation was carried out by three techniques: direct CPE observation (OIE reference), specific immunoenzymatic (IPMA) and immunofluorescence methods (IFA). The average VNT titer was 1,9 by CPE detection, 2,5 by IPMA detection and 2,4 by IFA detection (Table 2). Statistical analysis showed a significant difference between the gold standard CPE and the specific IPMA and IFA methods (Table 3).

4. Discussion

A number of immunological methods can be used for antibodies titration; however, VNT is the recommended test in the OIE Terrestrial Manual for post vaccination monitoring which test reflect conferred protection in animals. However, VNT is a labor intensive, time consuming and requires biosafety level 3 containment to handle the infectious virus (Tuppurainen, 2017). ELISA test is more convenient for large scale monitoring, easy to perform and results can obtained in 24 h. In the presented study, we investigated 4 different serological methods for the detection of specific antibodies against SPPV. Samples were collected from vaccinated and unvaccinated sheep and tested by ELISA and three variant of VNT method comparatively, in the objective to propose the best technique for antibody detection and quantification.

Currently vaccination against SPPV is the most effective way to control the spread of this virus and subsequently its economic damages (Madhavan et al., 2016) . Post vaccination monitoring is based on antibody detection in vaccinated animals, performed generally by VNT standard method using lamb testis cells. Recently, after LSD spread in Europe and Asia, an ELISA test has been developed and extensively used in the field for diagnosis or to evaluate conferred protection (Ochwo et al., 2019). Studies has proved a successful use of this test for the detection of antibodies against Lumpy Skin Disease (LSD) (Samojlovic et al., 2019). Although as indicated for the three capripoxviruses (SPPV, GTPV and LSD), this is the first time the ELISA commercial kit is evaluated for SPPV antibody detection regarding its sensitivity in comparison with the VNT gold standard.

Results showed that VNT is more sensitive than ELISA since 22% of positive tested sera were negative in ELISA. In addition, weak positive sera in VNT were either negative or doubtful in ELISA. This finding does not agree with that one reported by Nina et al. (2020) with test applied on LSD virus in cattle suggesting that despite cross-reaction between the three number of capripoxviruses there is a significant difference in ELISA results between SPPV and LSD viruses (Kresic, 2020).

In a previous study using an inactivated sheeppox virus as ELISA antigen, Babiuk and Wallace (2009) reported a sensitivity of 96% and specificity of 95% of the developed test, comparatively to VNT performed on OA3.Ts cells (Babiuk et al., 2009). This test is not available commercially; however, the high sensitivity and specificity could be explained by the use a specific SPPV antigen which probably not the case in our ELISA. Another similar ELISA test was developed by Bhanuprakash et al. (2006) based on a purified goat poxvirus antigen showed a sensitivity of 62% vs 48% in our study (Bhanuprakash et al., 2011).

The VNT as a gold standard technique in the OIE Manual is based on the use of primary lamb testis cells and the reading by CPE detection after neutralization with sera. Although, lamb testis are most sensitive cells for capripoxviruses replication it has been demonstrated recently that ESH-L of skin origin are more sensitive and can be maintained for several passages up to 40 (Rhazi et al., 2021). OA3.Ts have been also been used in VNT to avoid the hard work of preparation of primary cells but showed low sensitivity to SPPV compared to testis cells. In our study, sensitivity comparison between the three cells confirm that ESH-L are more convenient to perform VNT test since the global antibody titer obtained on those is higher than the titer on LT or OA3.Ts cells and CPE can be detect earlier an is more pronounced than in other cells. The final plate reading is up to 9 days in the OIE method on testis cells, while is 6 days on OA3.Ts cells, and only 4 days by the same technique on ESH-L, which is a great advantage.

To improve the VNT, we introduced a new method based on the use of anti-SPPV serum, which add specificity to the test. This technique has been used recently as a qualitative method to detect the presence or absence of antibody against SPPV by Haegman et al. (2019). In our study, sera where diluted to allow quantification of antibody, and this was the first time where the IPMA technique is applied as quantitative specific method for anti-SPPV antibody quantification. This method has been used for other viruses such as vaccinia virus, African swine fever virus, porcine circovirus type 2, swine influenza virus, swine hepatitis e virus (Afayoa et al., 2014; Gerber et al., 2012; Pileri et al., 2014; District, 2014) (Direksin et al., 2002) . Obtained results demonstrated the IPMA technique is more sensitive than the OIE standard method (antibody titer 2,5 vs 1,9). The method is specific detecting only the target virus and in addition, results can be obtained in 3 days versus 9 days in the VNT method.

IFA is also a specific method that has been used in our study to detect and quantify SPPV antibodies in sera of vaccinated sheep, which appeared to be a promising method for LSD virus (Abera et al., 2015). At our knowledge, VNT with revelation by IFA have never reported for

Table 1
Sensitivity and specificity of ELISA in comparison with VNT.

ELISA results	VNT results	
	Number of positives	Number of negatives
Number of positives	106 (TP)	0 (FP)
Number of negatives	15 (FN)	50 (TN)
Sensitivity %	87.60%	
Specificity %	100%	

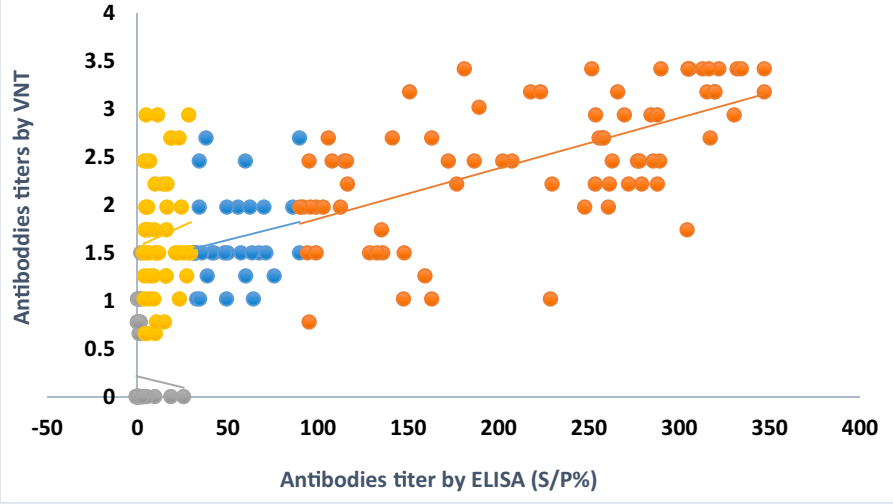

Fig. 1. Correlation between VNT and ELISA for detection of SPPV antibodies (strong positive (orange), weak positive (blue), doubtful (yellow) and negative (grey)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

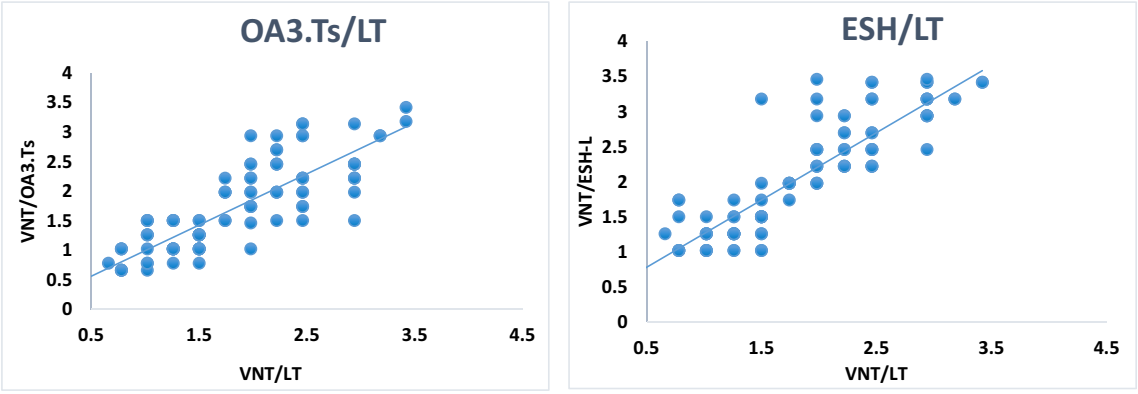

Fig. 2. Correlation between VNT results on OA3.Ts/LT and ESH-L/LT cells.

Table 2
Averages of antibody titer obtained by VNT, IPMA and IFA on 100 SPPV positive sera.

	VNT	IPMA	IFA
Weak positive	1.2408	1.9696	1.8296
Strong positive	2.5704	3.0216	2.96
Global	1.9	2.5	2.4

Table 3
Statistical significance calculated between tests.

	Test	*P* value
Weak positive	VNT/IFA	$p < 0.01$
	VNT/IPMA	$p < 0.01$
Strong positive	VNT/IFA	$p < 0.01$
	VNT/IPMA	$p < 0.01$

SPPV antibody detection and quantification. The sera tested positive by IFA were much higher than sera tested by VNT (2,4 vs 1,9), same results obtained by Milovanović et al. (2019) where it's found that the percentage of positive sera tested for LSD antibodies were obtained by IFA (Milovanovi et al., 2019).

In our study the IFA seem to be as sensitive as IPMA but required expensive reagents (conjugated antibody) and IF microscope, reading could influenced by operator. IFA has been used for detection of antibodies against cellular antigens for more than 50 years (Cytochemistry et al., 2019; Coons et al., 1941; Kaplan, 1951). This method was applied for large groups of antigens (Billiouw et al., 2005; Duarte et al., 2003; Groen et al., 1989).

5. Conclusion

The best method for detection and quantification of anti-SPPV antibody remain virus neutralization test, commercial ELISA showed poor sensitivity for SPPV compared to VNT. The use of primary cells for VNT is a heavy operation and time consuming and could be advantageously replaced by ESH-L cells of lamb skin origin. The immunoenzymatic revelation technique based on the anti-SPPV serum, added specificity to VNT with earlier results.

References

Abera, Z., Degefu, H., Gari, G., Kidane, M., 2015. Sero-prevalence of lumpy skin disease in selected districts of West Wollega zone. BMC Vet. Res. 1–9. https://doi.org/10.1186/s12917-015-0432-7.

Afayoa, M., et al., 2014. Comparison of Immunoperoxidase Monolayer Assay , Polymerase Chain Reaction and Haemadsorption Tests in the Detection of African Swine Fever Virus in Cell Cultures using Ugandan Isolates, vol. 6, pp. 36–45. https://doi.org/10.5897/JGMV2014.0062 no. 4.

American, T.H.E., 1938. The American, vol. 27, pp. 493–497 no. 3.

Babiuk, S., et al., 2009. Detection of Antibodies Against Capripoxviruses Using an Inactivated Sheeppox Virus ELISA, vol. 56, pp. 132–141. https://doi.org/10.1111/j.1865-1682.2009.01067.x.

Beard, P.M., Kingdom, U., 2019. Capripoxviruses , Parapoxviruses , and Other Poxviruses of Ruminants. Elsevier Ltd.

Bhanuprakash, V., Indrani, B.K., Hosamani, M., Singh, R.K., 2006. The Current Status of Sheep Pox Disease, vol. 29, pp. 27–60. https://doi.org/10.1016/j.cimid.2005.12.001.

Bhanuprakash, V., Hosamani, M., Juneja, S., Kumar, N., 2011. Detection of Goat pox Antibodies : Comparative Efficacy of Indirect ELISA and Counterimmunoelectrophoresis Detection of Goat pox Antibodies : Comparative Efficacy of Indirect ELISA and Counterimmunoelectrophoresis, vol. 2119. https://doi.org/10.1080/09712119.2006.9706613.

Billiouw, M., Brandt, J., Vercruysse, J., Speybroeck, N., 2005. Evaluation of the Indirect Fluorescent Antibody Test as a Diagnostic Tool for East Coast Fever in Eastern Zambia, vol. 127, pp. 189–198. https://doi.org/10.1016/j.vetpar.2004.09.028.

Causta, P.P.R.I., Kato, F., 1979. A New Freeze-Dried Living Virus Vaccine against Sheep-Pox, vol. 1, pp. 305–319.

Chand, P., Kitching, R.P., Black, D.N., 1994. Western Blot Analysis of Virus-Specific Antibody Responses for Capripox and Contagious Pustular Dermatitis Viral Infections in Sheep, pp. 377–385.

Coons, A.H., Creech, H.J., Jones, R.N., 1941. Experimental Biology and Medicine. https://doi.org/10.3181/00379727-47-13084P.

Cytochemistry, I., Cells, H., Dellavance, A., Eduardo, L., Andrade, C., 2019. Chapter 3, vol. 1901. https://doi.org/10.1007/978-1-4939-8949-2.

Direksin, K., Joo, H., Goyal, S.M., 2002. An Immunoperoxidase Monolayer Assay for the Detection of Antibodies Against Swine Influenza Virus, vol. 171, pp. 169–171.

District, T., 2014. Development of a Novel Immunoperoxidase Monolayer Assay for Detection of Swine Hepatitis E Virus Antibodies based on Stable Cell Lines Expressing the ORF3 Protein, vol. 62, pp. 243–256. https://doi.org/10.1556/AVet.2013.051 no. December 2013.

Duarte, P.C., Daft, B.M., Conrad, P.A., Packham, A.E., Gardner, I.A., 2003. Comparison of a Serum Indirect Fluorescent Antibody Test with Two Western Blot Tests for the Diagnosis of Equine Protozoal Myeloencephalitis, vol. 13, pp. 8–13.

Edition, S., 2008. World Organisation for Animal Health Manual of Diagnostic Tests and Vaccines (Mammals , Birds and Bees), , Sixth ed.vol. 2

Gari, G., Biteau-coroller, F., Legoff, C., 2008. Evaluation of Indirect Fluorescent Antibody Test (IFAT) for the Diagnosis and Screening of Lumpy Skin Disease using Bayesian Method, vol. 129, pp. 269–280. https://doi.org/10.1016/j.vetmic.2007.12.005.

Gerber, P.F., Matos, A.C.D., Guedes, M.I.M.C., Madureira, M.C., Silva, M.X., Lobato, Z.I. P., 2012. Validation of an Immunoperoxidase Monolayer Assay for Total Anti – Vaccinia Virus Antibody Titration. https://doi.org/10.1177/1040638711435231.

Groen, J., Groen, G., Hoofd, G., Osterhaus, A., 1989. Comparison of Immunofluorescence and Enzyme- Linked Immunosorbent Assays for the Serology of Hantaan Virus Infections, vol. 23, pp. 195–203.

Haegeman, A., et al., Mar. 2020. An Immunoperoxidase Monolayer Assay (IPMA) for the detection of lumpy skin disease antibodies. J. Virol. Methods 277, 113800. https://doi.org/10.1016/j.jviromet.2019.113800.

Jenson, T.A., 2014. Chapter 13 Agar Gel Immunodiffusion Assay to Detect Antibodies to Type A Infl uenza Virus, vol. 1161, pp. 141–150. https://doi.org/10.1007/978-1-4939-0758-8.

Kaplan, M.H., 1951. Localization of A N T I G E N I N Tissue Cells. no. 7.

Kresic, N., 2020. crossm Evaluation of Serological Tests for Detection of Antibodies against Lumpy Skin Disease Virus. no. May. https://doi.org/10.1128/JCM.00348-20.

Madhavan, A., Venkatesan, G., Kumar, A., 2016. Review article Capripoxviruses of small ruminants : current updates and future perspectives. Asian J. Anim. Vet. Adv. 11 (12), 757–770. https://doi.org/10.3923/ajava.2016.757.770.

Milovanovi, M., Dietze, K., Mili, V., Radoji, S., Val, M., Moritz, T., 2019. Humoral Immune Response to Repeated Lumpy Skin Disease Virus Vaccination and Performance of Serological Tests, pp. 1–9.

Ochwo, S., et al., 2019. Seroprevalence and Risk Factors for Lumpy Skin Disease Virus Seropositivity in Cattle in Uganda, pp. 1–9.

Office International des Epizooties, 2017. Manual of diagnostic tests and vaccines for terrestrial animals. In: Chapter 2.4.13. Paris: Lumpy Skin Disease. OIE.

Pileri, E., Cortey, M., Rodríguez, F., Sibila, M., Fraile, L., Segalés, J., 2014. Comparison of the immunoperoxidase monolayer assay and three. Vet. J. 1, 3–6. https://doi.org/10.1016/j.tvjl.2014.05.025.

Rao, T.V.S., Bandyopadhyay, S.K., 2000. A Comprehensive Review of Goat Pox and Sheep Pox and their Diagnosis, vol. 1, pp. 127–136.

Rhazi, H., Safini, N., Mikou, K., Alhyane, M., Lenk, M., 2021. Comparative sensitivity study of primary cells , vero , OA3 . Ts and ESH-L cell lines to lumpy skin disease , sheeppox , and goatpox viruses detection and growth. J. Virol. Methods 293, 114164. https://doi.org/10.1016/j.jviromet.2021.114164 no. April.

Samojlovic, M., Polacek, V., Lupulovic, D., Lazic, G., 2019. Detection of Antibodies against Lumpy Skin Disease Virus by Virus Neutralization Test and ELISA Methods. no. March. https://doi.org/10.2478/acve-2019-0003.

Shankar, H., Yadav, M.P., 1988. Detection of Goat Pox Antigen and Antibody by the Counter Immunoelectrophoresis Test, pp. 109–113.

Tuppurainen, E., 2017. Diagnostic Assays for the Detection of Lumpy Skin Disease Virus and Antibodies. no. October.

Tuppurainen, E.S.M., et al., 2017. Review : Capripoxvirus Diseases : Current Status and Opportunities for Control, vol. 64, pp. 729–745. https://doi.org/10.1111/tbed.12444.

Varshovi, H.R., Norian, R., Azadmehr, A., Ahangaran, N.A., 2021. Immune response Characteristics of Capri Pox Virus Vaccines Following Emergency Vaccination of Cattle Against Lumpy Skin Disease Virus. https://doi.org/10.22067/veterinary.v9i2.65381.