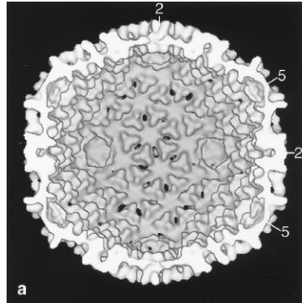


## Concept



**Infectious Bursal Disease Virus (IBDV) capsids are highly immunogenic**

Viral-like particles (VLP) based on the IBDV capsid may be engineered to allow the insertion of short amino acid sequences that will be expressed without affecting self-assembling.

Those VLPs may be produced in expression systems (yeasts, etc.)

# Concept

- ✓ For most **pestiviruses**, **protection** is mediated by neutralizing antibodies directed to the **E2 protein**
- ✓ The E2 neutralizing epitopes of BVDV are known
- ✓ Two BVDV genotypes are recognized (BVDV-1 and BVDV-2) several subgenotypes are included in each genotype
- ✓ The neutralizing epitopes in E2 are partially conserved although genetic diversity may affect cross-neutralization

By means of a bioinformatic analysis of available E2 sequences (Genbank), a set of potentially cross-reactive peptides with expected capability for inducing neutralizing antibodies were synthesized

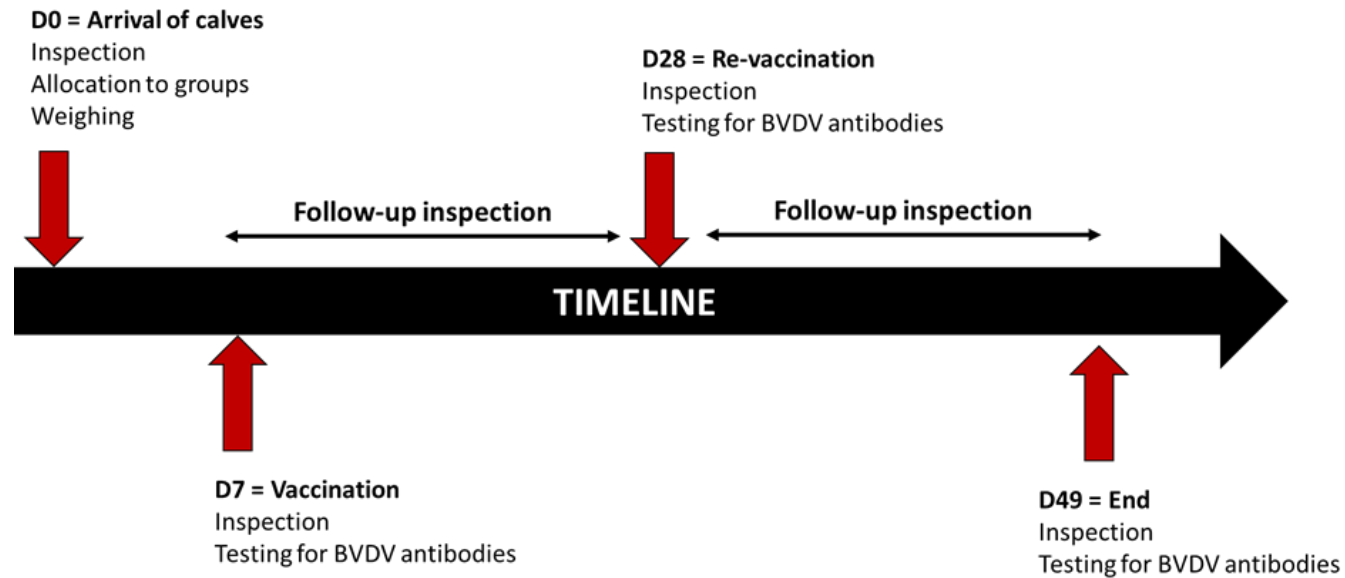
# Concept

- ✓ Peptides were tested against sera of BVDV-positive and negative animals in a peptide ELISA
- ✓ They were tested as well in a competition ELISA to assess whether depletion of peptide-specific antibodies reduced reactivity with BVDV virus

**Finally, two peptides P99 and P35 were identified as the most reactive with sera of BVDV-positive animals**

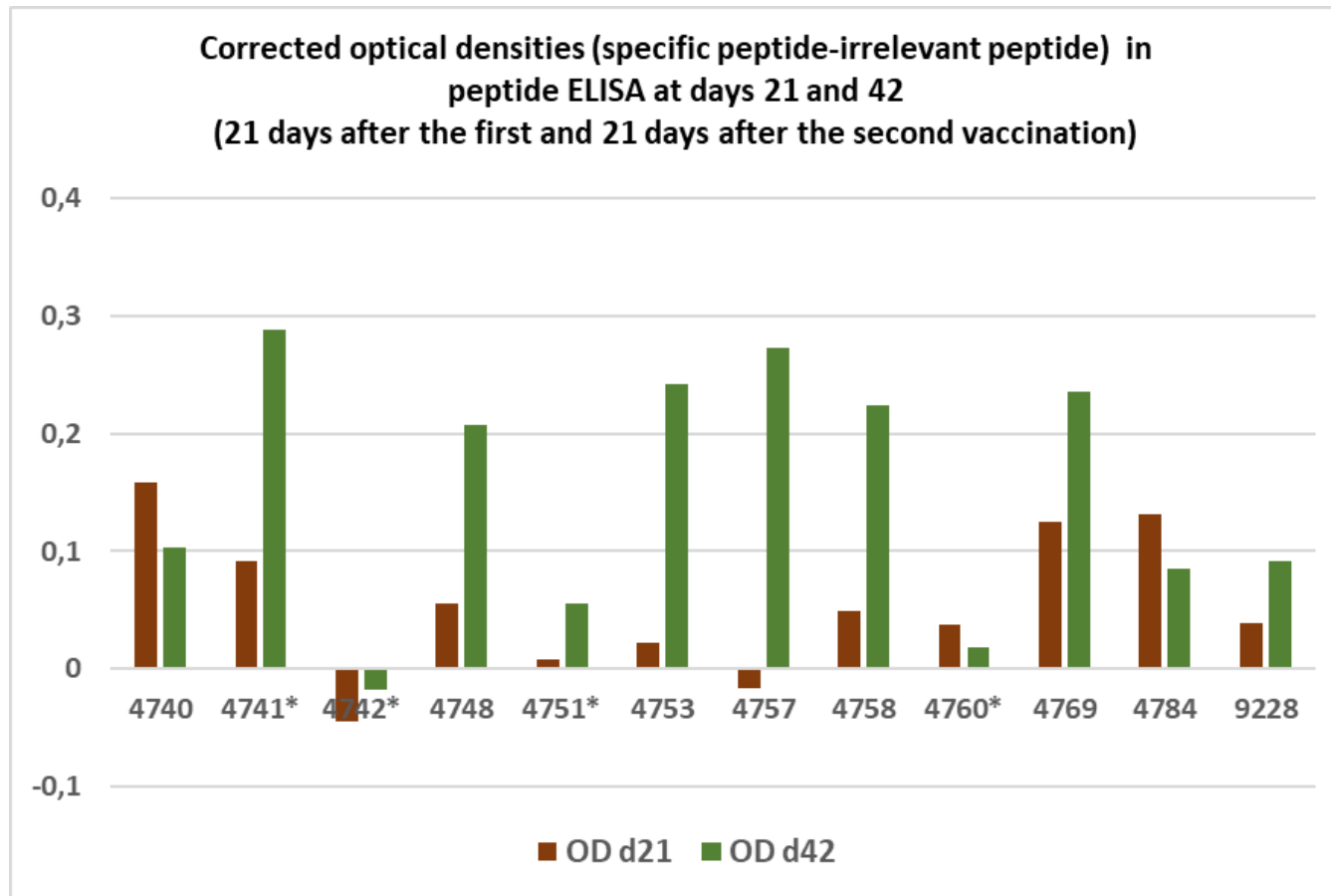
# Background

In a preliminary experiment, the immunogenicity and safety of the VLP-peptide constructions was tested in BVDV-free calves



# Background

The VLP-construct produced a **seroconversion against the BVDV-peptides** and **was safe** although the strength of such response was limited



# Background

A **new VLP-production process** was developed to increase the concentration of the VLP.

**The product is now re-tested on calves**

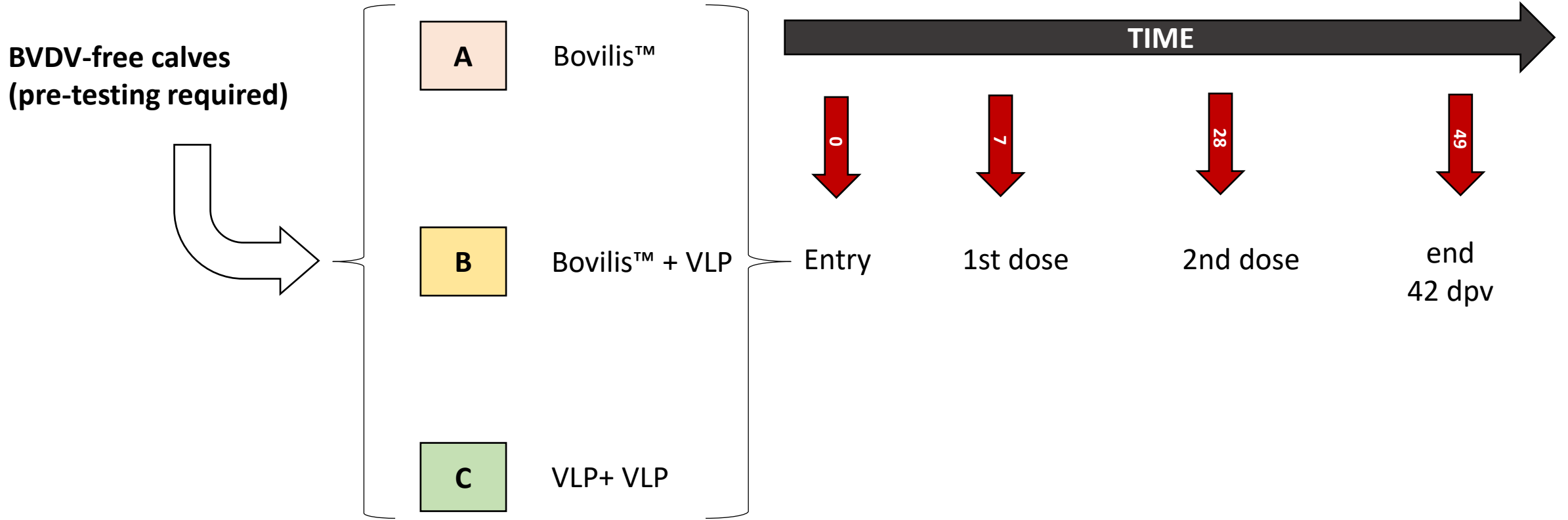


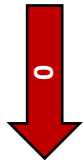
Universitat Autònoma  
de Barcelona



VLP-BVD  
AQUILON

# Experimental design





Entry



1st dose

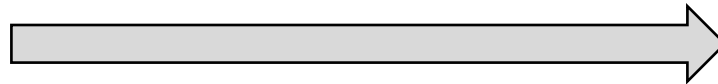


2nd dose



end  
42 dpv

**Bleeding**



- **Total BVD antibodies**
- **Anti-p80 antibodies**
- **P99 and P35v antibodies**
- **Anti-IBDV VP2 antibodies**
- **Neutralizing antibodies**



# Development of the experiment



Frisian x Aberdeen Angus crossbred calves were purchased for the study

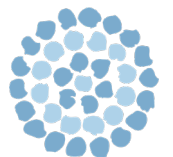
Animals were pre-tested for p80 BVDV-antibodies and BVDV-antigen (capture ELISA)

At the beginning of the study, the average weight was  $380 \pm 38$  Kg

## INMUNOLOGÍA BOVINA

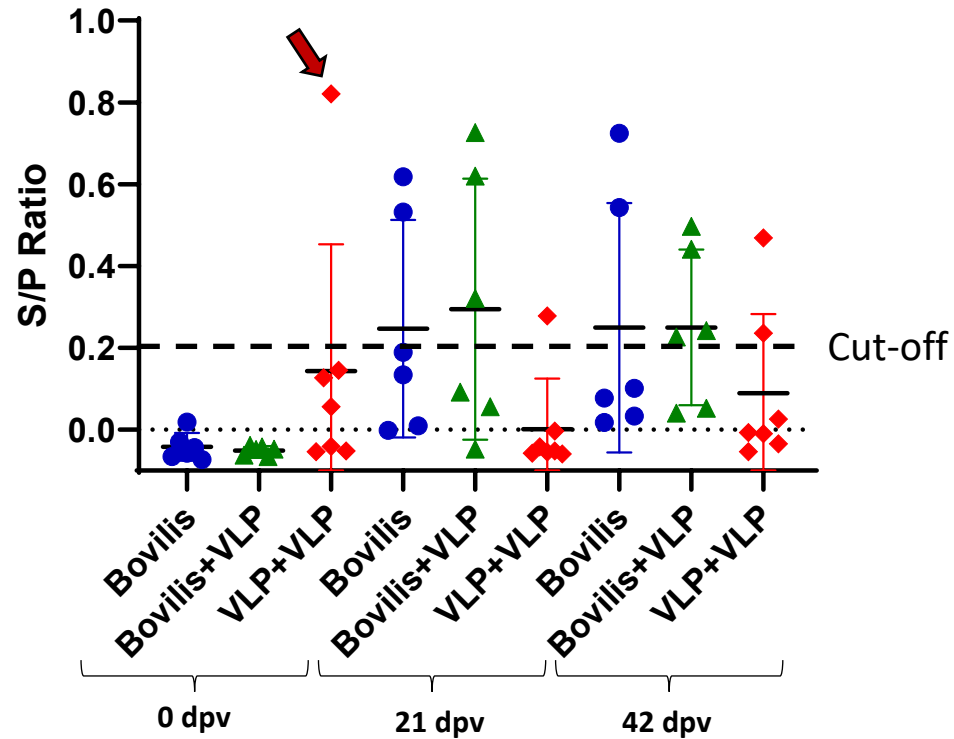
	REFERENCIA MUESTRA	BVD Ag	BVD p80
1	0682	-	-
2	0636	-	-
3	0634	-	-
4	0674	-	-
5	0677	-	-
6	0675	-	-
7	0689	-	-
8	0634	-	-
9	0628	-	-
10	0686	-	-
11	0639	-	-
12	0664	-	-
13	0645	-	-
14	0623	-	-
15	0629	-	-
16	0659	-	-
17	0697	-	-
18	0665	-	-

**All animals were negative for p80 antibodies and for BVDV antigen** as expected because of the BVDV-free status of the source farm





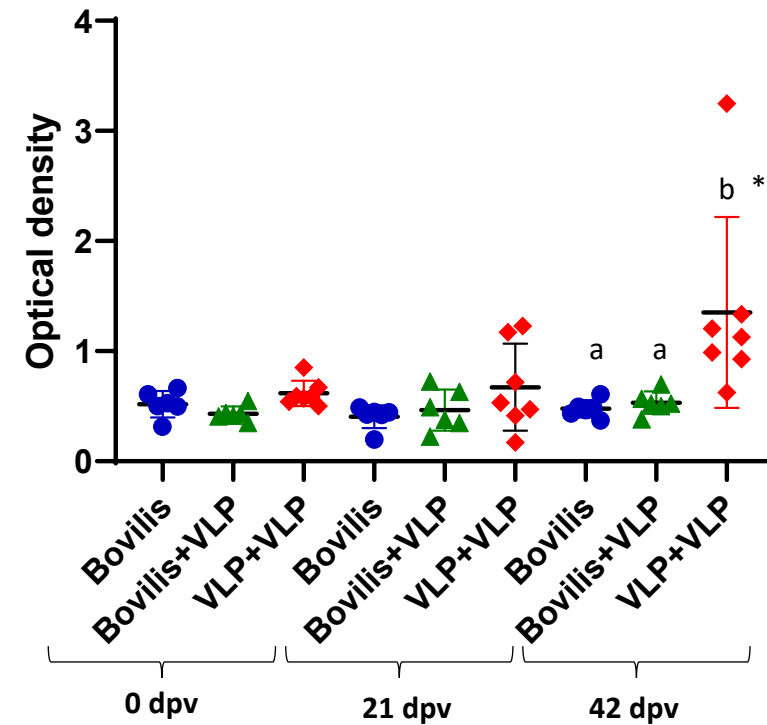
### Total BVDV antibodies



➔ This animal tested negative 5 days before.

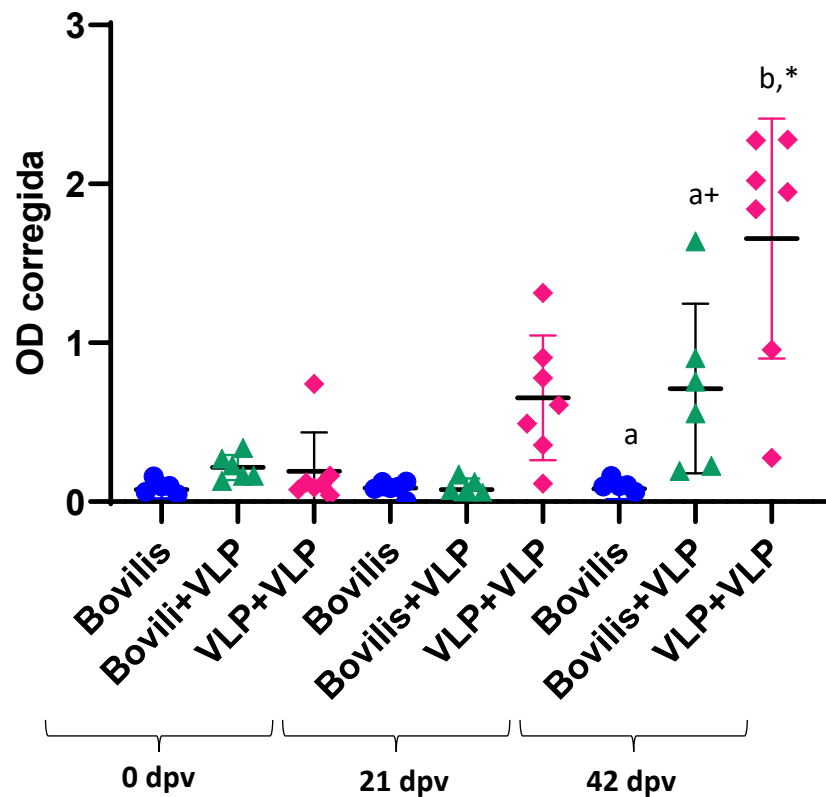
Non-significant differences between groups

### VP2 antibodies

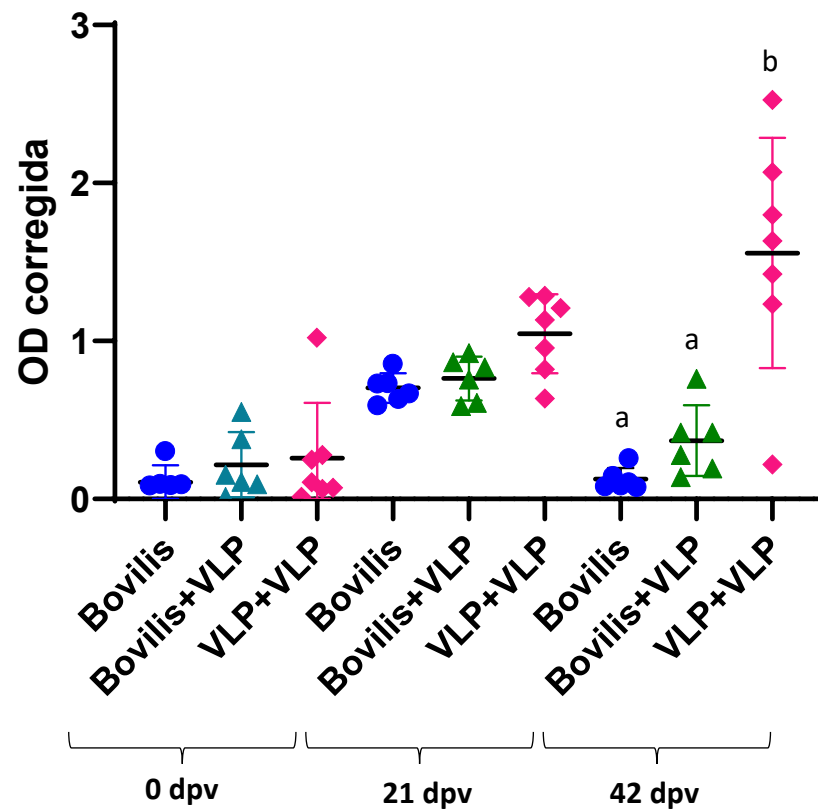


VP2 OD increased significantly in the VLP+VLP group at 42dpv

### Péptido 99



### Péptido 35



# Conclusions

Peptide 99 (BVDV-1b) was only recognized by VLP-vaccinated animals, indicating the specificity of this antigen

Peptide 35 (BVDV-1a) was recognized by animals vaccinated with VLP and with Bovilis+VLP (as expected since Bovilis is BVDV1a)

The insertion of BVDV epitopes was efficient to induce specific antibodies

Analysis of neutralizing antibodies is ongoing