31 WBC
WORLD BUIATRICS CONGRESS

SEPTEMBER 4TH TO 8TH
MADRID 2022

A synergy between influenza D virus (IDV) and *Mycoplasma bovis* in bovine respiratory disease (BRD)

Lion A, Rancon C, Secula A, Cassard H, Gaudino M, Ducatez M, Meyer G

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ECBHM
European College of Bovine Health Management

Bovine Respiratory Viruses

- Well known viruses

BRSV, BoHV-1, BVDV, BPI3, BCoV…..

- New technologies (NGS): identification of new viruses or better characterization of little known viruses (Hause *et al.*, 2011; Ng *et al.*, 2015; Mitra *et al.*, 2016, Elias *et al.*, 2019 ….)

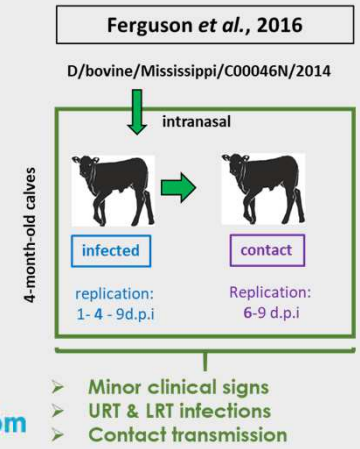
Bovine rhinoviruses A and B,
Bovine astrovirus,
Bovine adenovirus 3,
Influenza D virus……

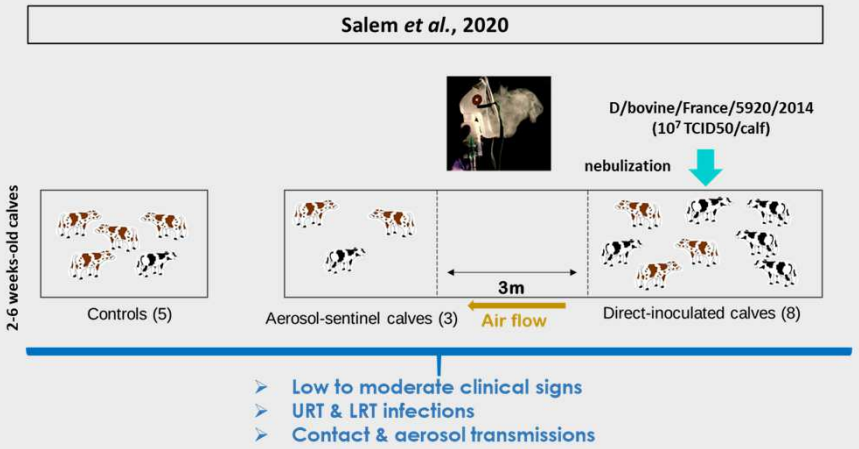
Involvement in respiratory diseases ?

Is IDV involved in BRD ?

- Statistical associations between the IDV detection in respiratory samples and BRD in calves (Ng *et al.*, 2015; Hause *et al.*, 2014)

- Experimental infections (Ferguson *et al.*, 2016; Salem *et al.*, 2019)
  - Both URT and LRT tropism
  - Local inflammatory response with infiltration of neutrophils and mononuclear cells
  - **Low to moderate pathogenicity**
  - Contamination by contact or aerosols (short distance)





Objective : is IDV a co-pathogen in BRD ?

- High frequency of coinfections in BRD (Gaudino *et al.*, 2022, In press Veterinary Sciences)
- Frequent association of IDV and *Mycoplasma bovis* in veal calves (France)

Experimental coinfection by nebulization of naïve calves (3-6 weeks old) with *M. bovis* and/or IDV

Control calves	IDV group	M. bovis group	Coinfected IDV+M.bovis group
DMEM, 5 calves	10^7 $TCID_{50}$/calf, 8 calves (3 euthanized at Day 6) D/bovine/ France /5920/2014	10^{10}CFU/calf, 8 calves (3 euthanized at Day 6) RM69 strain	IDV (10^7 $TCID_{50}$/calf) + M.bovis (10^{10}CFU/calf), 8 calves (3 euthanized at Day 6)

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Summary of the main results

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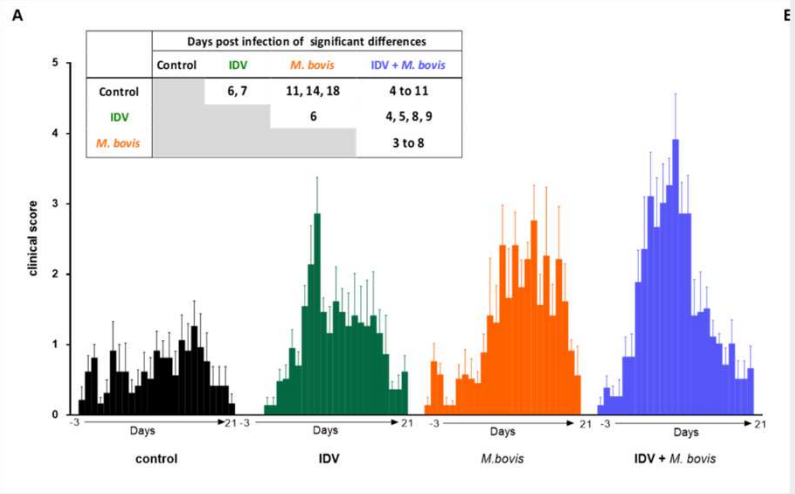
1. Coinfection shortens the time to appearance of clinical signs and increases their severity



2. macroscopic and microscopic respiratory lesions were more severe in coinfected calves

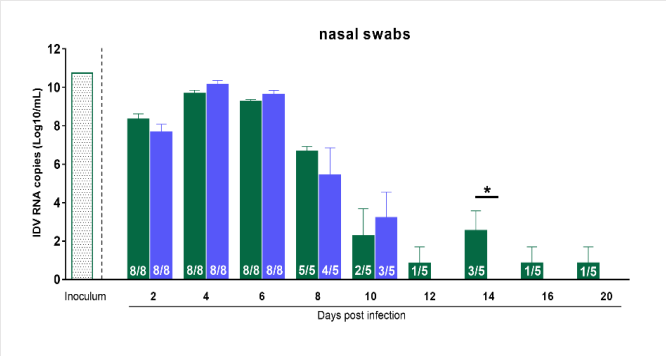
	D6	D21
IDV	minor atelectasis and interstitial pneumonia: right cranial and accessory lobes	No
M. bovis	No	nasal congestion, tracheitis and subacute interstitial bronchopneumonia of minimal extent
IDV+ M.bovis	+++ Severe tracheitis (necrosis and fibrino-purulent exudate) + interstitial pneumonia	tracheitis and interstitial bronchopneumonia of minimal extent

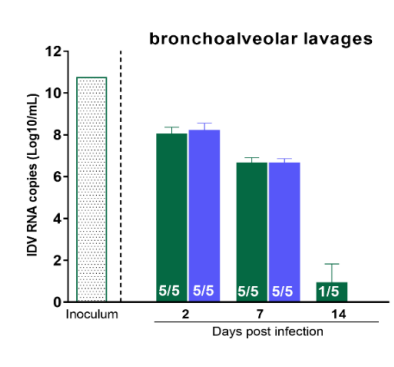
⇨ each pathogen may potentiate the clinical effect of the other

Summary of the main results

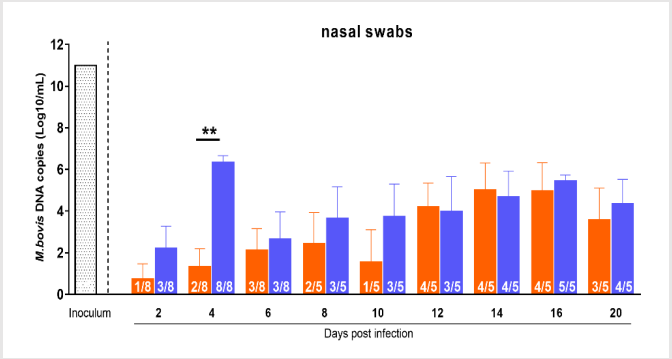
3. IDV infection promotes *M. bovis* colonization of the LRT

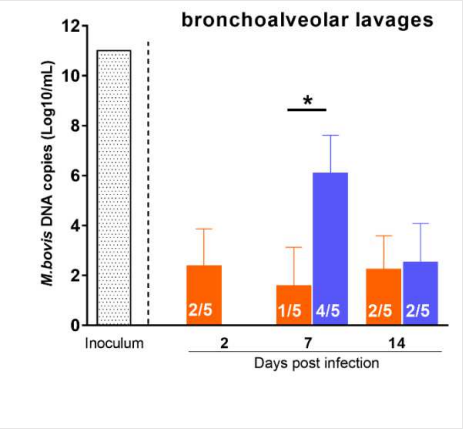
IDV ⇨ No correlation between the severity of disease and the levels of IDV replication





M. bovis ⇨ Significant higher loads in BAL fluid samples at 7 dpi and in the trachea and lungs *(not shown)* at 6 dpi in the coinfected group





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Summary of the main results

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⇨ In BAL fluids (lower respiratory tract)

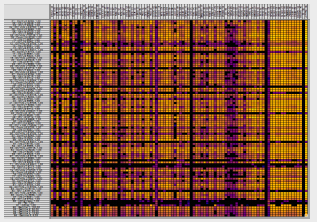
➢ **4.** IDV and *M. bovis* coinfection increases white cell recruitment to the airway lumen

(compared to D0)	IDV	*M. bovis*	IDV + *M.bovis*
White cells	D2, D7, D14: ↑	D2, D7, D14: ↑	D2, D7, D14: ↑
Neutrophils	D2 ↑	D2 ↑, D7 ↑↑	D2, D7, D14: ↑↑
Lymphocytes	→	D7 ↑	D7: ↑↑, D14 ↑
Macrophages	D2, D7, D14 : →	D7: ↓	D2 →, D7 D14: ↓

➢ **5.** IDV and *M. bovis* coinfection extends the innate immune response

Fluidigm RT-qPCR

52 genes: PRRs, cytokines, chemokines, antiviral molecules, growth factors proteases



Differentially expressed genes/D0	IDV	*M.bovis*	IDV+ *M.bovis*
D2	32	4	32
D7	12	17	24
D14	17	7	13

⇨ Quick immune response at D2 for IDV and IDV+ *M.bovis* groups, that extends to day 7 only for the coinfected group

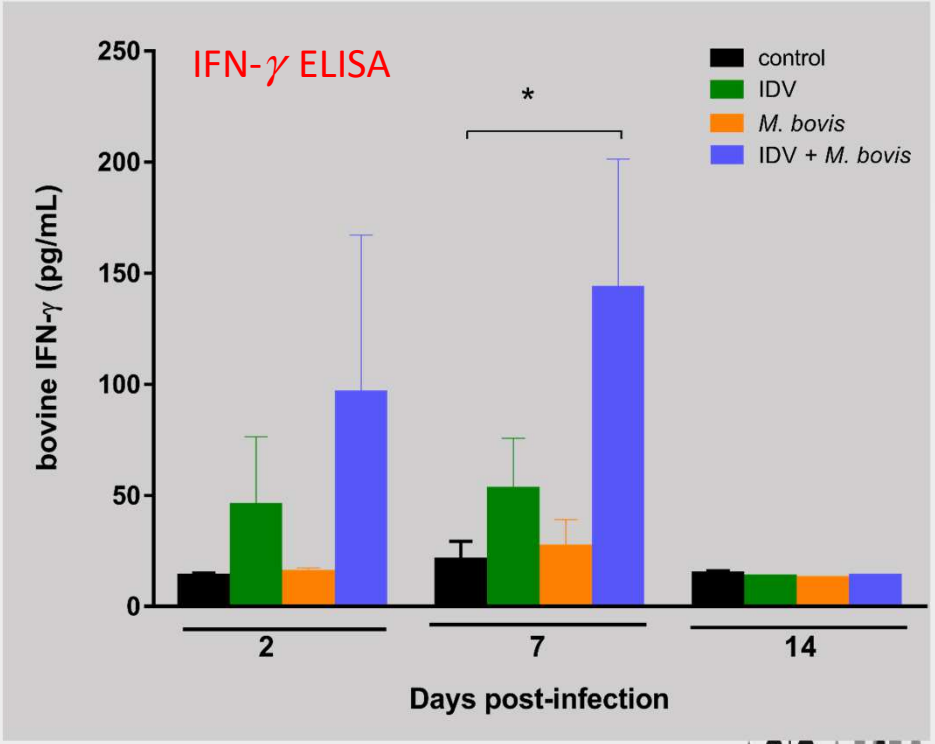
⇨ Delayed and low local host response for *M.bovis*

Disease Severity linked with overexpression of IFN-γ

⇨ Comparative statistical analyses between coinfected group and IDV or *M. bovis* groups

⇨ overexpression of IFN-γ quickly after infection (D2) with a peak at D7

- correlation with high level of lymphocytes in BAL at D7 dpi
- IFN-γ involved in the enhanced disease of coinfected animals ?
- INFγ $^{-/-}$ mice protected from IAV- *S. aureus* superinfection (Kudva *et al.*, 2011; Damjanovic *et al.*, 2013)



Conclusions

- Synergy between IDV and *M. bovis*
  - extension of the distribution of *M. bovis* in the lung,
  - exacerbated respiratory pathogenicity
  - strong and prolonged transcriptomic local response in the LRT
- IDV as co-pathogen in BRD ?

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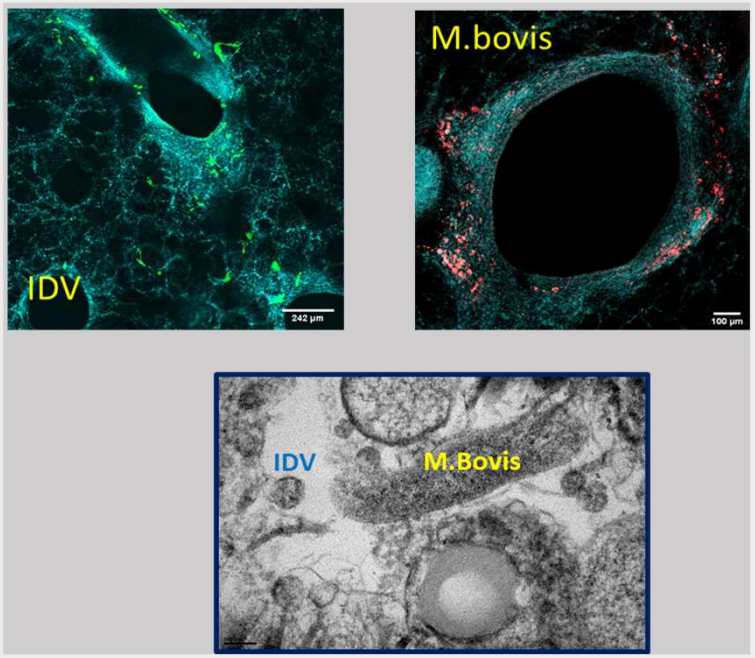
Enhanced Pathogenesis Caused by Influenza D Virus and Mycoplasma bovis Coinfection in Calves: a Disease SeverityLinked with Overexpression of IFN-g as a Key Player of the Enhanced Innate Immune Response in Lungs

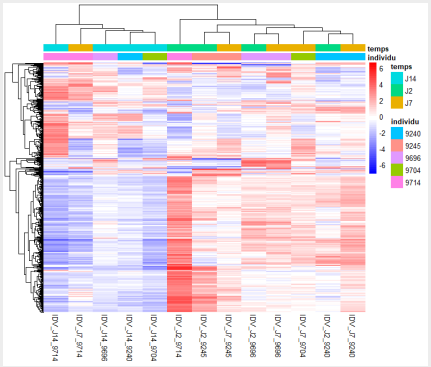
Perspectives

- current studies of interactions between respiratory pathogens

Precision-Cut Lung Slices (PCLS)



(M. Gaudino, submitted)



(A. Lion, in preparation)

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ENVT-INRA , UMR 1225 – Interactions Hosts-Pathogens, VIRéMIE Team, Toulouse, France

A. Lion, A. Secula, C. Rançon, E. Salem, H. Cassard, M. Gaudino, M. Delverdier, E. Baranowski, G. Foucras, M. Ducatez, G.Meyer

INRA, UE 1277, Experimental Infectiology Platform (PFIE), INRA-Val de Loire Research Centre, Nouzilly, France

O. Boulesteix, A. Pinard, A.Deslis.

Department of Virology, Immunobiology and Parasitology, National Veterinary Institute, Uppsala, Sweden;

S. Zohari

Swedish University of Agricultural Sciences, Host Pathogen Interaction Group, Department of Clinical Sciences, Uppsala, Sweden

S. Hägglund, K. Näslund, J-F. Valarcher

Istituto Zooprofilattico Sperimentale Della Lombardia e dell'Emilia Romagna Bruno Ubertini, Brescia, Italy

A. Moreno, E. Brocchi