


# Toxoplasma Vaccines

## *Status, Challenges And Future Directions*


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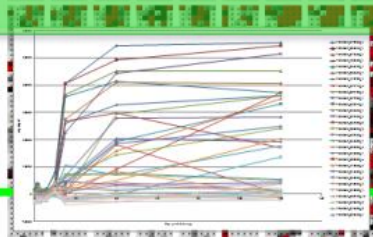
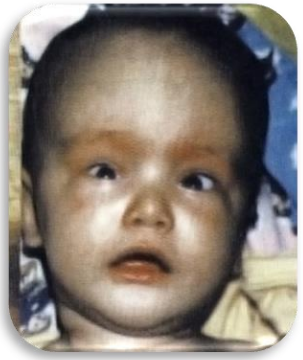


# Brief info about Toxo

***Toxoplasma gondii* is a protozoan parasite**



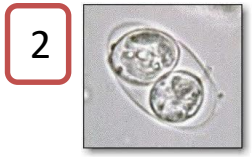
***Toxoplasma gondii***

- **Serious clinical presentations formed in the fetus and in immune compromised patients (AIDS, Cancer, Tranplantation)**
- **Linked to behavioral syndromes (schizophrenia or bipolar disorder)**
- **Animal health (economic loss)**

# Brief info about Toxo

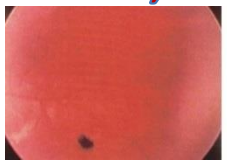
## Felidae cats are definitive host

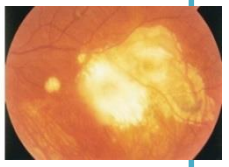
- **Cats disseminate resistant oocysts in their feces to enviroment (contagious up to 18 m)**
- **Eating food or drinking water contaminated with cat feces**
- **Ingestion of tissue cysts in raw or under-cooked meat prepared from infected hosts**


# Do we need a vaccine?

- 500 million people are estimated to be infected worldwide
- The rate of toxoplasmosis among pregnant women range from 37% to 58% in Europe and 10.8% in the US
- In the US,
  - each year 400–4000 congenital toxoplasmosis,
  - up to 1,26 million ocular toxoplasmosis





- Tainted water outbreaks
  - British Columbia Canada-7718 people
  - Coimbatore India-178 people
  - Izmir Turkey-171 Air Force recruits
- Bioterrorism agent (BSL-2 agent; CDC)

# Do we need a vaccine?

**Amount of domestic cats**

USA: 74 milyon
France: 11 million
UK: 8,5 million
Germany: 8 million

**Toxoplasmosis in cats**

USA: 31,6%
Germany, France, and Italy: 9-46%
Turkey: 35,6%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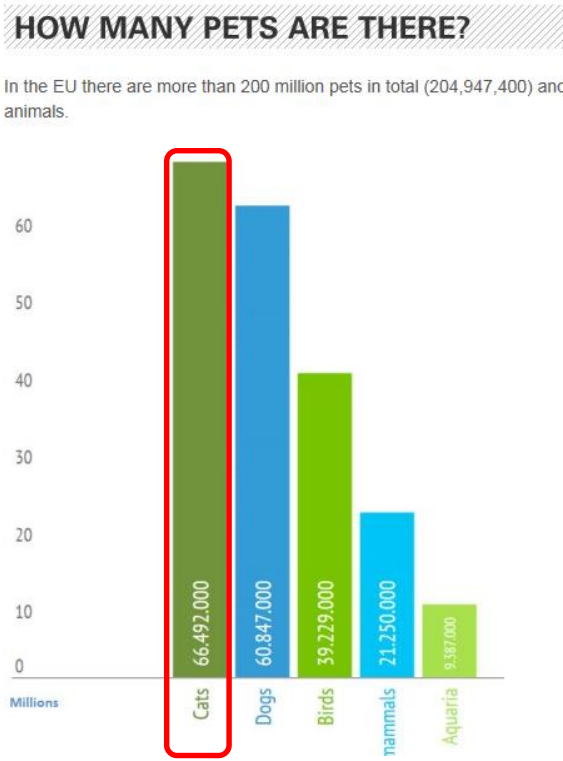
## U.S. Pet Ownership Statistics

Companion animals | Exotic animals | Formulas/Calculator

Source: *2012 U.S. Pet Ownership & Demographics Sourcebook*

### Companion animals

| | Dogs | Cats | Birds | Horses |
|---|---|---|---|---|
| Percent of households owning | 36.5% | 30.4% | 3.1% | 1.5% |
| Number of households owning | 43,346,000 | 36,117,000 | 3,671,000 | 1,780,000 |
| Average number owned per household | 1.6 | 2.1 | 2.3 | 2.7 |
| Total number in United States | 69,926,000 | 74,059,000 | 8,300,000 | 4,856,000 |
| Veterinary visits per household per year (mean) | 2.6 | 1.6 | 0.3 | 1.9 |
| Veterinary expenditure per household per year (mean) | $378 | $191 | $33 | $373 |
| Veterinary expenditure per animal (mean) | $227 | $90 | $14 | $133 |



# Do we need a vaccine?

- When we take in to consideration the population of US (over 300 million people) each family (of 4 individuals) owns approximately one cat (74 million)
- Overall, this friendship must be further improved by a protective vaccine against humans and/or cats.

# Vaccine against Toxo

## Current vaccine research against toxoplasmosis

- **Live vaccine for sheeps (incomplete strain-S48)**
- **More than 60 vaccine candidate antigens (Gedik et al. 2016, Trials in Vaccinology) almost always selected**
  - **Randomly**
  - **Based on biological properties (such as being a surface protein, having a role in pathogenesis, or high immunogenicity)**
- **None of them conferred the desired efficacy to be able to proceed to clinical trial**

Vaccine Development Pipeline

# Vaccine against Toxo

**IPROVE** (Innovation Partnership for a Roadmap on Vaccines in Europe; March 16th, 2016)

- One of the major knowledge gaps and challenge: selection of appropriate antigens
- Methods to select antigens take advantage of recent advances in vaccinology (e.g. *in silico* analysis, *in vitro* and *in vivo* immunoscreens)
- Significant risks of failure at relatively late stages of the development process
  - Disappointing results of RTS,S clinical trials

Hybrid protein, formulated in a multi-component adjuvant, showed only 39% protection in East African children (Hill 2011, Philos Trans R Soc Lond B Biol Sci)

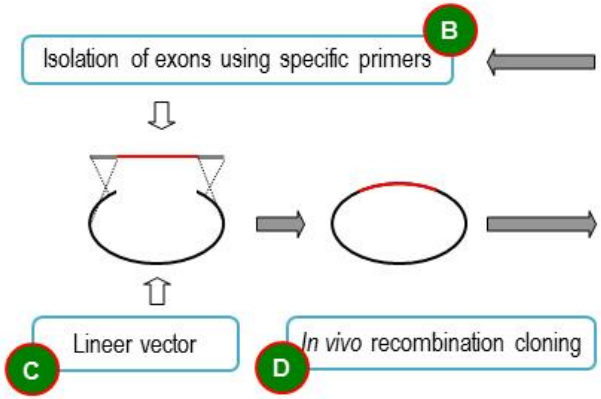
# Discovery of *Toxoplasma gondii* vaccine candidate antigens

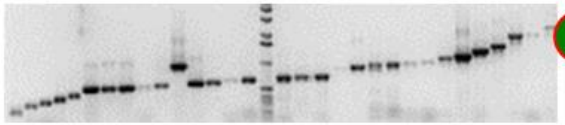
## Helpful info about *T. gondii*

- In the life cycle of *T. gondii*, tissue cysts (bradyzoites) and oocysts (sporozoites) are the main infective forms
- As they enter the host cell, bradyzoites and sporozoites convert to motile tachyzoites which invade the tissues
- Just as the immune response starts, tachyzoites change into slowly dividing bradyzoites and remain latent
- Thus, a successful vaccine against *T. gondii* is likely to contain antigens from all forms of the parasite, and selected using a rational approach

# Discovery of *Toxoplasma gondii* vaccine candidate antigens

## High throughput protein microarray screening approach

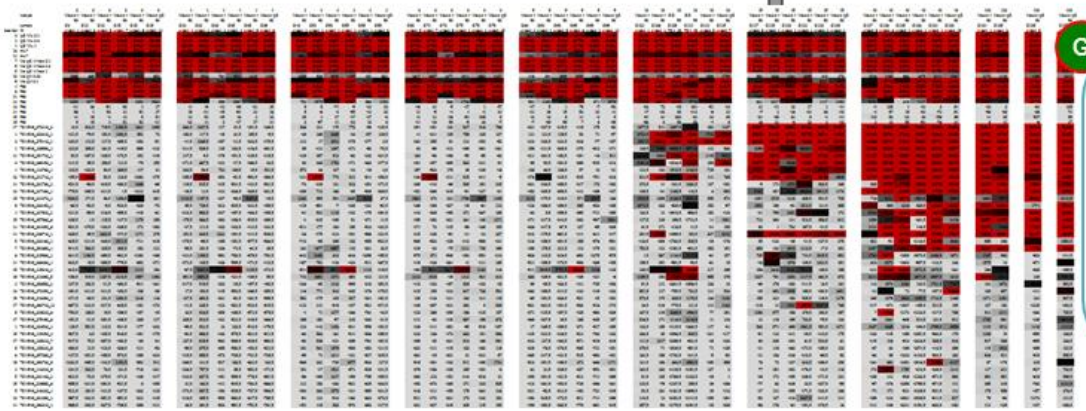


A
2870 proteins were down-selected from >8,000 genes (>43,000 exons)
(Liang et al. 2011, Moll. Cell Proteomics; Felgner et al. 2015, Parasitology)

E
✓ pXI plasmids with inserted exons

F
✓ Recombinant proteins are expressed by by *in vitro* transcription/translation system and printed on microarray slides.
✓ Presence of HIS tagged protein expression is shown by anti-HIS antibody probing



G
**HEAT MAP**
✓ Protein mikroarray chips are probed with
1) Well categorized patient sera
2) Sera obtained from murine models infected with tissue cysts and oocysts
✓ **Vaccine antigen discovery**

# Discovery of *Toxoplasma gondii* vaccine candidate antigens

## Importance of sera

**Sera of patients with acute and chronic toxoplasmosis**

- **D: Determination of the initiation of the infection is not possible**
- **A: Recently acute samples from the Izmir Outbreak (showing the beginning of infection)**

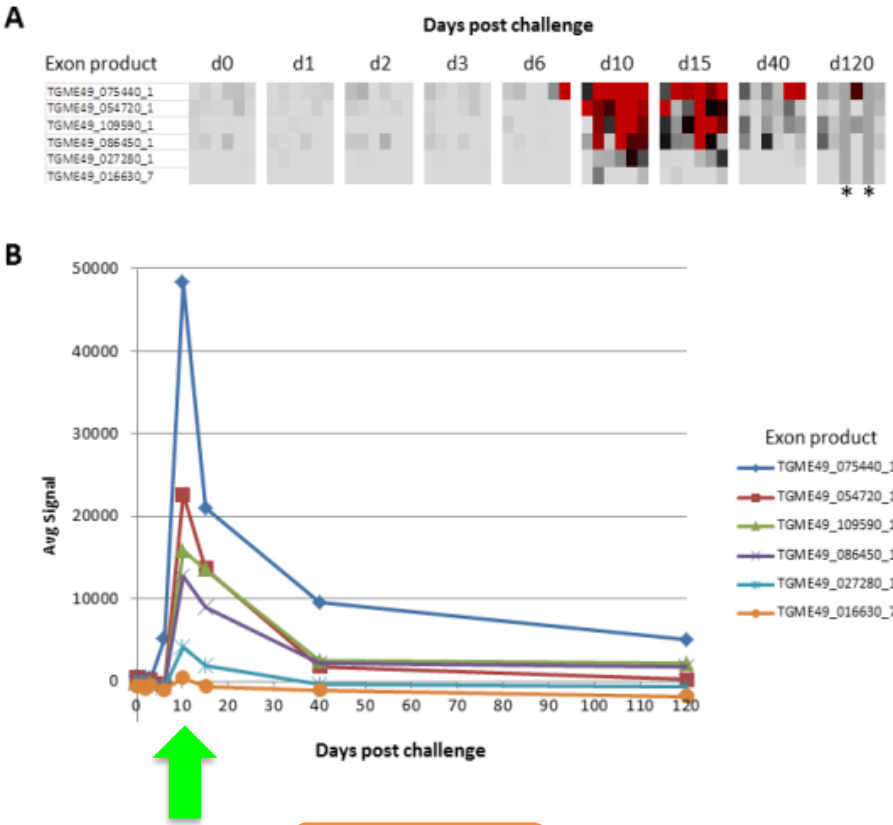
**Sera of mice orally infected tissue cysts and oocysts**

**(collected at day 0, 1, 2, 3, 6, 10, 15, 40, and 120)**

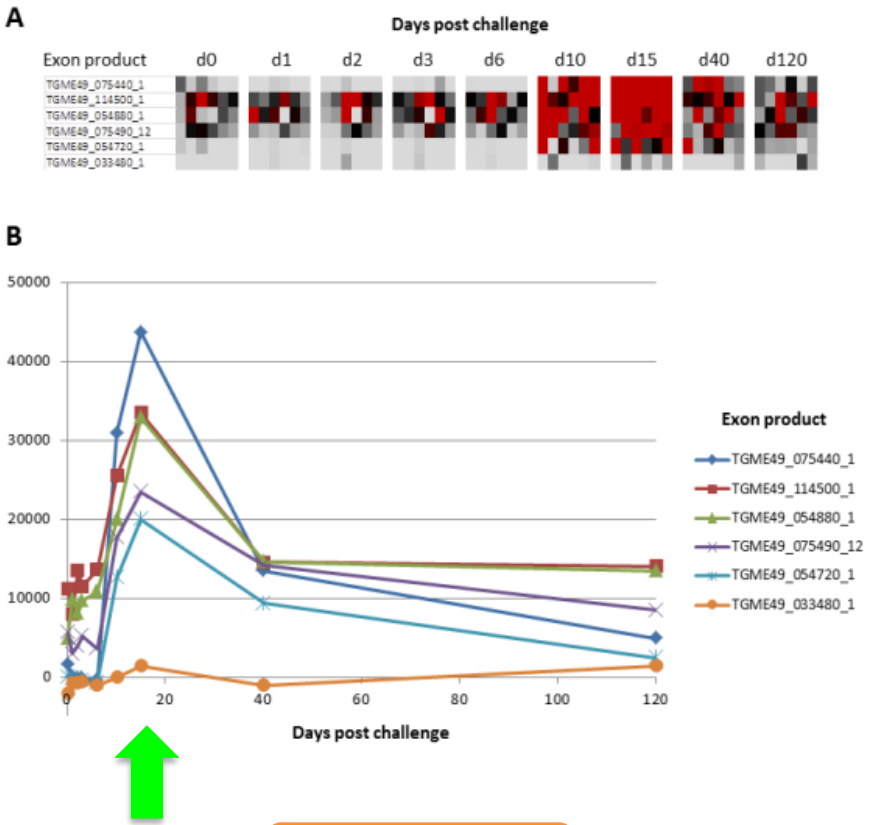
- **A: Determine the dominant antigens presented by tissue cysts and oocyts**
- **A: Enable to identify Ab kinetics**
- **A: Compare the murine antibody profiles with human profiles**

# Discovery of *Toxoplasma gondii* vaccine candidate antigens

## IgM kinetics of mice infected with oocysts and tissue cysts



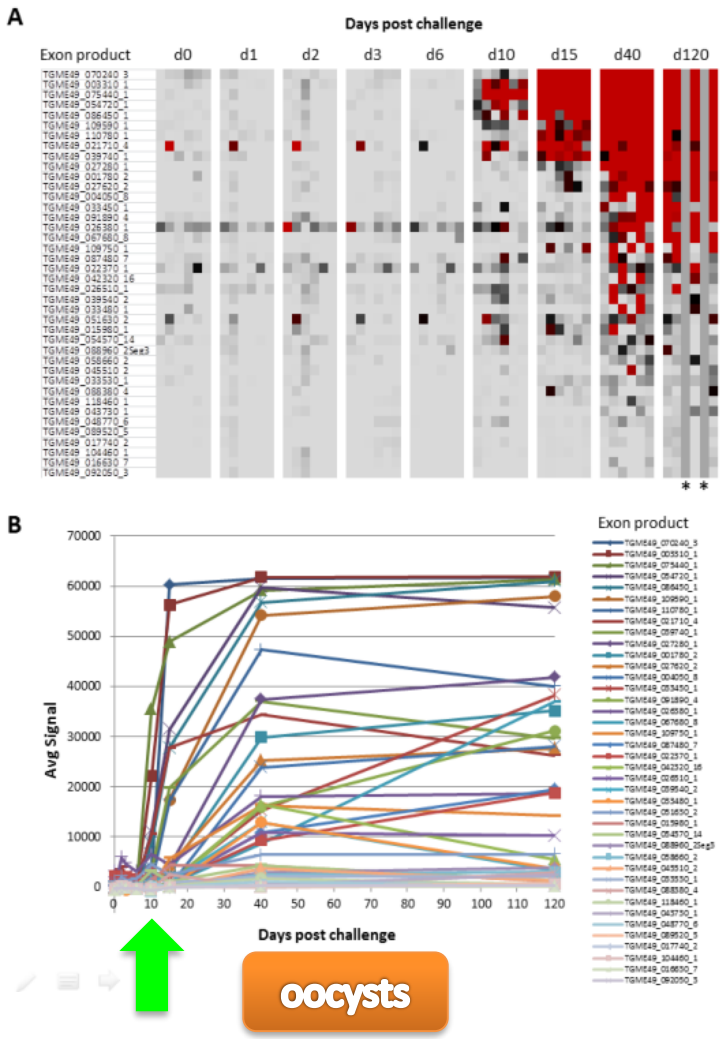

oocysts

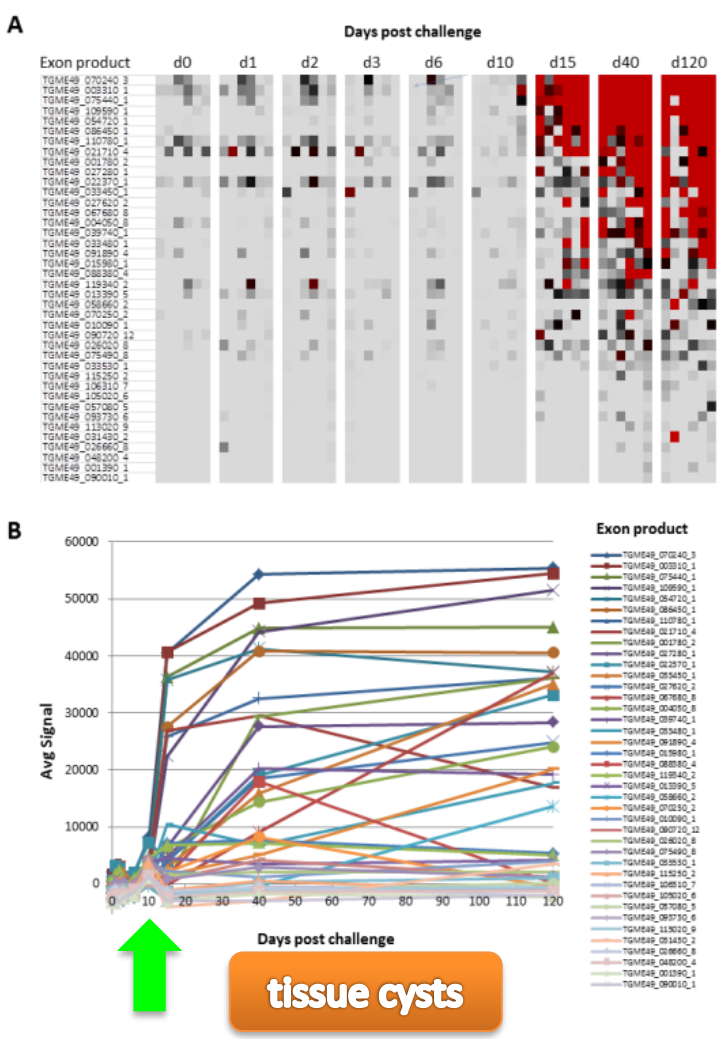



tissue cysts

# Discovery of *Toxoplasma gondii* vaccine candidate antigens

## IgG kinetics of mice infected with oocysts and tissue cysts



# Discovery of *Toxoplasma gondii* vaccine candidate antigens

## Define the properties of vaccine antigen(s)

- ✓ **actively induce strong immune response at the very beginning of the infection** (i.e. strong IgM response at the first 10-15 days of infection)
- ✓ **induce long lasting immunity** (i.e. strong IgG response until day 120)
- ✓ **must be antigenic in both forms of the parasite** (because transmission can occur through oocysts or tissue cysts)
- ✓ **preferably not structural proteins**

# Discovery of *Toxoplasma gondii* vaccine candidate antigens

## Candidates from murine sera screening

- Two antigens GRA6 and GRA8 have all of the above mentioned properties
- GRA3, GRA5, ROP1, and SRS29C have similar properties excluding antigenicity in IgM response in oocyst infected mice
- Besides, there are 11 more antigens that induce long lasting IgG response in both oocyst and tissue cyst infected mice MAG1; GRA2, GRA4, GRA7, GRA14; MIC1, MIC2, MIC12; SRS13, SRS29A; ROP6

# Discovery of *Toxoplasma gondii* vaccine candidate antigens

## Matching candidates with previous studies

- When we match these antigens with the human screening data from our previous studies, all of the antigens showed strong immune response in human sera (except SRS29C) (Liang et al. 2011, Mol Cell Proteomics; Felgner et al. 2015, Parasitology)
- Two studies about transcriptomic and proteomic analyses of *T. gondii* confirmed that:

  Among the 17 candidates 14 of them (except MAG1, SRS13, and SRS2A) showed elevated expression levels in sporozoites at days 4 and 10 of the infection (Fritz et al. 2012a, PlosOne; Fritz et al. 2012b, PlosOne)

# Discovery of *Toxoplasma gondii* vaccine candidate antigens

**Were they used as vaccine candidate antigen previously?**

**Antigens tested**

- GRA2
- GRA4
- GRA5
- GRA6
- GRA7
- ROP1
- MIC1
- MIC2
- MAG1

**Antigens not tested**

- GRA3
- GRA8
- GRA14
- MIC12
- ROP6
- SRS29A
- SRS29C
- SRS13

# Discovery of *Toxoplasma gondii* vaccine candidate antigens

## Take home messages

- **The 9 antigens previously tested have proven efficacy and validate our results**
- **8 new vaccine candidates** (dominant in the antibody response, long lasting and present against both forms of parasite)
- **Multiplexing antigens in vaccine formulation increase the immune response**
- **A multivalant vaccine using all/a part of these antigens will be of great interest in toxoplasmosis vaccinology field**

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