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Tiergesundheit / Animal health **Applied Research and Development**

Differenzierung von Antikörpern gegen BVDV und BDV

Differentiation of antibodies to BVDV and BDV

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Key words
Border disease (BD), bovine viral diarrhea (BVD), cattle, eradication, serum neutralisation test (SNT), cross-neutralisation, species specificity, antibody differentiation, LIPS assay, diagnostics.

Aim of the study
The aim of the project was to develop a method that allows serum antibodies against BVDV and BDV to be differentiated more quickly than with the cross-serum neutralisation test (cross-SNT) currently in use. Faster differentiation of antibody-positive sera detected during the serological surveillance in the Swiss BVD eradication scheme might help to identify the source of infection as quickly as possible, which is especially important in the final phase of eradication and for maintaining a BVD-free status.

Material and methods
The newly developed method for the antibody (Ab) differentiation is based on a LIPS (luciferase immunoprecipitation system) assay [1]. The E2 proteins of the three virus strains that allow differentiation in the cross-SNT (BVDV-1a, BVDV-1h, BDV-8 [BDswiss]; representing the ruminant pestivirus species currently circulating in Switzerland) were used as antigens [2]. For that purpose, expression plasmids were constructed that encode for a fusion protein of the E2 envelope glycoproteins of these three pestivirus strains with a nanoluciferase. In order to achieve efficient protein expression in cell culture supernatants, the E2 membrane anchors were removed, and a secretion signal was added. An additional Twin-Strep-tag® was incorporated that allowed for affinity-purification of the chimeric proteins from the cell culture supernatants. Another version (v2) of the E2-luciferase fusion protein encoding for an additional dimerisation domain did not provide any advantage and, thus, was not further pursued.

In preliminary experiments, an amount of antigen providing 10^7 light units / 10µl and a serum dilution of 1:10 turned out to be optimal in the LIPS assay to quantify possible differences in their reactivity to the BVD or BD antigen. Samples displaying a signal (LU) higher than the mean of the blank plus five times its standard deviation were considered as being positive.

To devise a system to differentiate BVD- and BD-antibodies by the new LIPS assay, 52 sera were tested that were collected in our diagnostic division in 2021 to 2025. Thereof, 48 were previously tested Ab-positive in the NS3-ELISA. These Ab-positive sera were classified as BVDV-Ab positive (22 sera), BDV-Ab positive (25 sera) or indeterminate (1 serum) by cross-SNT. Each serum was tested in triplicates in the LIPS assay using all three antigens, i.e., BVDV-1a, BVDV-1h, and BDV-8, and the ratio of the mean of the sample signal to the mean of the blank was calculated per antigen. If the *relative* ratio (the ratio of the serum to a BVDV antigen divided with the ratio to the BDV-8 antigen) was above 1, the serum was assigned to contain BVD-Ab, whereas sera with relative ratios below 1 were classified BD-Ab containing samples.

[1] Burbelo, P.D., Goldman, R., and Mattson, T.L. (2005) A simplified immunoprecipitation method for quantitatively measuring antibody responses in clinical sera samples by using mammalian-produced Renilla luciferase-antigen fusion proteins. BMC Biotechnol 5, 22.
[2] Kaiser, V., Nebel, L., Schüpbach-Regula, G., Zanoni, R.G., and Schweizer, M. (2017) Influence of border disease virus (BDV) on serological surveillance within the bovine virus diarrhea (BVD) eradication program in Switzerland. BMC Vet Res 13, 21.

Results and significance

Western blot analyses demonstrated that monomeric and dimeric forms of the E2 antigen were present in purified and unpurified preparations, and upon addition of the luminescence substrate, both preparations emitted a strong signal (light units, LU) confirming the proper expression and folding of the luciferase domain of the chimeric proteins. As purification did not provide an advantage, the unpurified supernatants were further used to simplify the system.

All sera previously tested Ab-positive in the NS3-ELISA were also positive by the LIPS assay using the BD-antigen and either the BVD-1a or the BVD-1h antigen. Thus, two of the three antigens, i.e., the BD- and one of the BVD-antigens, were sufficient for all these samples to be determined as Ab-positive. All blanks were indeed negative, whereas one out of the four sera that were negative in the NS3-ELISA were clearly positive in the LIPS assay. The source of this difference is not known and requires further investigations.

By calculating the relative ratios between the results using the E2 antigen of BVDV-1a to E2 of BDV, 96% of all sera were assigned by the LIPS assay identically as they were classified by the LIPS assay. Thus, only one serum was assigned to BVD and one to BD in the LIPS assay, whereas they were inversely classified by cross-SNT. By contrast, classification by the LIPS assay using the E2 antigen of BVDV-1h to E2 of BDV was only 89% compliant with the data from the cross-SNT.

In summary, by testing 52 cattle sera, the newly designed LIPS assay based on the E2-antigens of BVD-1a, BVDV-1h and BDV-8 is able to detect sera for the presence of antibodies to ruminant pestiviruses (BVDV or BDV) that are congruent in 98% of the cases to the results of an NS3-based ELISA, whereby only one BVDV antigen would be sufficient next to the BDV-antigen. In addition, differentiation of the sera for antibodies to BVDV and to BDV could be achieved by using the E2 antigen of BVDV-1a and of BDV-8 in the LIPS assay, which provided results congruent with the cross-SNT in 96% of the sera tested. The reason for the few divergent results is not yet known. Thus, the new assay appears to be a useful tool for differentiation of antibodies to BVDV and BDV in cattle sera that is faster than the currently applied cross-SNT, and which does not require cell cultures. This might support the veterinary authorities in achieving BVD freedom in Switzerland and is particularly relevant in maintaining BVD freedom, as the Swiss cattle population is highly susceptible to re-infection by pestiviruses, while BDV remains endemic in the sheep population. However, for further validation of the method, more sera need to be analysed and compared to their results in the cross-SNT, including more sera that were tested negative or indeterminate in the NS3-ELISA and in the cross-SNT, respectively.
Notably, this LIPS assay might also be a useful tool to develop serological assays for other viral disease, as (i) it is very versatile and can be adapted to a variety of different antigens, (ii) expression plasmids can be chemically synthesized based on available nucleotide sequences, e.g., for newly emerging viruses, and (iii) as it might be used as surrogate assay for neutralisation tests (provided the corresponding antigens are known) even in the absence of infectious virus or a suitable cell culture model.

Publications, posters and presentations

Moser, K. (2024) Differentiation of BVDV & BDV Antibodies Using an E2 Luciferase Immuno-precipitation System (LIPS) Assay", 22nd Münchenwiler Meeting for Virology Students, Oct 31st 2024 (oral presentation).
Moser, K. (2025) Differenzierung von BVDV & BDV Antikörpern anhand eines E2 Luciferase Immunopräzipitation System (LIPS) Assay. 12. Schweizerische Tierärztetage, Basel April 24th 2025 (oral presentation).
Moser, K., Ezzat, S., Oberli, A., Stalder H.P., and Schweizer M., Serological Differentiation of BVDV and BDV Antibodies by an E2-based LIPS Assay (working title), manuscript pending.

Project: *1.24.02: Differentiation of antibodies to BVDV and BDV*

Project duration: *01.05.2024 – 31.08.2025*