

Experimental inoculation of calves with EHEC O157:H7 MC2 strain isolated from cattle



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Introduction and Objectives



EHEC O157:H7 MC2

- Persistent strain in a French cattle farm ¹
- Large arsenal of virulence determinants ^{1,2}: *stx_{1a}*, *stx_{2c}*, *eae-γ*, *ehxA*, *iha*, *toxB*, *lpf1*, *lpf2* ...
- Large number of colonization factors ²

Inoculation of MC2 to calves ► better characterization of ...

Fecal excretion of MC2

Digestive colonization by MC2

Host response

... to identify strategies aimed at reducing EHEC O157:H7 shedding.

¹ Auffret et al., 2017, Draft genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 strain MC2 isolated from cattle in France. *Genome Announc.* 5:e01087-17.

² Segura et al., 2018, Factors involved in the persistence of a Shiga toxin-producing *Escherichia coli* O157:H7 strain in bovine feces and gastro-intestinal content. *Front. Microbiol.* 9:375.

Materials and Methods

- Approval of Ethics Committee and of the French Ministry of Higher Education and Research (number APAFIS#4704-2016032517325815 V4)

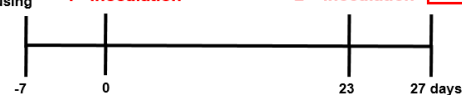
- Two groups of 3 month-old calves:

- 5 calves per group
- Inoculated group: 2 inoculations of 10¹⁰ CFU MC2 RfR
- Control group: inoculation of NaCl

- Quantification / detection of MC2 RfR :

- Media supplemented with rifampicin
- CFU and MPN counts

Housing 1st Inoculation 2nd Inoculation Necropsy



MC2 RfR counts / detection in intestinal contents and tissues

Histopathological changes

Production of pro-inflammatory cytokines (ELISA)

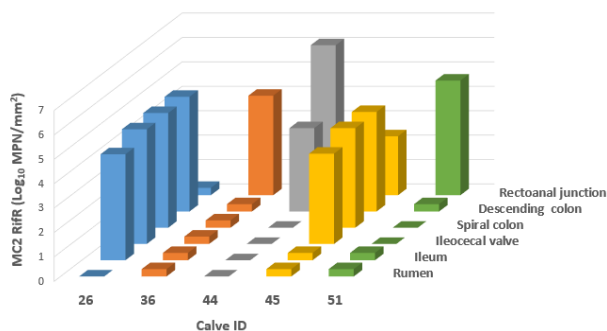
Fecal excretion of MC2 RfR

Results

Clinical health status of inoculated calves

- MC2 inoculation did not induce significant clinical disease.
- No differences were observed in follow up of hematological and biochemical parameters between inoculated and control calves.
- No visual abnormalities (digestive tract, kidney ...) were observed in any of the inoculated calves during necropsy.

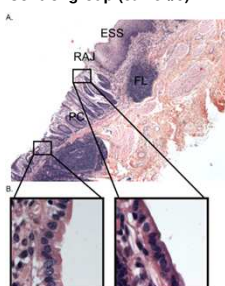
Recovery of EHEC O157:H7 MC2 from intestinal tissues



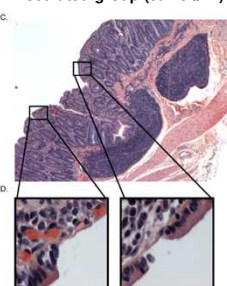
	Calve ID				
	26	36	44	45	51
Detection of MC2 RfR in:	+	+	+	+	+
Tonsils	+	-	+	+	-
Lymph nodes (ileocecal and caudal mesenteric)	-	-	-	-	-
Hide and ears	+	+	+	+	+

Histopathological changes at the rectoanal junction

Control group (calve #9)



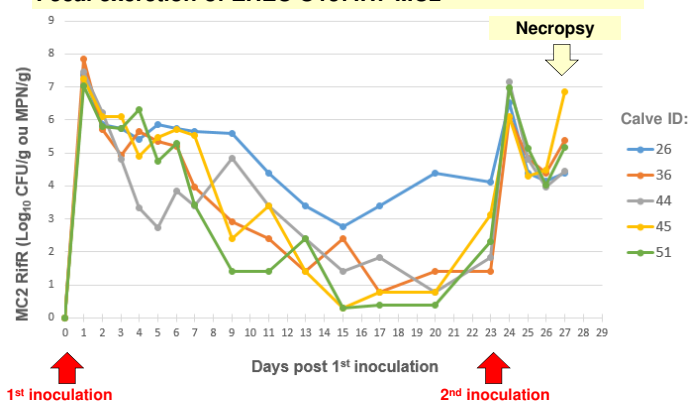
Inoculated group (calve #44)



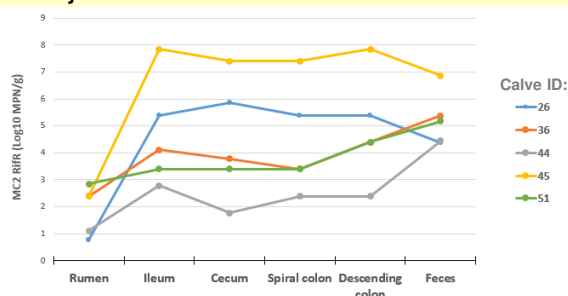
Hematoxylin eosin stained sections; ESS: stratified squamous epithelium; FL: lymph follicle; PC: crypt; RAL: rectoanal junction; A, C x40, B, D x1000

Effaced microvilli, detached squamous epithelial cells, hyperemia and eosinophilic infiltration

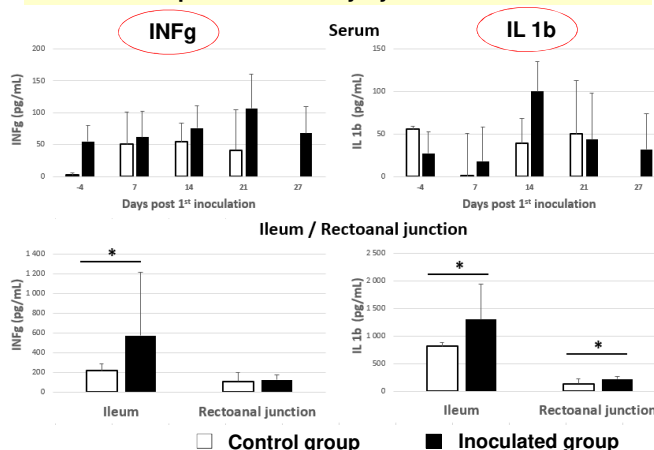
Fecal excretion of EHEC O157:H7 MC2



Recovery of EHEC O157:H7 MC2 from intestinal contents



Production of pro-inflammatory cytokines



Conclusions

- All calves excreted MC2 23 dpi.
- All compartments of the digestive tract were colonized by MC2.
- MC2 was not invasive.

- MC2 did not induce clinical disease.
- Inoculation of MC2 induced histopathological changes and local production of pro-inflammatory cytokines.