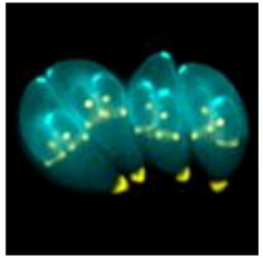
Kurdistan Region Iraq
Cihan University -Erbil
College of Science
Department of
Biology

Dec 4, 2018

Virulence factors and gene polymorphism in *Toxoplasma gondii*



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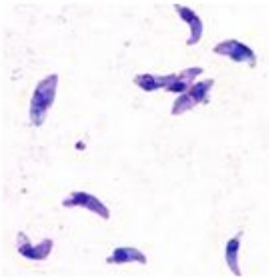
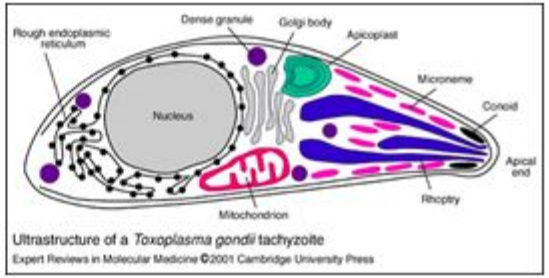
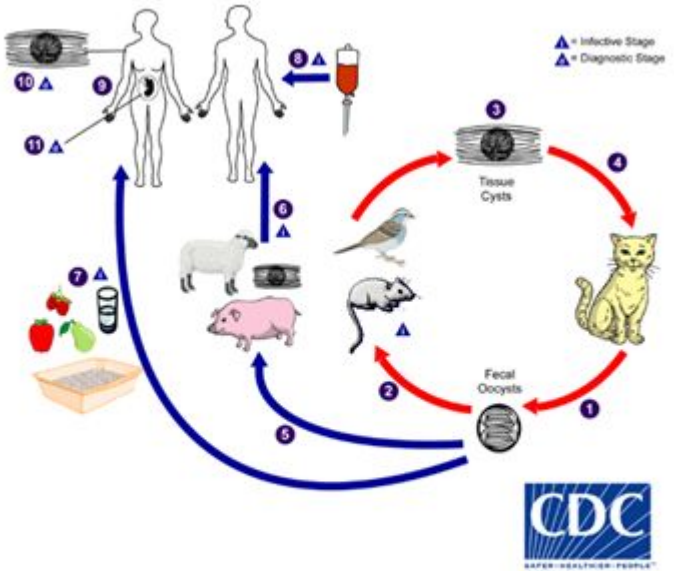
- Toxoplasmosis is a worldwide infectious disease caused by the obligate intracellular protozoan parasite, *Toxoplasma gondii* (*T. gondii*).

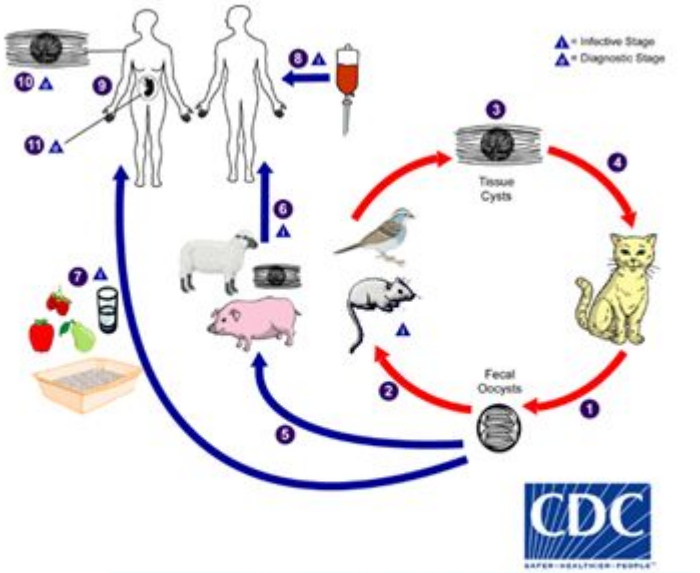




Toxoplasma gondii **tachyzoite**



Life cycle of Toxoplasma gondii



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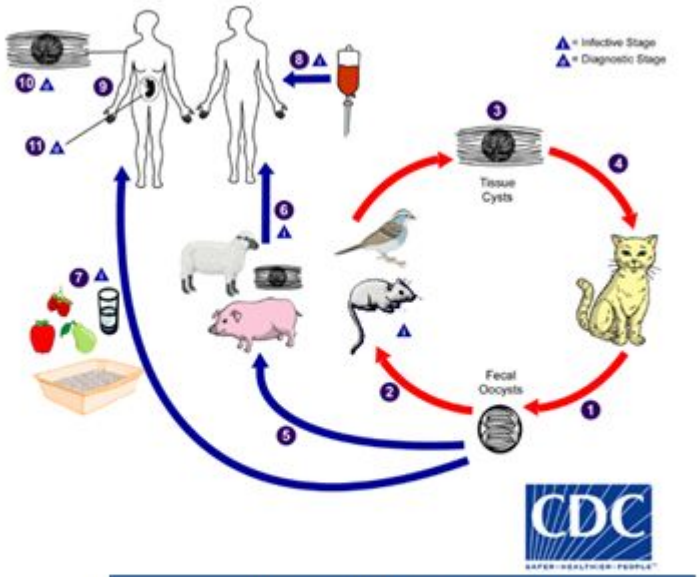


Life cycle of Toxoplasma gondii

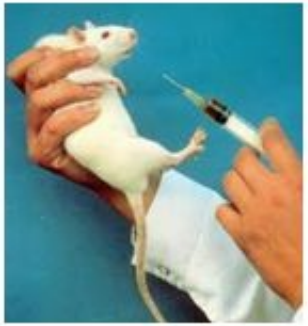
Strain variation (>100 strains)

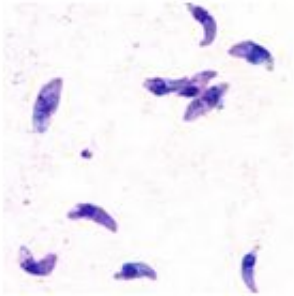
- *Toxoplasma gondii* displays a highly clonal population structure, with >95% of the strains that have been isolated from North America and Europe grouped into one of three lineages, types I–III.

T. gondii strain	Genetic type (ToxoDB PCR-RFLP genotype #)	No. of tissue cysts measured	Size (diameter) in µm	
			Mean	SD
TgGoatUS4	Type III (#2)	550	36.14	4.22
GT1	Type I (#10)	688	38.15	2.95
TgNmBr1	Type II (#1)	373	40.78	3.06
CT1	Type I (#10)	204	40.92	8.83
VEG	Type III (#2)	900	42.48	2.24
TgCTPrC3	Atypical (#18)	625	43.54	1.57
TgBbUS1	Atypical (#147)	625	49.19	3.41
TgRabbitBr1	Atypical (#19)	177	49.23	8.38
ME49	Type II (#1)	632	49.65	2.54
TgCatCo1	Type I (#28)	476	51.39	4.91
TgPigUS15	Atypical (#8)	12	65.33	11.34

Hermanns et al. 2015. Cellular Microbiology published by John Wiley & Sons Ltd, Cellular Microbiology, 18, 244–259

- Even though strains show approximately 98% genetic homology, they display dramatic differences in virulence.
- All acutely virulent strains in mice, with LD100 of <10 organism, belong to the type I lineage.

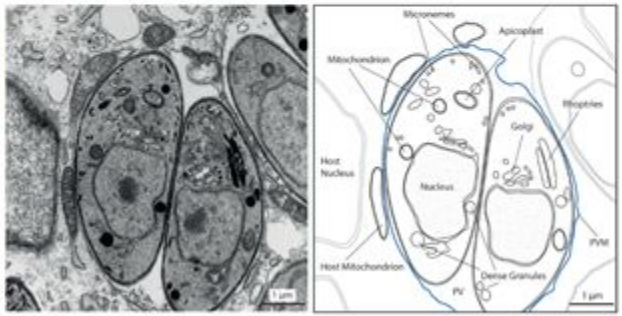




- Type II and III strains are relatively non-virulent or mildly virulent in mice, with an LD50 ranging from 102 to 105 infectious organisms.
- Type II strains are most common in human infection, while type III strains are most common in wild animals and are rarely isolated from humans.
- Type I strains are rare both in the wild and in human patients; however, several severe clinical cases of toxoplasmosis in immunocompetent individuals were found to be associated with type I strain infections

- Analysis and fine mapping of Toxoplasma genome narrowed the responsible region to 140kb containing 21 genes on chromosome VII.
- Analysis of the 21 genes in this region revealed *ROP18* to be the only gene polymorphic enough to be a viable candidate responsible for the virulence phenotype.

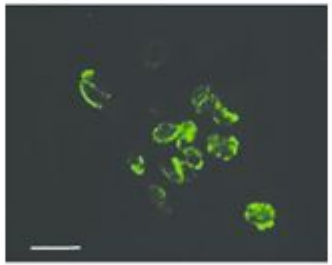
ROP18 stand for Rhoptry protein 18



- The genome of more than 60 strains of *T. gondii* have been sequenced. Most are 60-80 Mb in size and consist of 11 - 14 chromosomes.
- The major strains encode 7800-10,000 proteins , of which about 5200 are conserved across RH, GT1, ME49, VEG.

Toxoplasma genome info.

- The type I allele of the ROP18 gene was sufficient to confer the acutely virulent phenotype, as type III transgenic parasites expressing the type I ROP18 allele showed a 4–5 log increase in virulence compared with the type III parental strain.



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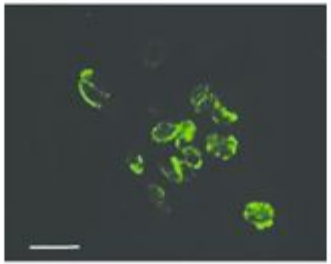
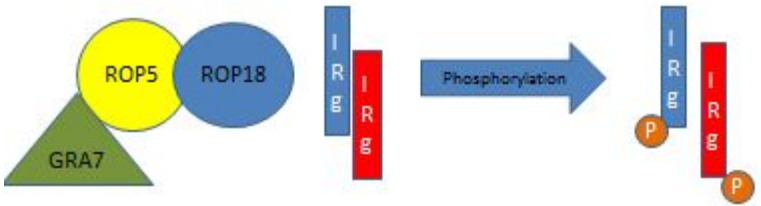
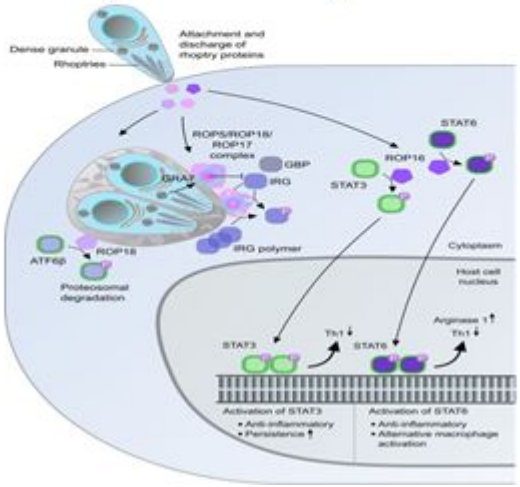


- ROP18 targets the interferon-inducible immunity related GTPase (IRG).
- ROP18 phosphorylates and inactivates, IRga6 and Irgb6, blocking their recruitment to the parasitophorous vacuole membrane and preventing destruction of the parasite.

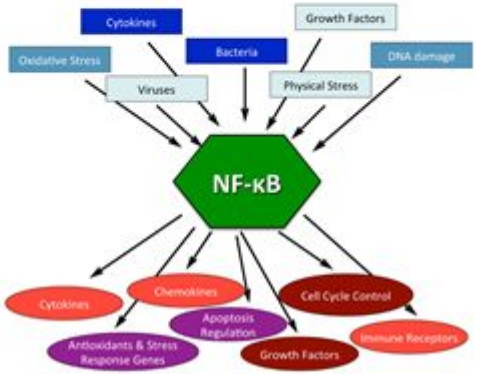
- Loading of IRG proteins onto the parasitophorous vacuolar membrane (PVM) is required for vacuolar rupture resulting in parasite clearance.
- In virulent strain (e.g. type I) infections, polymorphic effector proteins ROP5 and ROP18 cooperate to phosphorylate and thereby inactivate mouse IRG proteins to preserve PVM integrity.



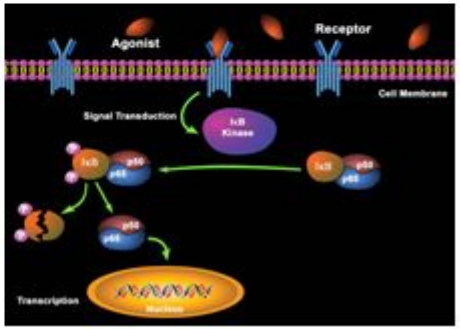
- A study confirmed that dense granule protein GRA7 as an additional component of the ROP5/ROP18 kinase complex and identified GRA7 association with the PVM by direct binding to ROP5.



- NF-κB is a family of dimeric transcription factors and central components of innate and adaptive immunity, responsible for the activation of many genes required in infection, stress and injury.
- The NF-κB family of transcription factors comprises five members: p50 (NF-κB1), p52 (NF-κB2), p65 (Rel A), (Rel B), and (Rel C).



- In the absence of inflammatory stimuli, NF-κB is maintained in an inactive form through binding to an inhibitor of NF-κB (IκB).
- Once the cells are stimulated, IκB is rapidly phosphorylated, ubiquitinated, and degraded, facilitating the subsequent translocation of NF-κB to the nucleus to induce a wide array of genes critical in the immune response and inflammation.



- Some studies have shown that type I strains inhibit NF-κB pathway and the recruitment and activation of immune cells, resulting in the enhanced survival of the parasites.
- These studies showed that infection of mammalian cells with the type I strain results in the activation of IKK and degradation of IκB.

- However, despite the initiation of NF- κB signaling, infection with *T. gondii* did not lead to the activation but termination of NF- κB. The reason for disabling NF-κB is associated with blocking of p65 translocation to the nucleus.
- ROP18 phosphorylates p65 at Ser468 and targets this protein to the ubiquitin- dependent degradation pathway.

- ROP18 kinase- deficient type I parasites displayed inability to inhibit NF-κB, with enhanced production of IL-6, IL-12 and TNF-α in infected macrophages.
- Other studies have shown that transgenic parasites deficient of ROP18 induced M1-biased activation.
- These findings demonstrate that the virulence factor ROP18 in type I strains is responsible for inhibiting the host NF-κB pathway and for suppressing pro-inflammatory cytokine expression, thus providing a survival advantage to the infectious agent.


Thank You!