

Host Defense Peptides-based nanoparticles for the treatment of Bovine Respiratory Disease

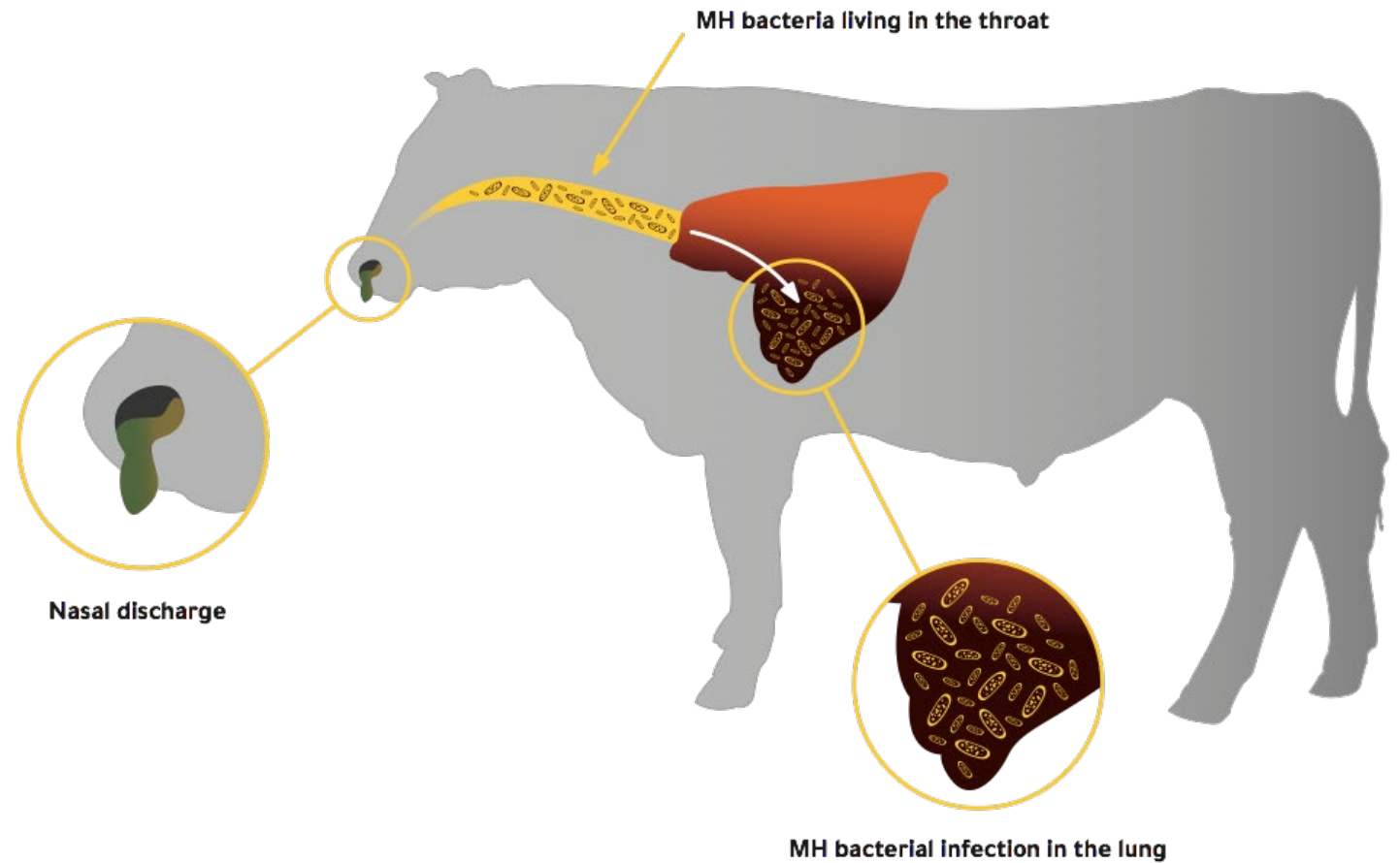
R. Balta-Foix, J. Seras-Franzoso, F. Andrade, D. Rafael, J. Atienza, N. Ferrer-Miralles, I. Abasolo, S. Martí, E. Garcia-Fruitós and **A. Arís**

Bacterial pathogens:

- *Pasteurella multocida*
- *Mannheimia haemolytica*
- *Histophilus somni*
- *Mycoplasma bovis*

Viral pathogens:

- Bovine herpesvirus type 1 (BHV-1)
- Parainfluenza-3 virus (PI3),
- Bovine viral diarrhea virus (BVDV)



GOAL

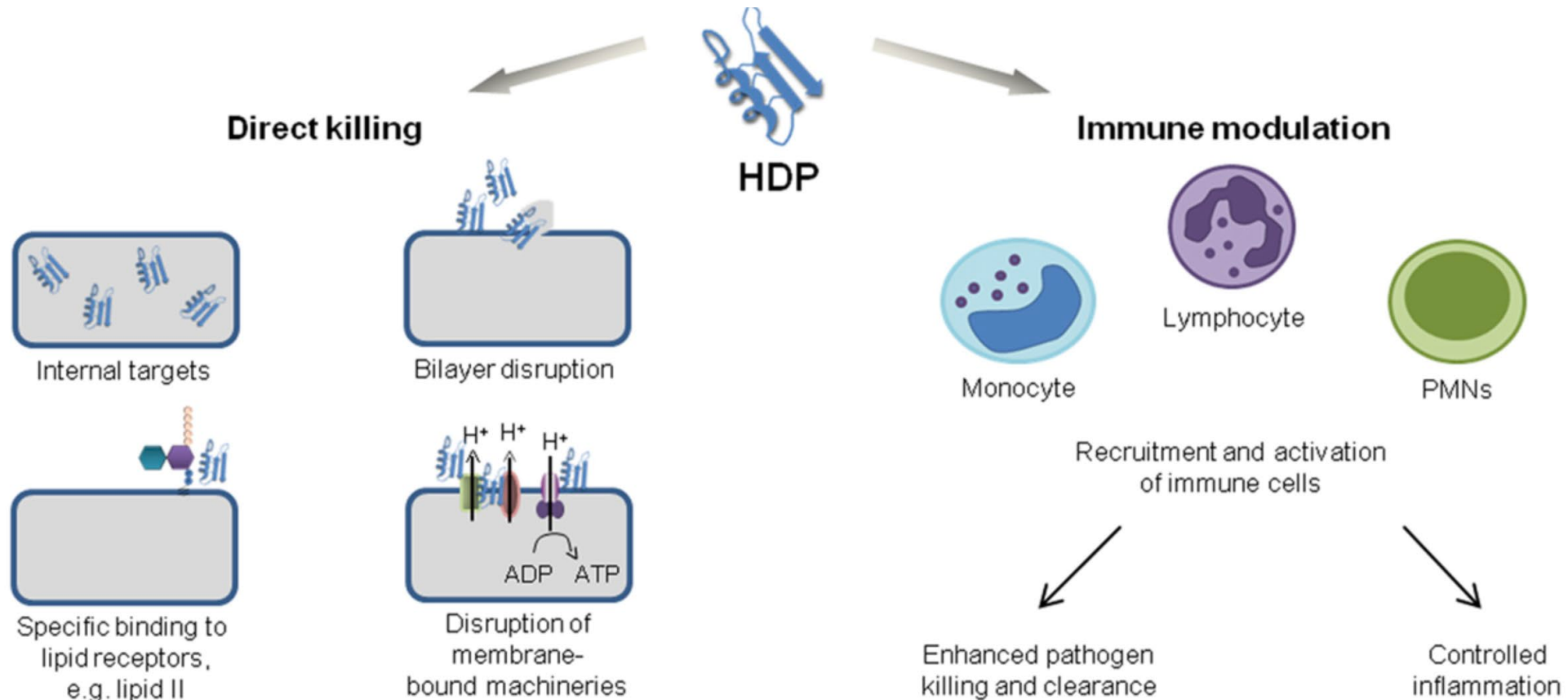
“ To find new drugs to treat bacteria and virus involved in Bovine Respiratory Disease (BRD) ”



STRATEGY

“ Use of **Host Defense Peptides** from the Innate Immunity to produce recombinant proteins with broad-spectrum antimicrobial and antiviral function ”

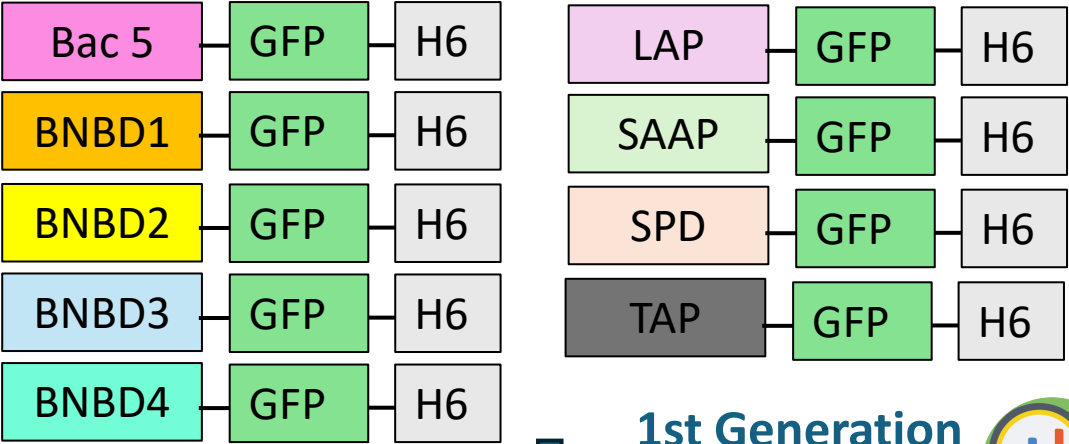




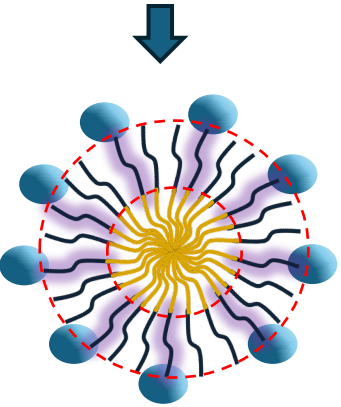
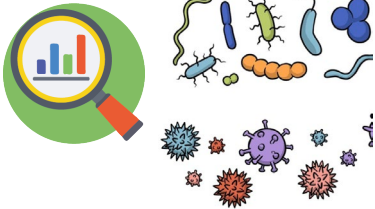
STRATEGY

Table 3.1. Host Defense Peptides used in the study.

Peptide name	Abbreviation	Category
Bactenecin 5	Bac5	Cathelicidin (HDP)
Bovine β -defensin 1	BNBD-1	β -defensin (HDP)
Bovine β -defensin 2	BNBD-2	β -defensin (HDP)
Bovine β -defensin 3	BNBD-3	β -defensin (HDP)
Bovine β -defensin 4	BNBD-4	β -defensin (HDP)
Lingual antimicrobial peptide	LAP	β -defensin (HDP)
Surfactant-associated anionic peptide	SAAP	Surfactant-associated peptide
Surfactant protein D	SPD	Surfactant protein
Tracheal antimicrobial peptide	TAP	β -defensin (HDP)



1st Generation molecules

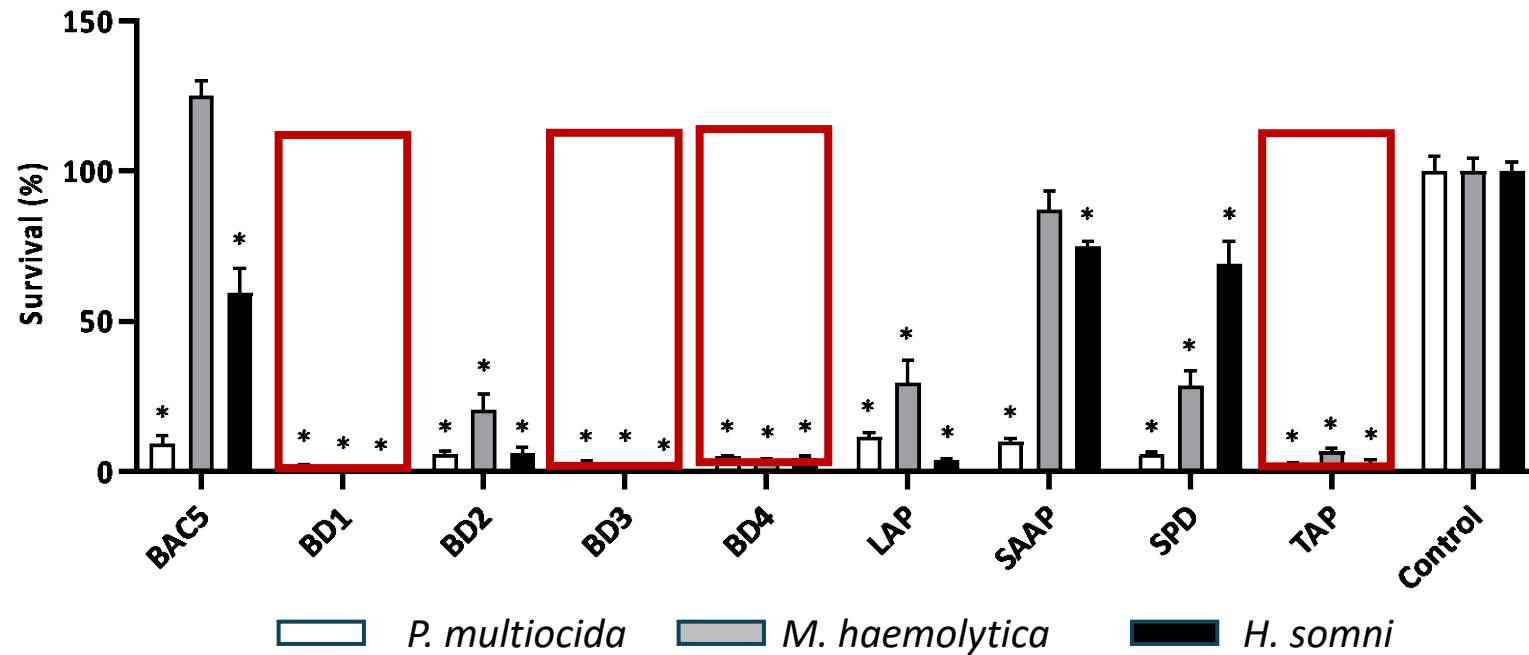
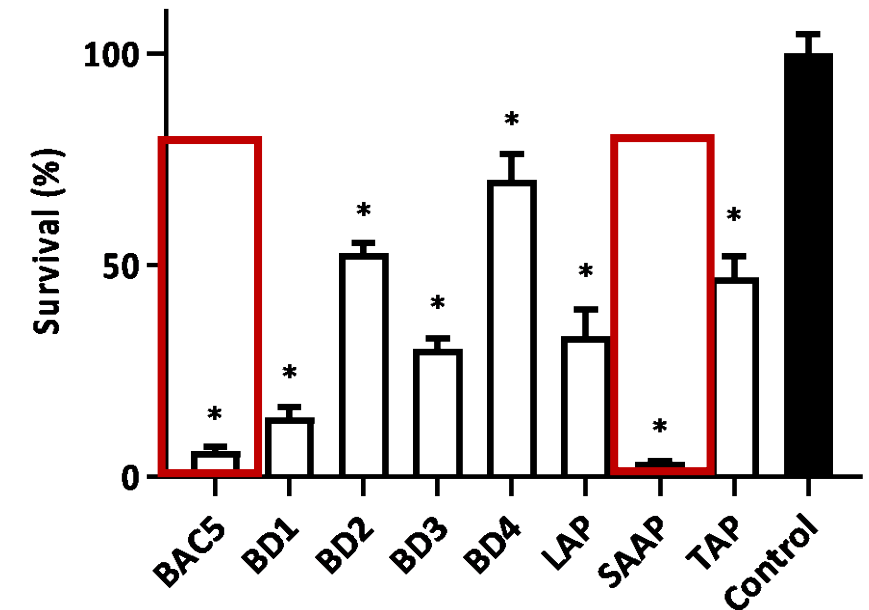


Nanoparticles



- ① BACTERICIDAL ACTIVITY: BNBD1, BNBD3 and BNBD4, TAP kill 3 gram-negative bacteria and BAC5 and SAAP kill *M. bovis*

Gram-negative bacteria

*Mycoplasma bovis*

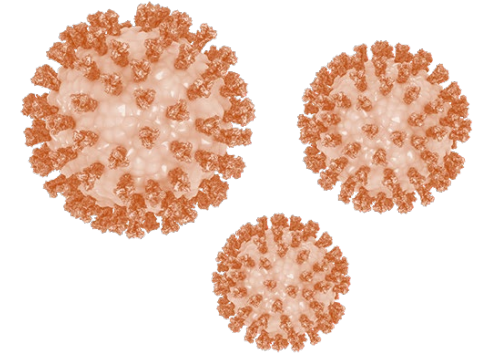
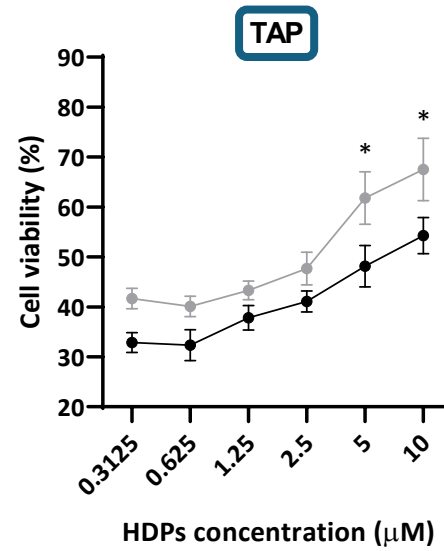
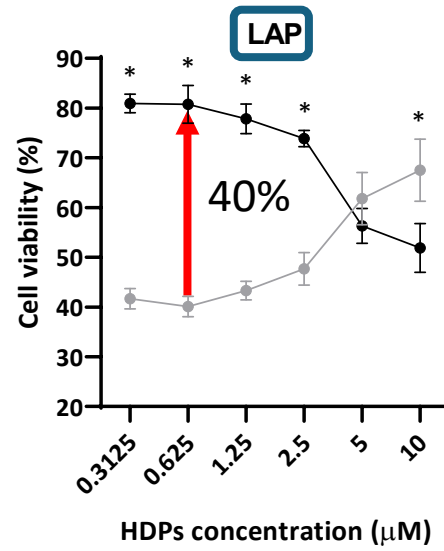
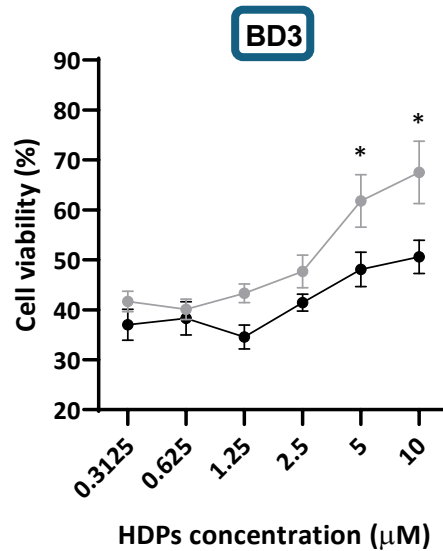
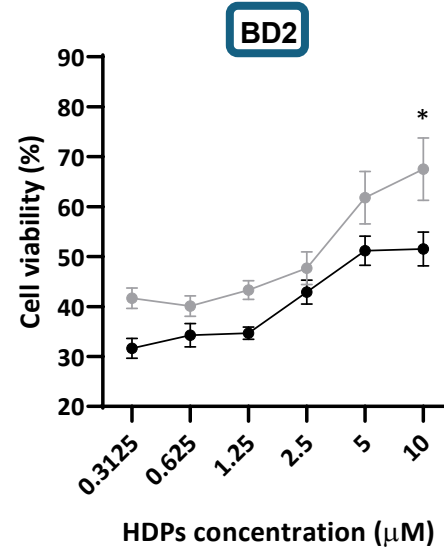
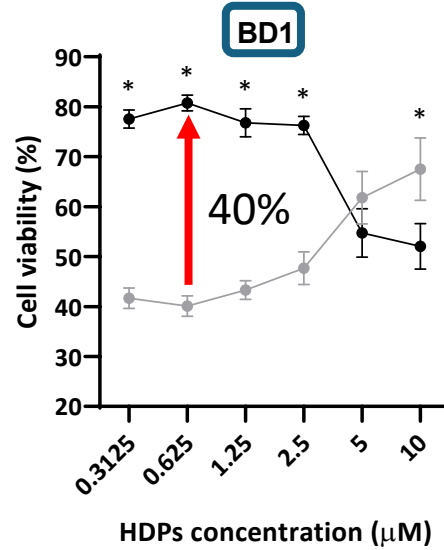
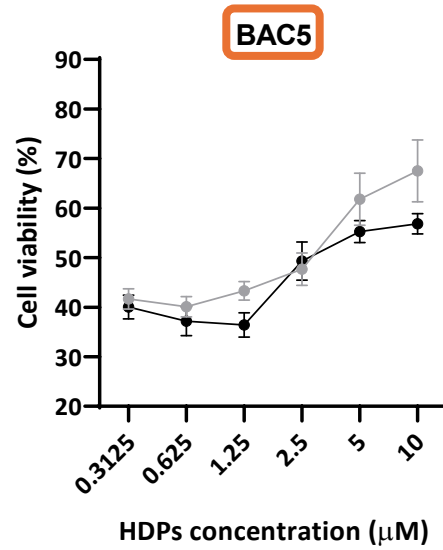
② BACTERIOSTATIC ACTIVITY (MIC):

BNBD1 and BNBD3 presented good performance for 2 gram-negative bacteria

Gram-negative bacteria

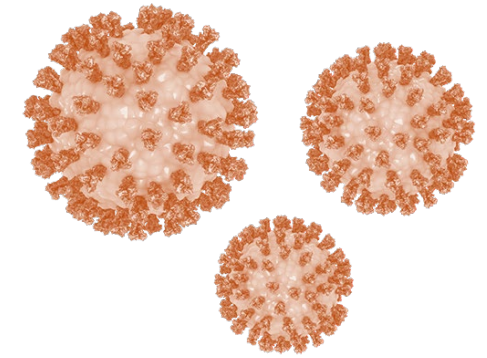
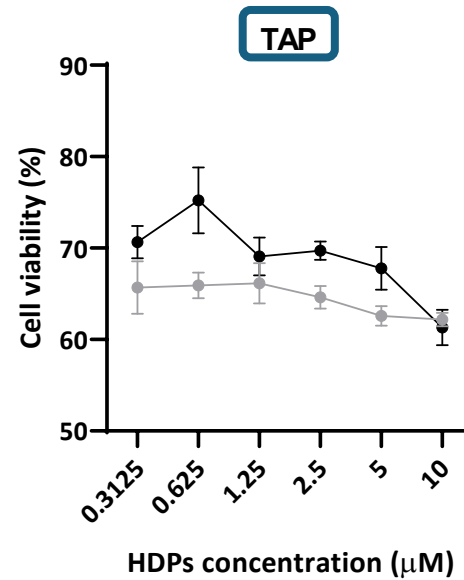
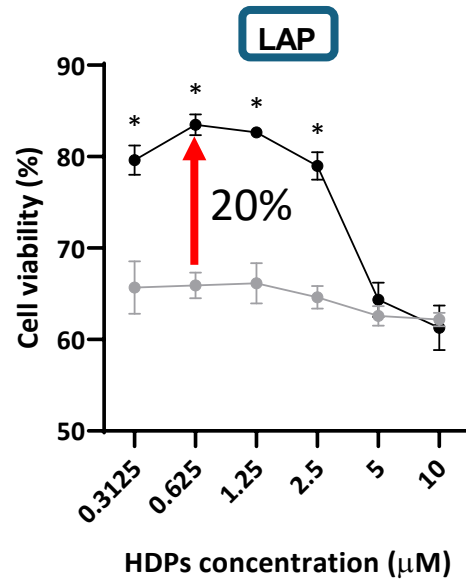
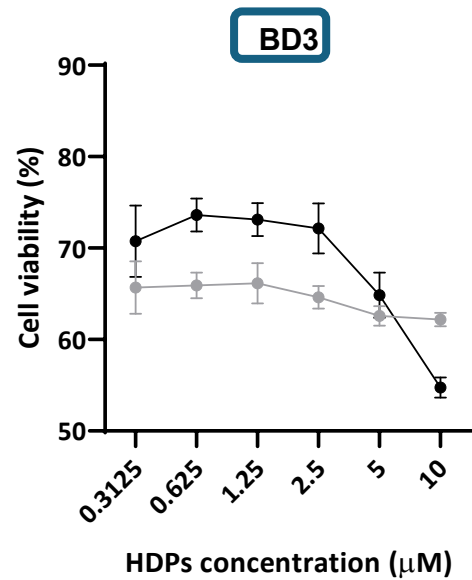
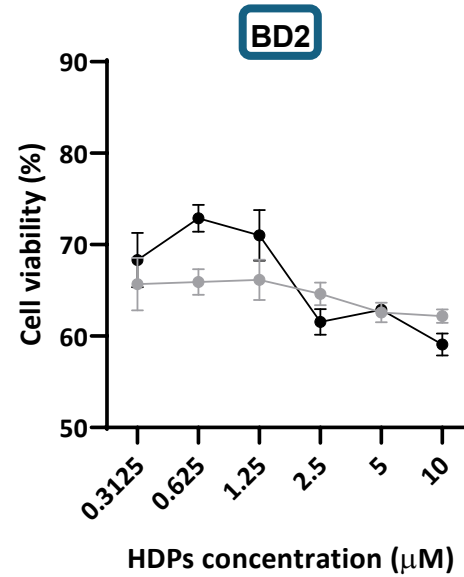
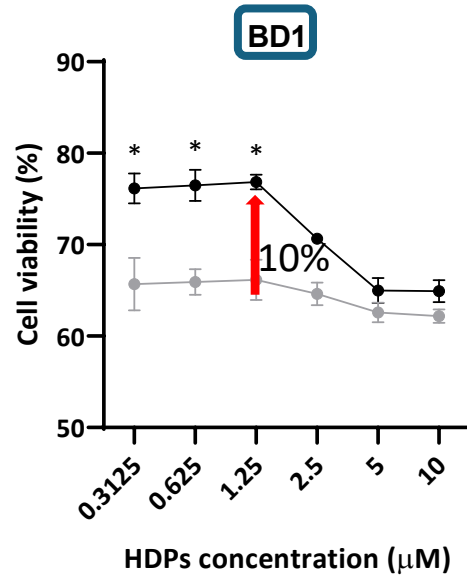
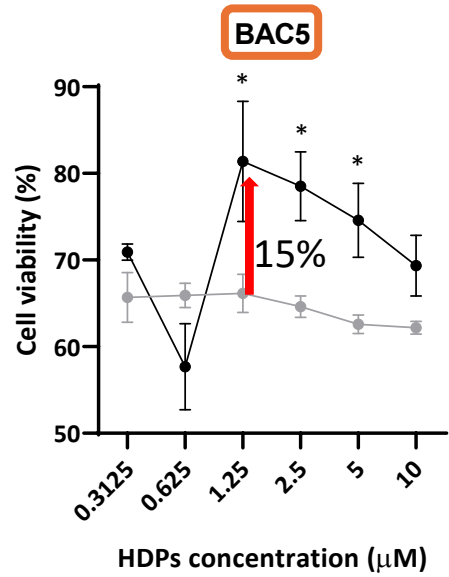
	<i>Pasteurella multocida</i>		<i>Mannheimia haemolytica</i>		<i>Histophilus somni</i>	
	µg/ml	µM	µg/ml	µM	µg/ml	µM
BNBD-1	128	3.93	64	1.97	>256	>7.87
BNBD-2	>256	>7.78	>256	>7.78	>256	>7.78
BNBD-3	64	1.93	64	1.93	>256	>7.73
BNBD-4	128	3.87	>256	>7.75	>256	>7.75
LAP	>256	>7.82	>256	>7.82	>256	>7.82
TAP	>256	>7.90	128	3.95	>256	>7.90

③ ANTIVIRAL EXPERIMENTS AGAINST BHV-1



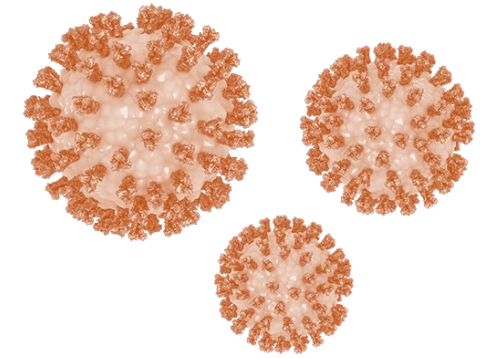
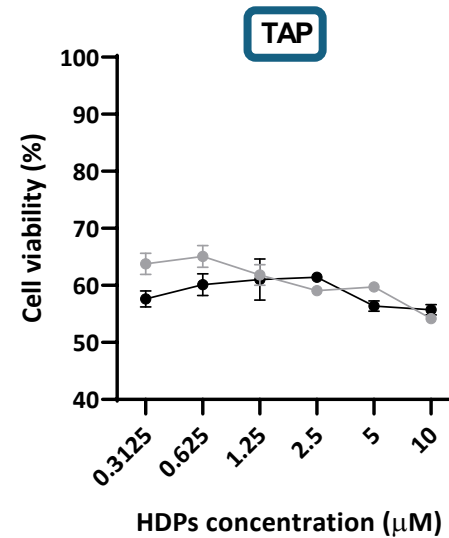
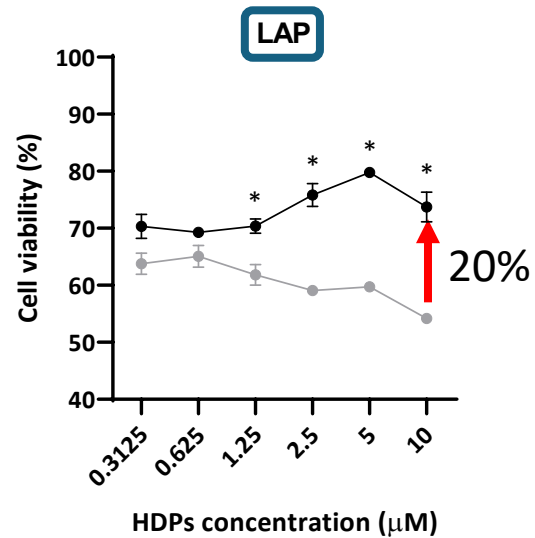
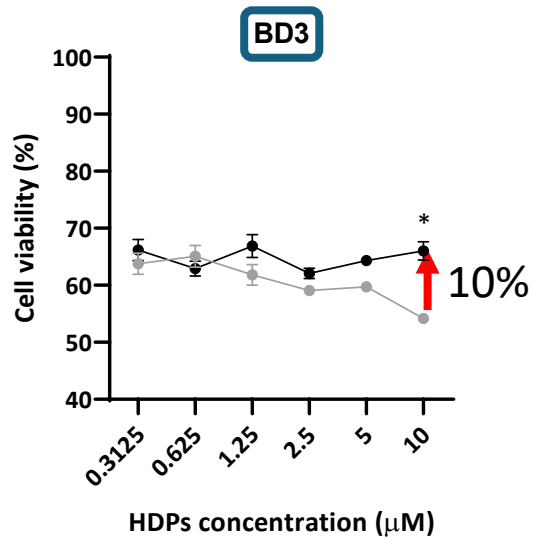
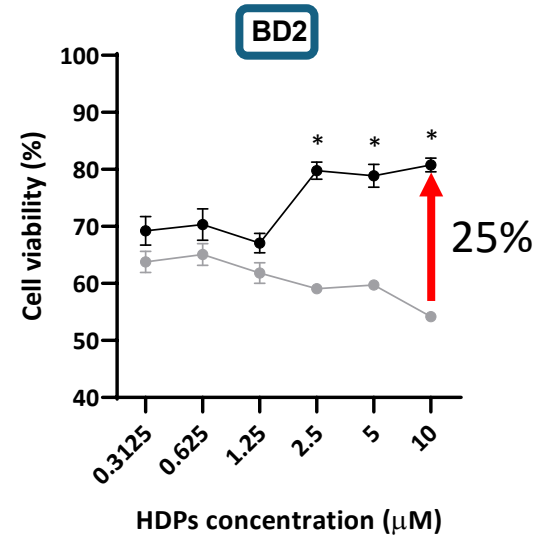
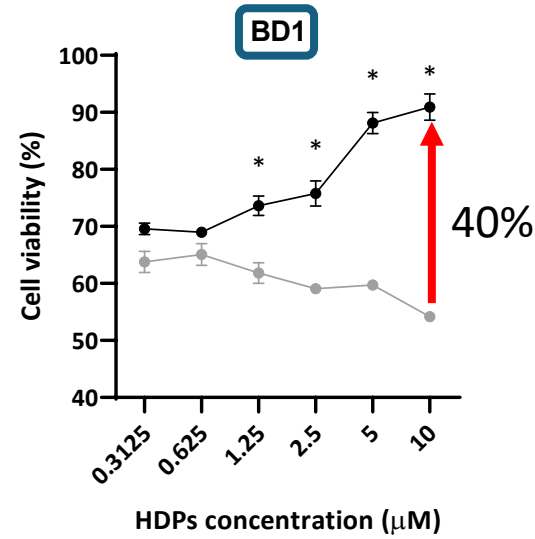
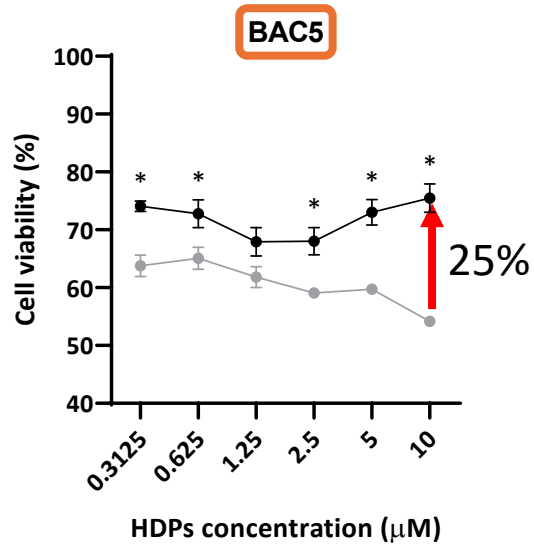
Bovine herpesvirus type 1 (BHV-1)

③ ANTIVIRAL EXPERIMENTS AGAINST PIV-3:



Parainfluenza-3 virus (PI3)

③ ANTIVIRAL EXPERIMENTS AGAINST BVDV

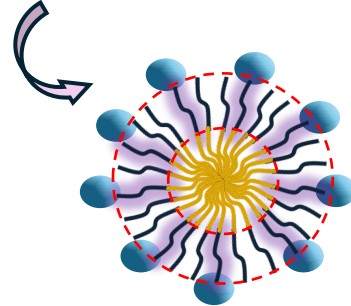


Bovine viral diarrhea virus (BVDV)

Selection of best
1st generation molecules



Pluronic® F127



Nanoparticles



IN VIVO EXPERIMENT

BAC5

Activity against *M. bovis*, BHV-1 and PIV-3

BD1

Activity against bacteria and viruses

BD3

Activity against gram-negative bacteria
and BVDV

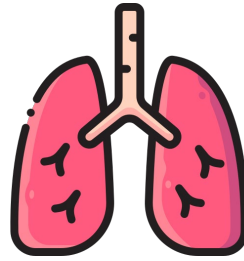
LAP

Activity against gram-negative bacteria
and viruses PIV-3 i BVDV



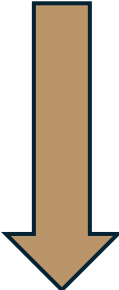


42 Crossbreed
unweaned calves



Pneumonia monitorization
by Ultrasonography Score
(TUS)



TUS 1		Healthy Animal
TUS 2		
TUS 3		
TUS 4		Sick Animal

Evolution of animals at TUS 2 for 1 week

n=21 Control Calves (Saline Solution)
n=21 Treatment Calves (Nanoparticles)

Qualitative variable
distribution and analyzed
by LR-Chisquare.

Day 1:

TUS Score (=2)



Health Score

Naso-pharyngeal swab → Metagenomic studies



Blood samples

Intranasal Treatment

Day 6:

TUS Score

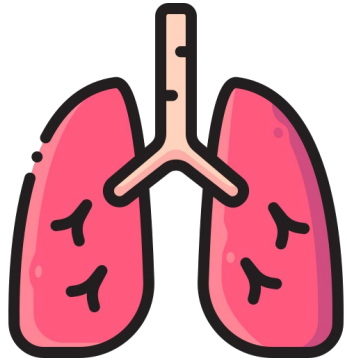


Health Score

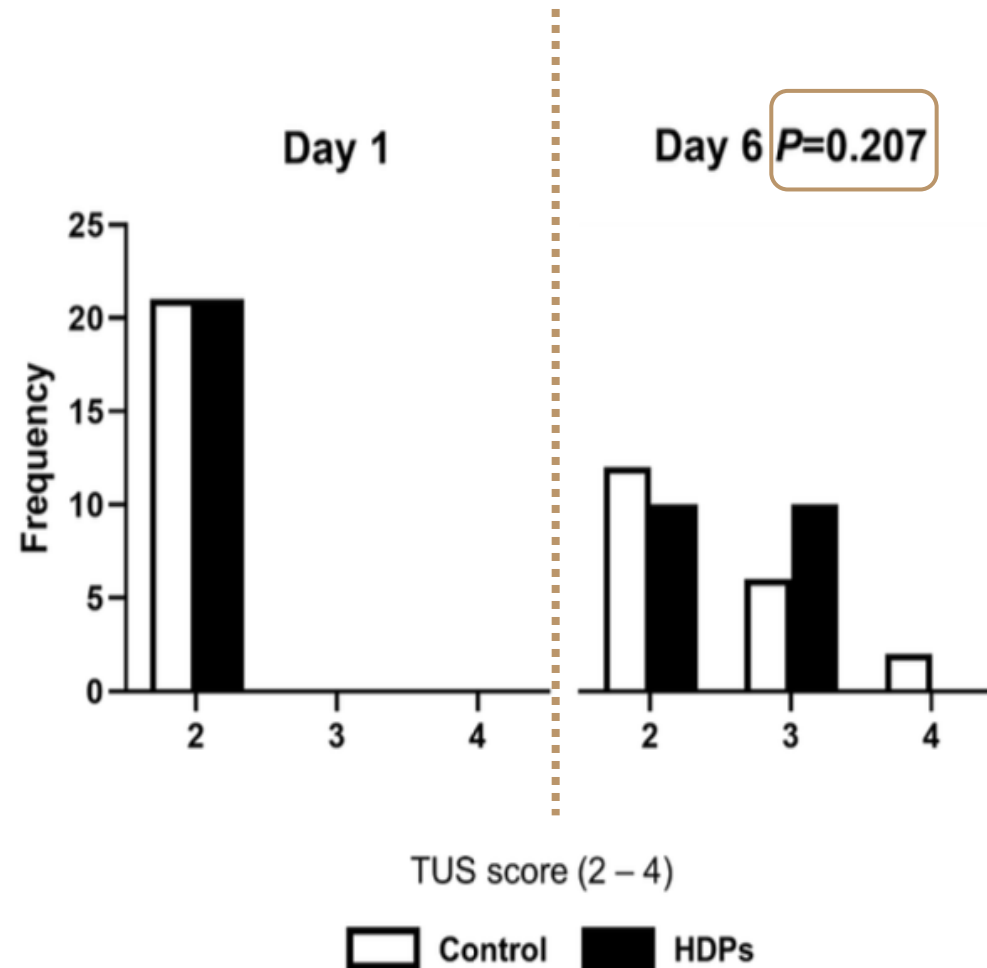
Naso-pharyngeal swab → Metagenomic studies



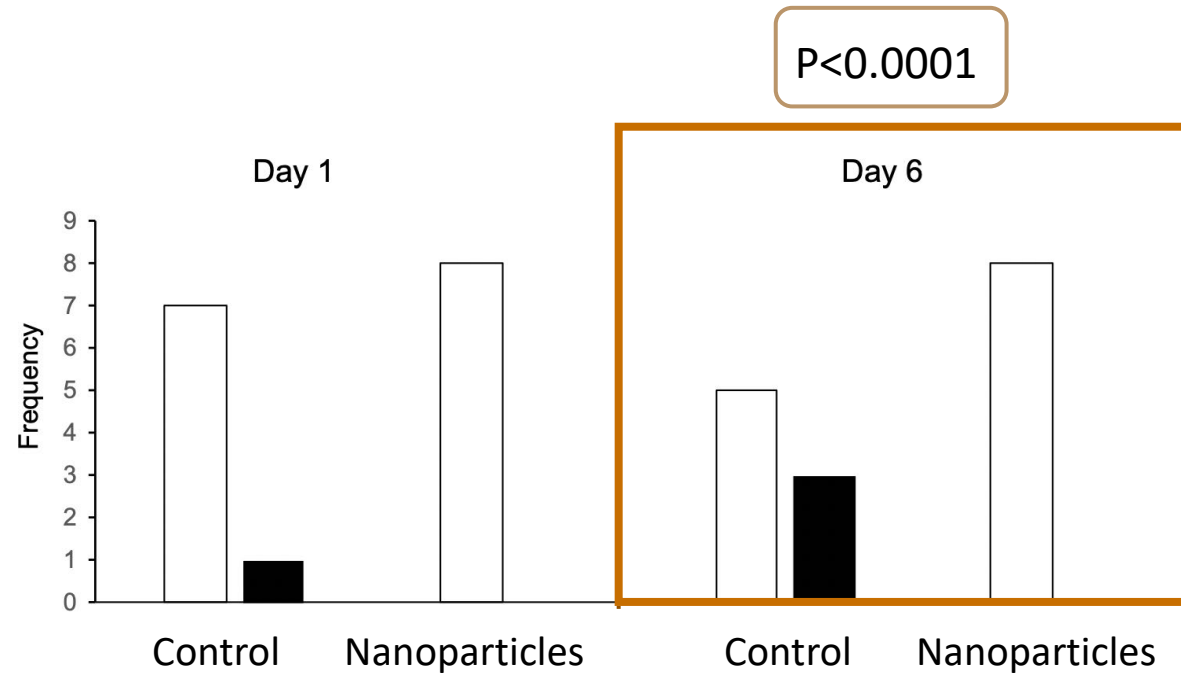
Blood samples



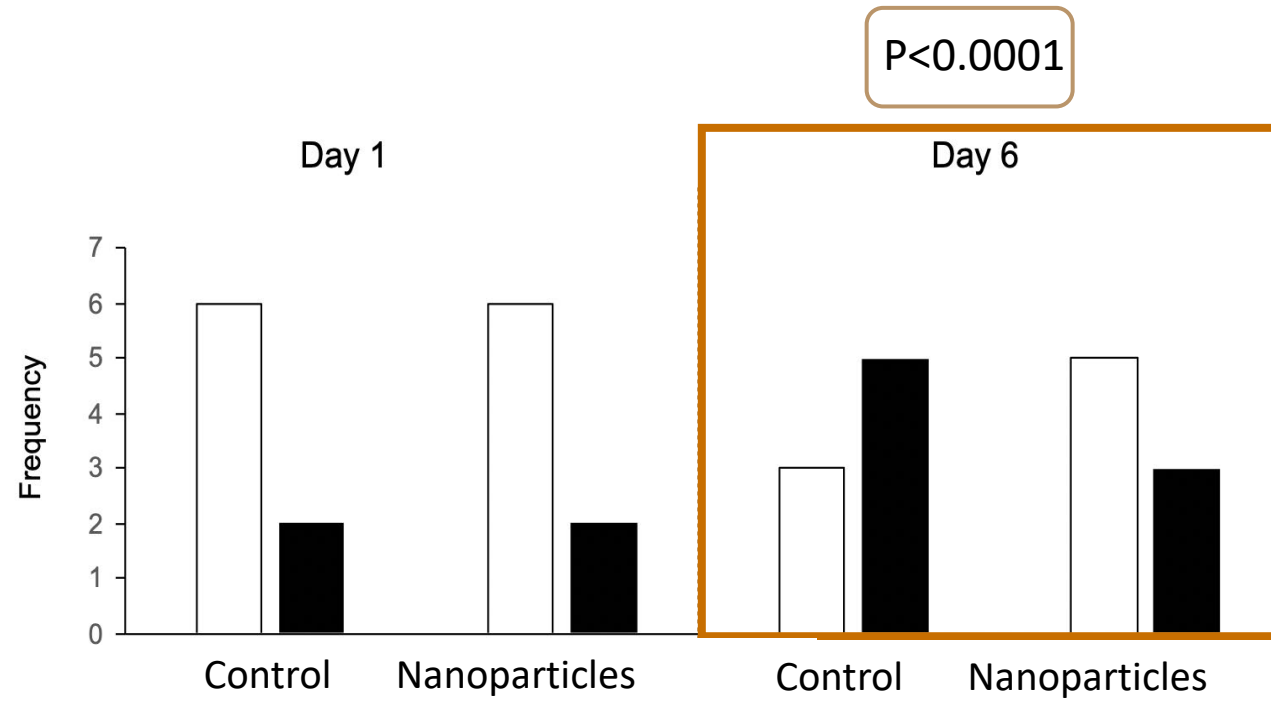
Evolution of Pulmonar Ultrasonography



Evolution of Pathogens (*M.bovis*)



Evolution of Pathogens (*P.multocida*)



-
- ✓ In vitro analyses demonstrate that HDPs could control bacterial and viral pathogens involved in BRD.
 - ✓ Preliminar results of in vivo experiment indicates promising potential of HDP-intranasal nanoparticles therapy to control the presence of *M. bovis* and *P. multocida* in calves

Ruminant Production



Institute
of Agrifood Research
and Technology

