Mucosal immune responses to Johne's disease in cattle

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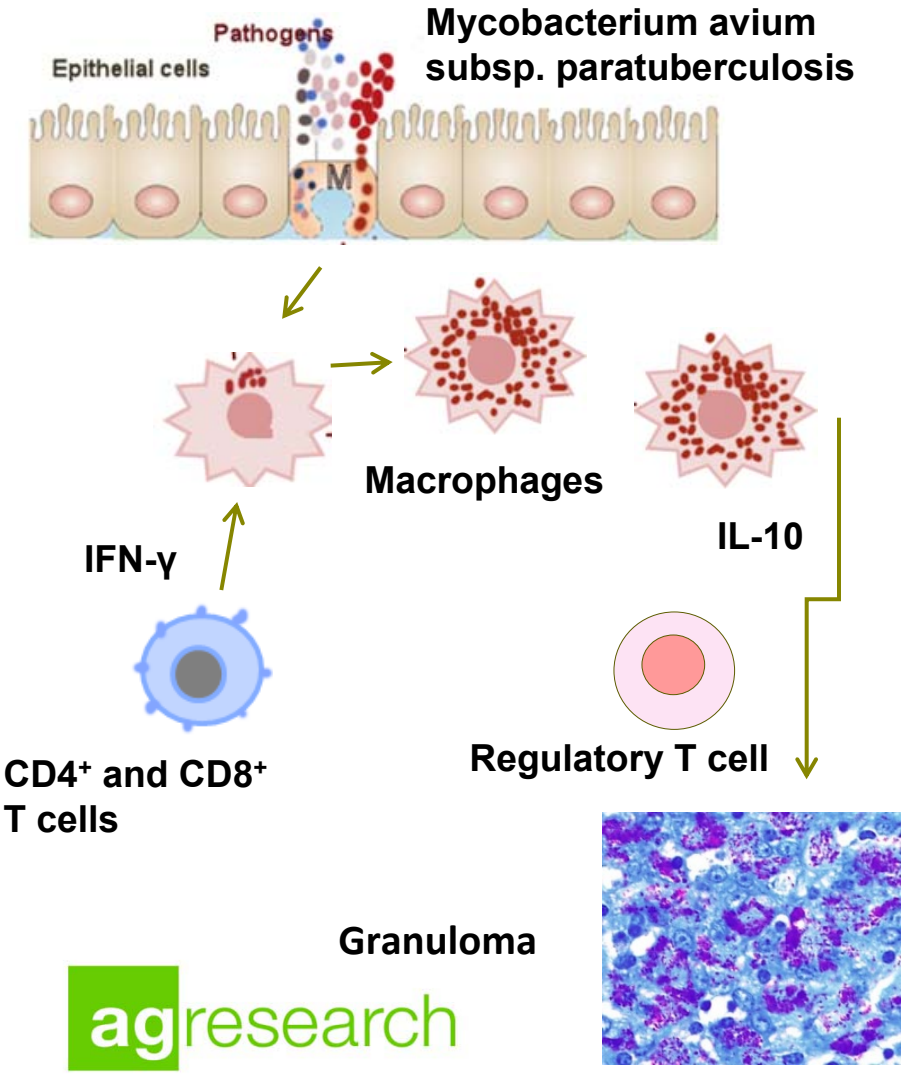
Introduction

Host / pathogen interaction → disease



Possible mechanisms how MAP subverts the gut immune system

- **Regulatory T cells (Subsets of WC1$^+$ γδ T cells)**
- **Immunoregulatory pathways**
- **Toll-like receptors (TLRs) which recognize foreign pathogens**

<u>Aims</u>

Identification of gene markers for susceptibility / resistance

Development of improved diagnostic tests (blood vs. gut immune response)

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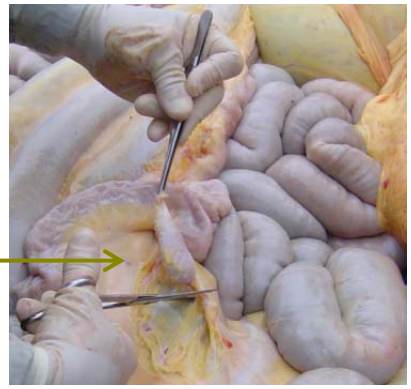
Study 1: Naturally infected cows
Methods

Naturally infected cows (20 + 9), control cows (5)

1. **Bacteriology and histopathology**

Mesenteric lymph node



Terminal ileum

2. **Immune cell separation**
   - Blood (PBMCs)
   - Mesenteric lymph node (MLN)
   - Terminal ileum

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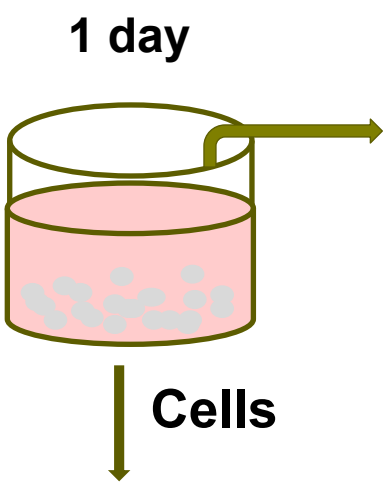
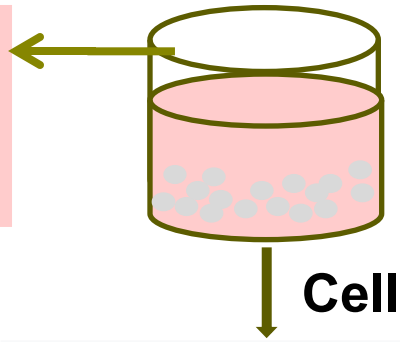
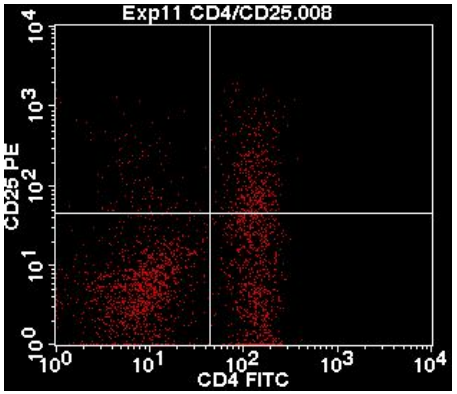
Cells were cultured with MAP sonicate



Supernatant - cytokines release
IFN-γ, IL-10



FACS analysis: CD4+, CD8+ and WC1+ γδ



mRNA expression:
- Cytokine genes
- Immune regulatory genes
- TLR1, 2 and 4 genes

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Results:

Classification, based on MAP culture, JD-ELISA and histopathology scores

Classification	n	MAP	Sero	Histopathology score (max. 24)
Severe disease	10	+	+	≥ 20
Moderate disease	8	+	+	8 - 19
	2	+	-	< 8
Control	5	-	-	< 8

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Terminal ileum of a severe disease cow, thickened intestinal wall, swollen mucosa

Clinically infected cow, very poor body condition

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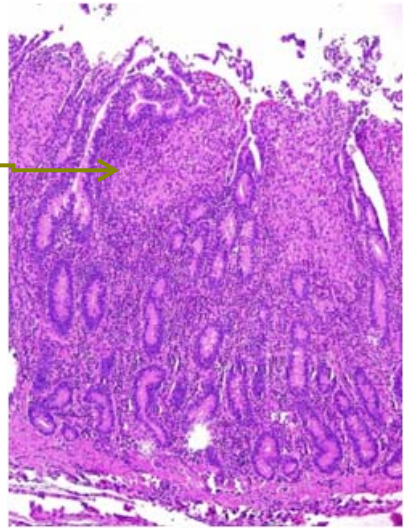
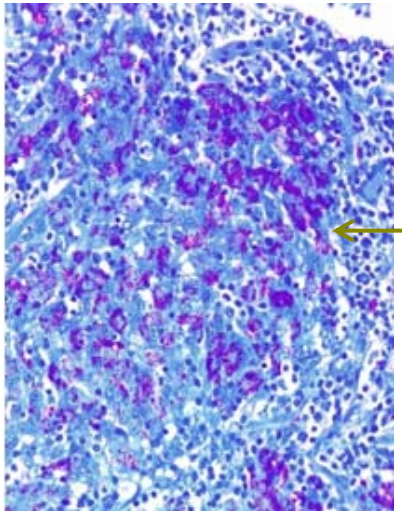
Histology of the mucosa of the terminal ileum from naturally infected cow

Diffused granuloma

Severe disease

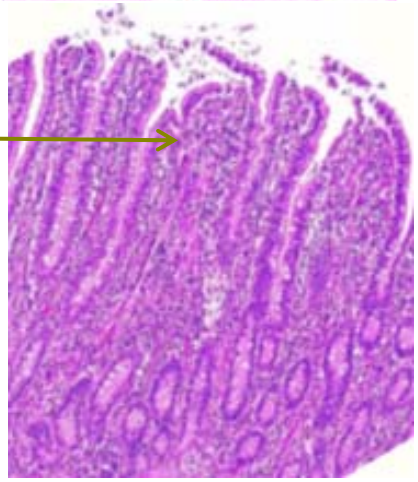


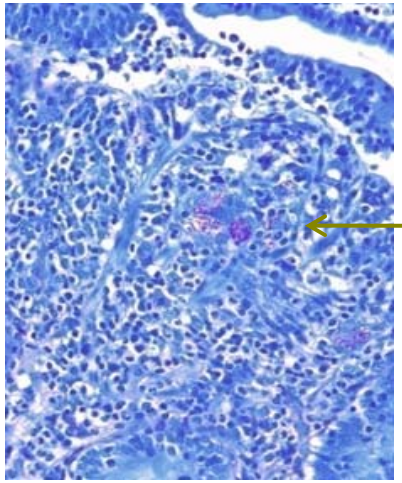


Acid fast organisms

Focal granuloma

Moderate disease





Acid fast organisms

HE stain

Ziehl Neelsen stain

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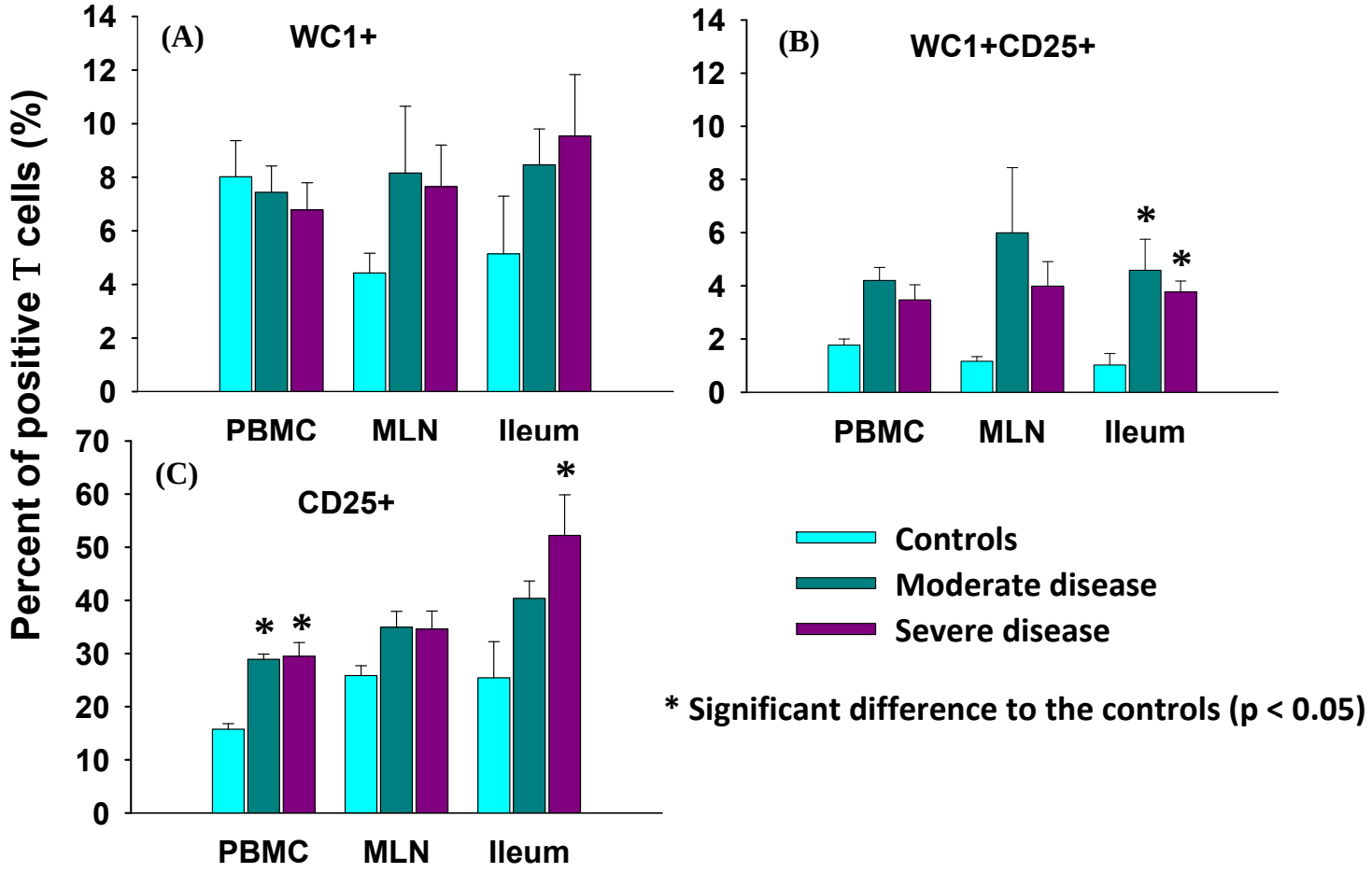
Percentages of WC1+, CD25+, and WC1+CD25+ lymphocytes of PBMC, MLN and ileal cultures, stimulated with MAP Ag



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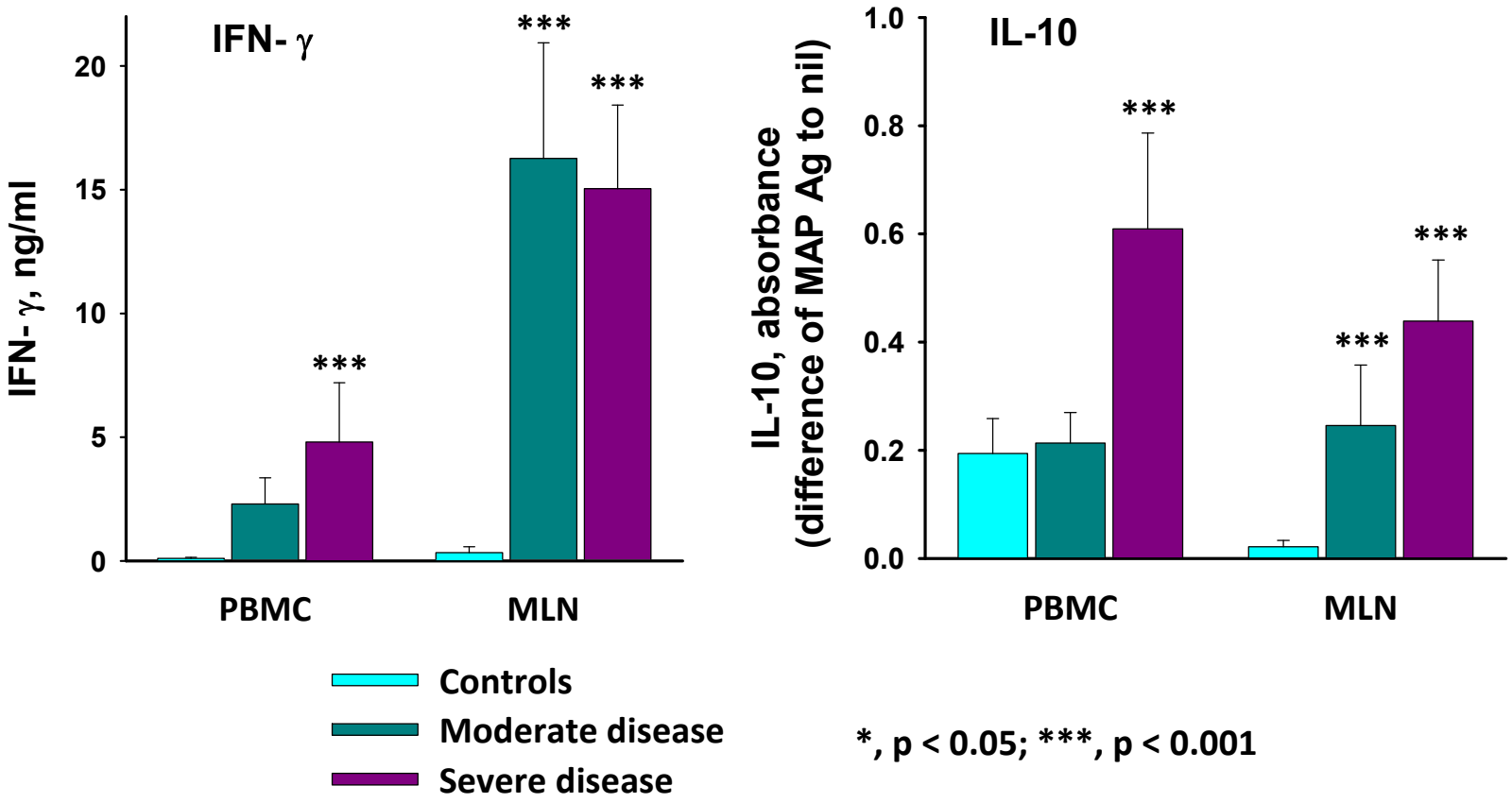
Cytokines released from PBMC and MLN cultures, stimulated with MAP Ag



*, $p < 0.05$; ***, $p < 0.001$

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Relative gene expressions of Toll-like receptors: PBMC and MLN cultures, MAP stimulation vs medium controls

Gene	Severe		Moderate		Control	
	Relative expression	P-Value	Relative expression	P-Value	Relative expression	P-Value
PBMC						
TLR1	0.38	0.039*	0.84	0.74	2.93	0.14
TLR2	0.21	0.026*	0.51	0.3	0.58	0.3
TLR4	0.5	0.26	0.34	0.24	1.03	0.94
MLN						
TLR1	1.11	0.83	0.77	0.66	27.95	0.08
TLR2	0.64	0.49	1.17	0.8	50.99	0.001*
TLR4	1.38	0.45	0.87	0.81	2.73	0.29

Relative expression <1 = down regulation, >1 = up regulation

* P<0.05

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Study 2: Experimental challenge of calves

31 calves, 5 – 8 wks old

Sampling at 2 months intervals:
faeces and blood
- **MAP culture (faecal)**
- **JD antibody ELISA (serum)**
- **MAP specific IFN-γ (whole blood)**

11 Controls, 20 challenge
- **MAP 10^9 bacilli / oral**
- **3 times at weekly intervals**

Kill at 7 & 18 months PI:
Measure blood and gut immune response

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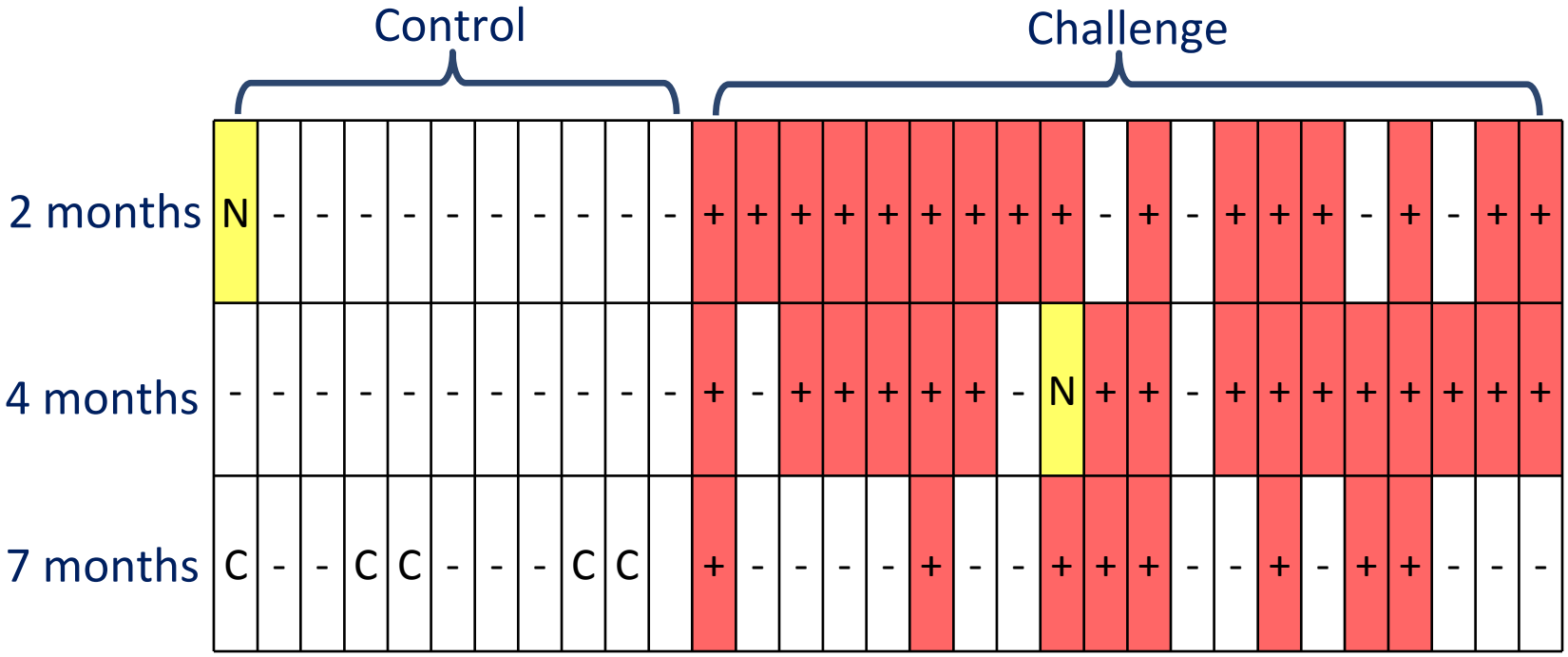
Faecal culture



+ faecal culture positive for MAP (19/20 challenge calves)

19/20 challenge calves elevated MAP-specific IFN-γ

All calves negative for JD-ELISA at 2, 4, 7 and 9 months

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Calve killed at 7 months (10 Challenge, 5 Control)

Tissue collection (ILN, MLN, IC and Ileum)

Challenge calves:

4/10 enlarge LN, 3/10 thickening of ileum

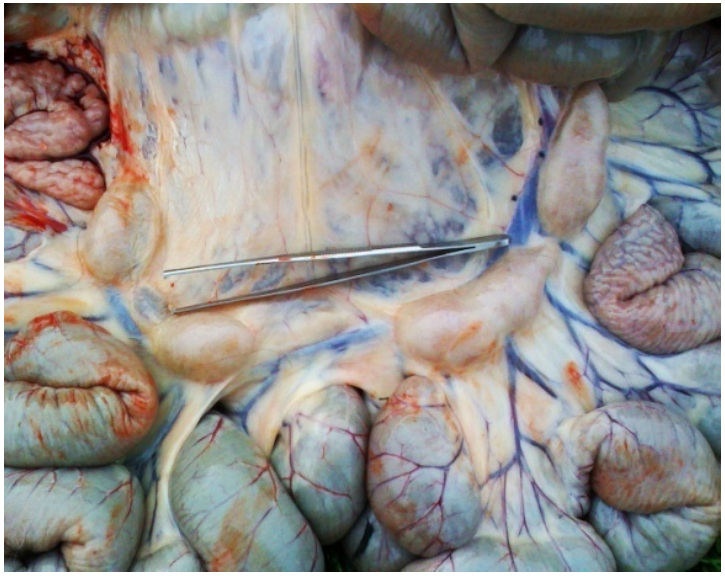
Histopathology lesions 3/10 (very mild)

10/10 tissue culture positive

Control calves:

No gross and histopathology lesions

No culture positive



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Summary

Naturally-infected cows

- Antigen-specific IFN-γ ↑
- Antigen-specific IL-10 ↑
- Regulatory T cells (WC1$^+$ γδ) ↑
- TLR 1 and 2 genes in PBMCs of severely affected animals ↓
- TLR 1 and 2 genes in MLN cells of control cows ↑

Experimentally-infected calves

- Successful experimental challenge model
- MAP culture +ve, MAP-specific IFN-γ +ve
- Gross and histopathology lesions in challenge calves

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