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EVALUATION OF *EIMERIA* SPP. PARASITES AS SURROGATES FOR THE STUDY OF *TOXOPLASMA GONDII* OOCYST INACTIVATION

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Introduction

- Foodborne diseases** are a global public health concern [1]
- T. gondii* : one of the **most important parasites to consider in the food domain** (multiple routes of infection, high prevalence, worldwide distribution) [2]
- Oocyst** of *T. gondii* : **resistant** to adverse environmental conditions for **very long periods** [3]. Soil and water can represent **sources of contamination of fresh produce** (i.e vegetables, fruits, shellfish) during primary production and **drinking water** [4]
- Implementation of control measures** by food industries and/or consumers to **reduce contamination levels and/or inactivate parasites in foods** → Need to assess the efficiency of control measures
- Problematic** : studies conducted on *T. gondii* are hampered by **financial and ethical constraints**
→ Efforts are underway to identify and characterize organisms that could be used as surrogates for *T. gondii* oocysts.

Objectives

- To determine whether oocysts of the genus *Eimeria* can be used as *T. gondii* surrogates by comparing the response of the two parasites to chemical and physical treatments in an agri-food context
- Impact of the treatments on the structure (permeability) of oocysts

Methods

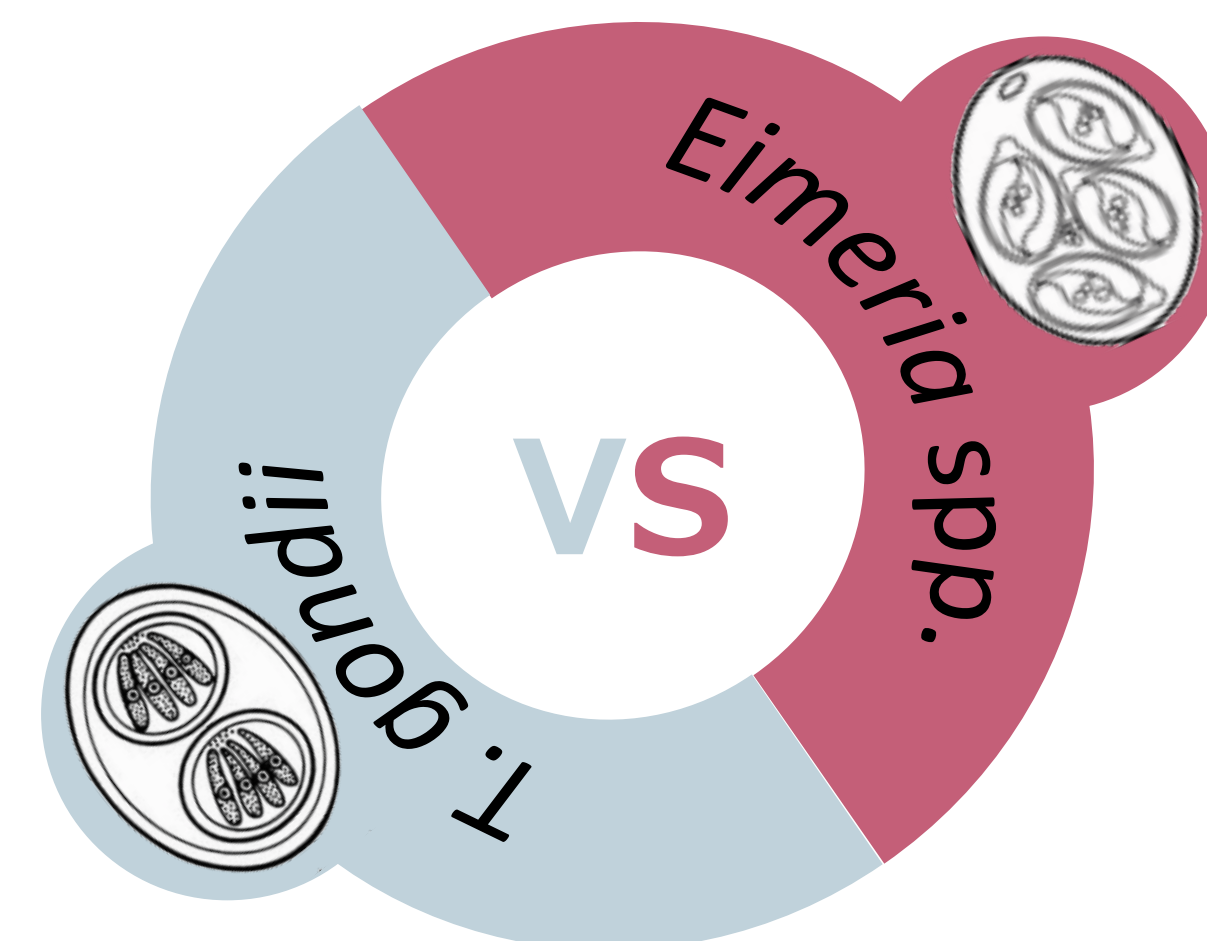
Oocyst suspensions	Treatments	Lectin markers	Analyses
<i>T. gondii</i>	<ul style="list-style-type: none"> NaOH 20% 1h Bleach 3.6% 30min 	<ul style="list-style-type: none"> <i>Bauhinia purpurea</i> (BP) 	Flow cytometry BD Accuri C6
<i>E. acervulina</i>	<ul style="list-style-type: none"> Heat 95°C 5min Freezing -80°C 48h Freezing/thawing cycle x6 	<ul style="list-style-type: none"> <i>Maclura pomifera</i> (MP) <i>Wheat germ agglutinin</i> (WGA) 	Fluorescence microscopy ZEISS LSM 210 (Data not shown)

Conclusions and perspectives

- Eimeria acervulina* has been previously described as a surrogate of *Cyclospora cayetanensis* in some studies [5], [6], [7] → oocysts are **safe to handle and appear to be an economical model for most studies on coccidian contamination**
- The response to different physical and chemical treatments of both parasites was evaluated by measuring the oocysts permeability to different lectins that mark specific layers of the oocysts. **Our results showed that bleaching, NaOH, freezing, 6 freezing cycles have similar effect on *E. acervulina* and *T. gondii* oocysts structure. However, heat treatment impacts differently. This suggest that *E. acervulina* could be representative of *T. gondii* behaviour upon certain treatments but not all. Hence, different surrogates could be required depending on the treatment that has to be assessed. Other *Eimeria* species are currently tested.**
- To validate *Eimeria* oocysts as surrogates of *T. gondii*, the next essential step is to compare the inactivation kinetics of both parasites upon different treatments. Such assessment requires to measure viability and/or infectivity of *Eimeria* oocysts. Finally, molecular responses (transcriptomic, proteomic) could also be evaluated to describe the potential surrogate with accuracy.

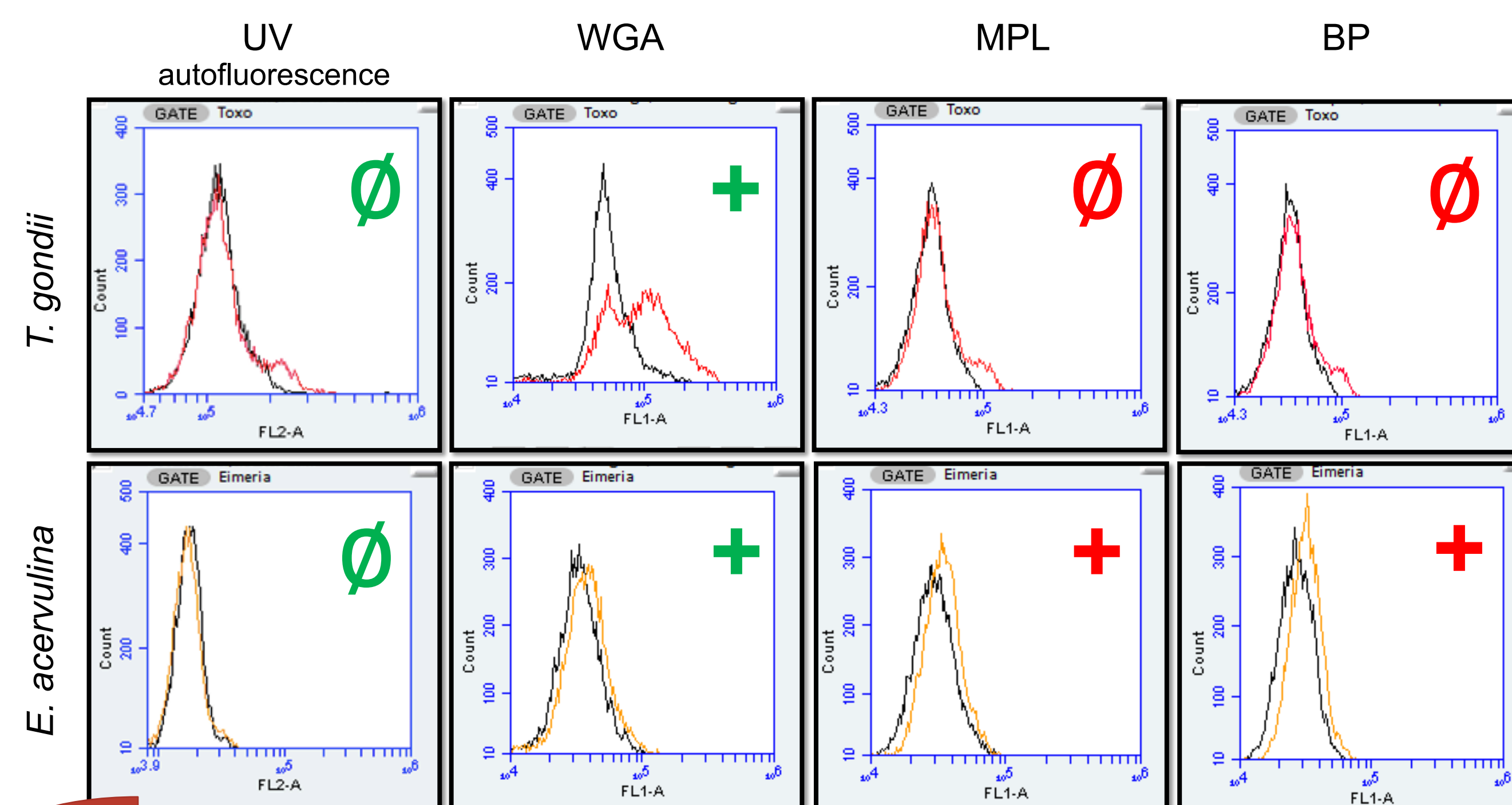
Why *Eimeria* spp. as putative surrogates of *T. gondii* ?

Oocyst structure 2 sporocysts x 4 sporozoites Walls : 2 layers, 90% proteins Few lipids and sugars	Oocyst structure 4 sporocysts x 2 sporozoites Walls : 2 layers, 90% proteins Few lipids and sugars
Size 11 µm x 13 µm	Size 14-21 µm x 15-31 µm
Mode of transmission Fecal-oral route	Mode of transmission Fecal-oral route
Pathogenicity to humans Yes	Pathogenicity to humans No
Definitive host Felids	Definitive host Depends on the species Ex: chicken, mouse,...
Production Difficult to implement Quantity depends on the animal	Production Easy to implement Large quantity



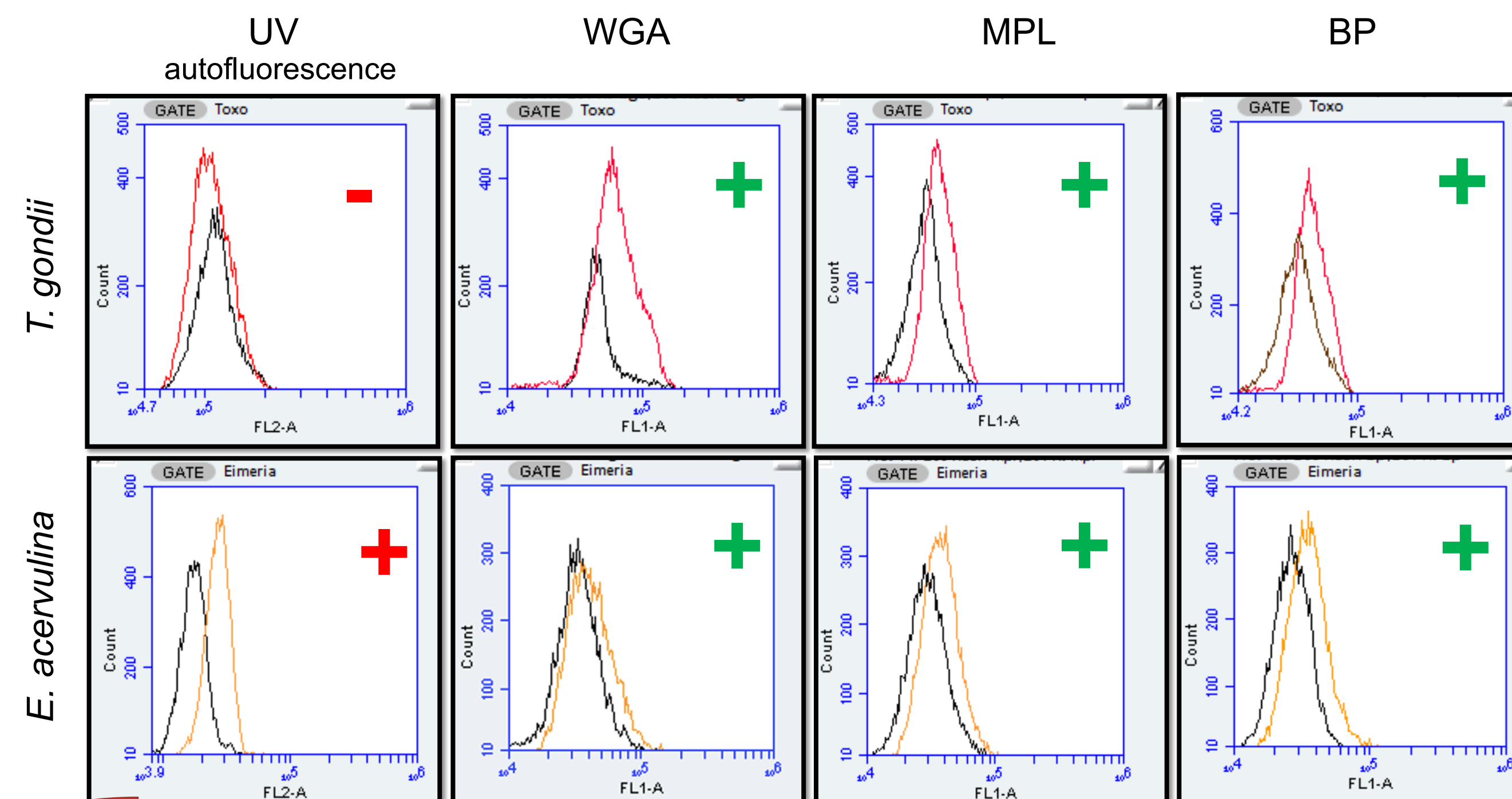
Results

Heat treatment



Heat treatment impacts on the inner structure (MPL, BP) of *E. acervulina* but not *T. gondii* oocysts

NaOH treatment



NaOH has a similar effect on *E. acervulina* and *T. gondii* oocysts structure

Fig 1 : Impact of the different treatments on the structure and permeability of *E. acervulina* and *T. gondii* oocysts obtained by flow cytometry (examples of Heat and NaOH treatments)
 - : Decrease in fluorescence vs. Control, + : Increase in fluorescence vs. Control, Ø : No change in fluorescence vs. Control, — Control (untreated, marked), — *T. gondii* (treated, marked), — *E. acervulina* (treated, marked), ■ Difference between the two parasites, ■ No difference between the two parasites

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