Bivalent protection against BTV in sheep by combination of MVA viral vectors expressing proteins VP2 of BTV-4 and BTV-8

Luis Jiménez-Cabello[1,*], Juana Sánchez-Puig[2], Eva Calvo-Pinilla[1], Sergio Utrilla-Trigo[1], Julio Benavides[3], Rafael Blasco[2], Aitor Nogales[1] and Javier Ortego[1]

[1] Centro de Investigación en Sanidad Animal (CISA-INIA/CSIC), Valdeolmos, Madrid, Spain.
[2] Departamento de Biotecnología, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC), Ctra. de la Coruña km 7.5, 28040 Madrid, Spain.
[3] Instituto de Ganadería de Montaña (CSIC-Universidad de León), 24346 León, Spain.

SUMMARY

Available vaccines against BTV are effective tools to counteract BT but they confer serotype-specific protection and do not permit to stablish a DIVA strategy. Here, we present a novel DIVA vaccination strategy based on the widely used recombinant MVA vaccine vector. We engineered recombinant MVAs expressing proteins VP2 of serotype 4 or 8 and confirmed their potential to confer robust bivalent protection in natural BTV hosts. This new vaccination regime offer bivalent protection, solves safety concerns of classic vaccine approaches and allows to implement a DIVA strategy. Our work also offers future guidance for the design of novel vaccines against orbivirus regarding the formulation of the VP2 antigen.

GENERATION OF rMVAs

We generated a rMVA viral vector co-expressing proteins VP2 of BTV-4 and BTV-8 (MVA-$VP2_{A4}$-$VP2_{F8}$). We also designed rMVAs individually expressing proteins VP2 of BTV-4 (MVA-$VP2_{A4}$) and BTV-8 (MVA-$VP2_{A8}$). We confirmed the efficient expression of the BTV antigens cloned in the rMVAs by immunofluorescence assay (IFA) (Fig. 1)

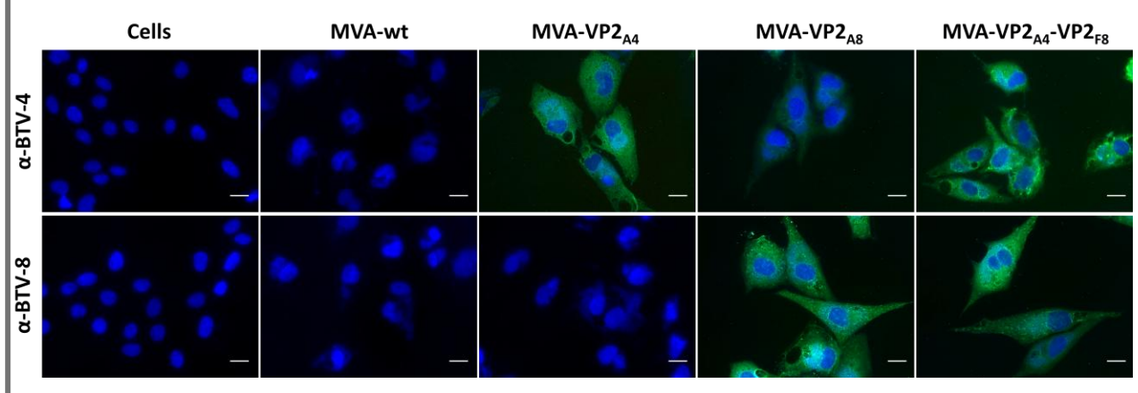

Figure 1. Expression analysis of heterologous BTV proteins by rMVA.

IMMUNOGENICITY OF rMVAs IN IFNAR(-/-) MICE

We evaluated the capacity of the rMVA viral vectors to induce a humoral neutralizing immune response against BTV in IFNAR(-/-) mice. Immunization with MVA-$VP2_{A4}$ or MVA-$VP2_{A8}$ induced high titers of homologous nAbs. We could not detect nAbs against either BTV-4 or BTV-8 after immunization with MVA-$VP2_{A4}$-$VP2_{F8}$. No immune interference occurred when the MVA-$VP2_{A4}$ and MVA-$VP2_{A8}$ were inoculated in different sites of injection (group MVA-$VP2_{A4}$/MVA-$VP2_{A8}$). nAbs titers were lower when these two rMVAs mixed in the same inoculum (group MVA-$VP2_{A4}$+MVA-$VP2_{A8}$).

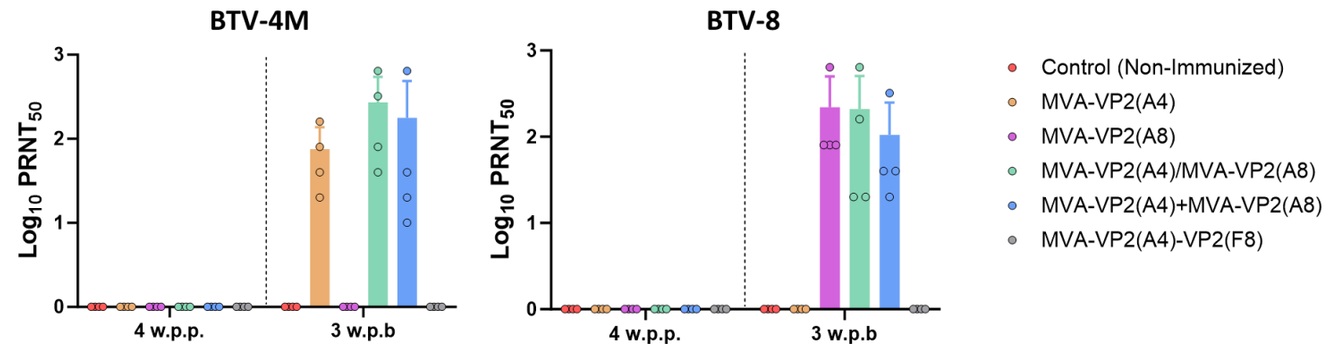

Figure 2. Neutralizing humoral immune response induced by the rMVAs.

PROTECTION AGAINST BTV

IFNAR (-/-) MICE

IFNAR(-/-) mice were immunized with two doses (separate inoculation) of 10^7 PFU of MVA-$VP2_{A4}$ and MVA-$VP2_{A8}$ and challenged with a lethal dose of BTV-4M (10 PFU) or BTV-8 (100 PFU). Control mice succumbed to BTV-4 and BTV-8 infection and displayed high levels of RNAemia. Immunization with the rMVAs conferred protection against BTV, preventing death and blocking BTV-4M and BTV-8 replication.

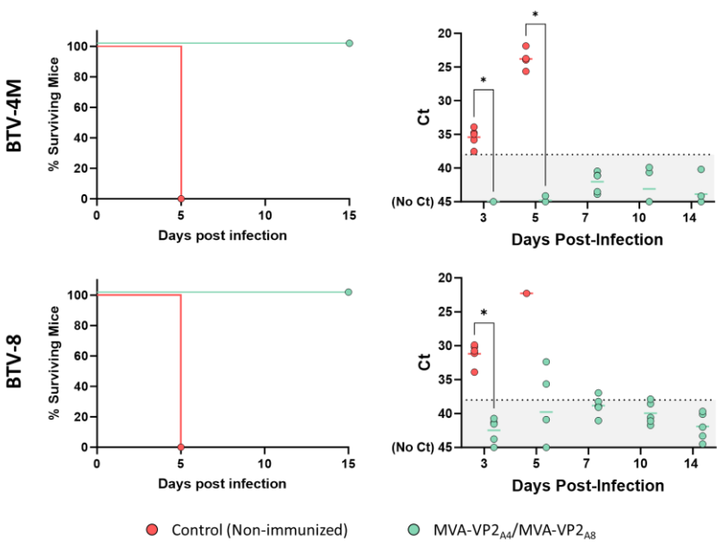

Figure 3. Protection of immunized IFNAR(-/-) mice against a lethal challenge with BTV-4M or BTV-8.

SHEEP

BTV-4M CHALLENGE

Sheep were immunized with two doses (separate inoculation) of 10^8 PFU of MVA-$VP2_{A4}$ and MVA-$VP2_{A8}$ and challenged with of BTV-4M (10^5 PFU). Control sheep developed increased rectal temperatures, detectable viremia and RNAemia levels, and lymphopenia and neutrophilia throughout the experiment. Immunized sheep presented steady rectal temperatures, attenuated lymphopenia and neutrophilia, and undetectable (Ct ≥ 38) levels of RNAemia and absence of infectious virus in blood after BTV-4M challenge.

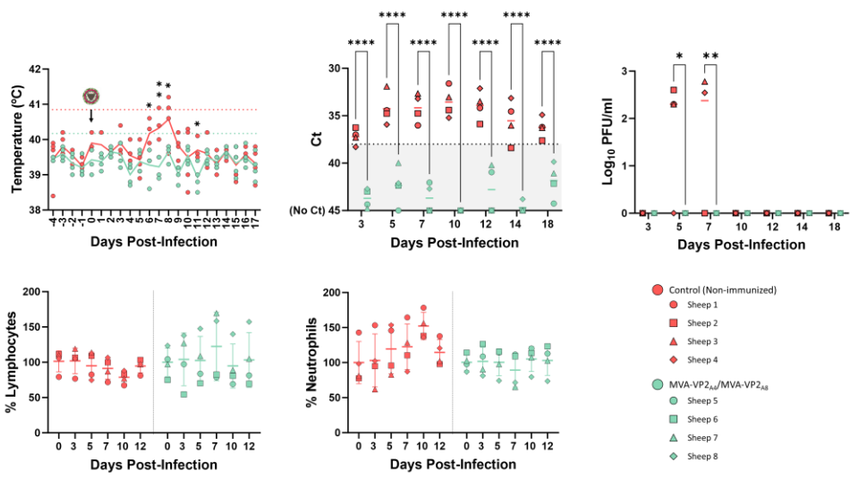

Figure 4. Protection of immunized sheep against a virulent challenge with BTV-4M.

BTV-8 CHALLENGE

Sheep were immunized with two doses (separate inoculation) of 10^8 PFU of MVA-$VP2_{A4}$ and MVA-$VP2_{A8}$ and challenged with of BTV-8 (10^5 PFU). Control sheep also developed increased rectal temperatures, detectable viremia and RNAemia levels, and lymphopenia and neutrophilia after challenge. Immunized sheep presented steady rectal temperatures, mild lymphopenia and neutrophilia, and undetectable (Ct ≥ 38) levels of RNAemia (except for day 5 post-infection) and absence of infectious virus in blood after BTV-8 challenge.

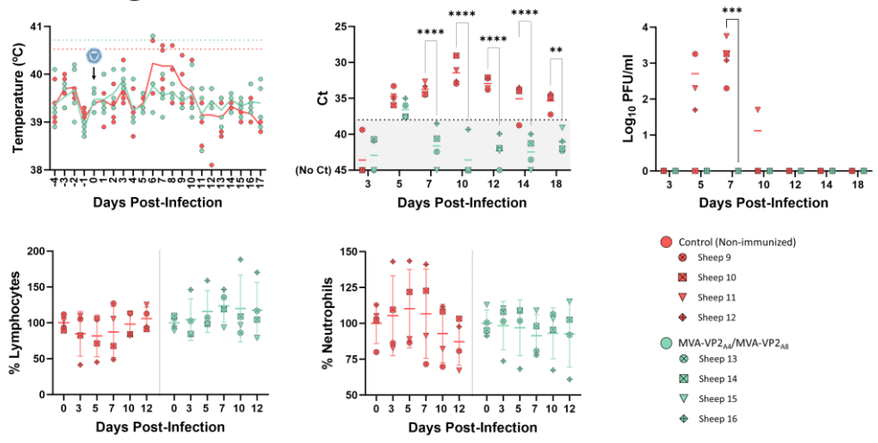

Figure 5. Protection of immunized sheep against a virulent challenge with BTV-8.

CONCLUSIONS & FUTURE PERSPECTIVES

We have developed recombinant MVA expressing proteins VP2 of serotype 4 and/or 8:

- Co-expression of proteins VP2 of serotype 4 and 8 by a single rMVA leads to immunological interference, impairing the antigenicity and protective potential of both BTV antigens.
- The combined immunization with rMVA expressing protein VP2 of serotype 4 and 8 constitutes an efficacious bivalente DIVA vaccination strategy against BTV.

The work was supported by grants PID2020-112992RR-I00, PID2023-147304OR-I00 and PRE2021 097320 funded by MCIN/AEI/10.13039/501100011033 and “ESF Investing in your future”,

MINISTERIO DE CIENCIA E INNOVACIÓN · AGENCIA ESTATAL DE INVESTIGACIÓN · CSIC Consejo Superior de Investigaciones Científicas · INIA Centro Nacional Instituto de Investigación y Tecnología Agraria y Alimentaria · CISA Centro de Investigación en Sanidad Animal · Instituto de Ganadería de Montaña igm · IOCVS 2025 Conference