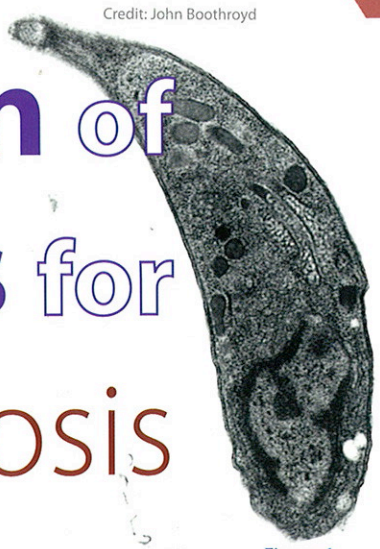
Identification of Virulence Factors for Toxoplasmosis



Credit: John Boothroyd

Figure 1

by Sandeep Ravindran

Somewhere between 20 and 60% of the world's population is thought to be infected with the parasite *Toxoplasma gondii*, though most people never even realize they're infected.

Although usually harmless, this single-celled protozoan parasite is capable of causng severe disease. Recently, researchers in Jon Boothroyd's lab in the Department of Microbiology and Immunology at Stanford have identified some of the factors that determine why *Toxoplasma* is sometimes harmless or deadly.

The Ubiquity of *Toxoplasma*

Toxoplasma (see Figure 1) is able to live inside the cells of all warm-blooded animals including humans, making it an extraordinarily widespread parasite. It is found all over the world (see Figure 2), and is thought to infect over 60 million people in the United States alone.

Upon initial infection, this parasite causes a disease called toxoplasmosis that is usually no more severe than the flu. Then, the parasite lies dormant, usually in the person's brain. However, if a person becomes immunocompromised by a disease such as AIDS, the parasite may then reactivate and cause severe damage to the brain, eyes and other organs (see Figure 3). In mothers infected during early pregnancy, *Toxoplasma* can cause terrible birth defects and lead to the spontaneous abortion of fetuses.

Researchers study *Toxoplasma* in order to improve early detection of infection, find a treatment for toxoplasmosis, and to help understand related parasitic diseases. *Toxoplasma* is closely related to *Plasmodium*, the parasite that causes malaria. Researchers hope that the study of *Toxoplasma* will shed light on Plasmodium and aid the fight against malaria.

Toxoplasma occurs in nature as three distinct strains: Types I, II and III. Interestingly, these strains differ in the severity of the disease they cause. It takes only a single Type I parasite to kill a mouse, whereas it takes nearly 100,000 Type III parasites to produce the same effect. It was unclear to

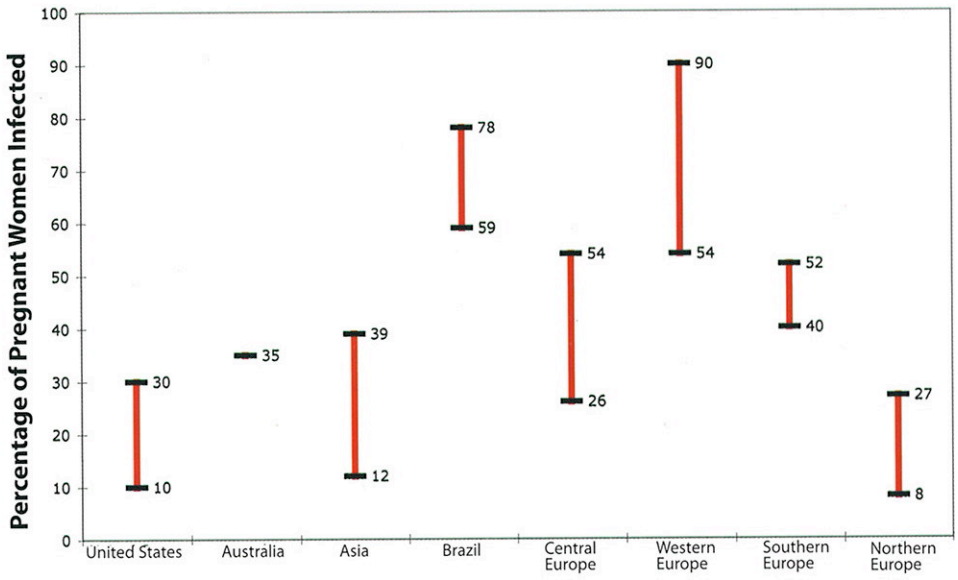


Figure 2: Ranges of *Toxoplasma* prevalence in pregnant women around the world

Credit: Sandeep Ravindran, Stanford University Data from Gross, U. 2004.

scientists how infection by different strains of the same parasite could lead to such wildly different disease outcomes. This question inspired researchers in the Boothroyd lab to undertake a detailed analysis of how these three parasite strains differ from one another.

Finding Differences Between Strains

Researchers received their first clue from the observation that similarly, variances existed between strains of other microbes, such as bacteria and viruses. For example, in bacterial pathogens such as *Escherichia coli* and *Salmonella*, some strains are harmless while others cause deadly disease in humans. Both the harmless and harmful strains inject proteins into the host cells. The difference in virulence can be attributed to differences in the proteins injected. Slight changes in these proteins can allow an otherwise harmless bug to cause a deadly disease.

> *Toxoplasma* is found all over the world, and is thought to infect over 60 million people in the United States alone.

Previous work had shown that, like bacteria and viruses, *Toxoplasma* was able to manipulate its host cell. It seemed likely that it also did this by protein injection. The Boothroyd lab hypothesized that the more aggressive strains might have different versions of these proteins and that those differences might be responsible for the variation in the disease caused by the three strains.

To test their hypothesis, they shifted their attention from proteins to the genes that encode them. The Boothroyd lab used microarrays to monitor the activity of human and *Toxoplasma* genes in a human cell infected with one of the three *Toxoplasma* strains. Based on the activity of the genes, the researchers could infer how abundant a protein may be during parasitic infection. By looking for genes that were more abundant during infection with the deadly type I strain, they were able to identify two *Toxoplasma* genes, ROP16 and ROP18, that coded for the proteins associated with the strain-specific changes to the host cell.

Both ROP16 and ROP18 are kinases, a class of enzymes involved in regulating a wide range of cellular processes. This finding was one of the first examples of a parasite injecting its own kinases into a host cell during invasion. It's like *Toxoplasma* is sending its own workers in disguise into the vast factory that is the cell. The disguised workers take control of the machines already present in the host and use the machines to produce what the pathogen needs to survive. It is of particular significance that upon injection into the host cell, ROP16 actually ends up in the host's nucleus (see Figure 4). The nucleus is the command center of the cell, and is an ideal place for a parasite protein to take control. The proteins *Toxoplasma* sends into the cell might not just be workers, but in fact managers, taking control of the whole factory itself.

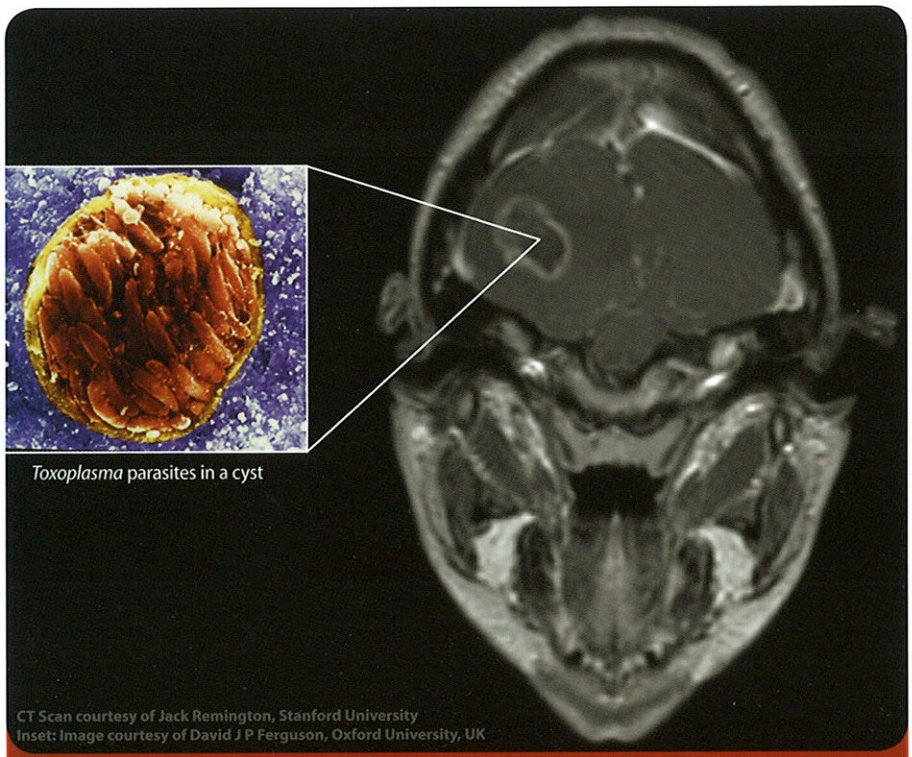


Figure 3: Computerized Tomography (CT) scan showing brain lesions caused by *Toxoplasma* parasites coming out of dormancy.
Inset: Electron micrograph of *Toxoplasma* parasites in their dormant phase

The researchers found that it's not just a simple case of strain I possessing ROP16 and ROP18 and the other strains lacking them. The different strains actually have their own specific versions of ROP16 and ROP18.

The Boothroyd lab believes that these different versions of the proteins have evolved to optimize infection of different host species. A parasite like *Toxoplasma* has evolved to take over its host cell in a finely regulated manner. If it is not forceful enough, the host is able to fight it off; if it is too forceful, it kills the host cell, and as a result, the parasite dies too. Thus, severe disease-causing strains actually may be a result of the strain injecting a protein optimized for one host into a different host that is not adapted to it.

Professor Boothroyd clarified, "The negotiator proteins that [*Toxoplasma*] injects into a cell of one host species can just be too forceful in the 'wrong' host, sort of like sending a SWAT team to handle a kindergarten disagreement." Boothroyd continued, "What gets me really excited is that we now know how different strains of

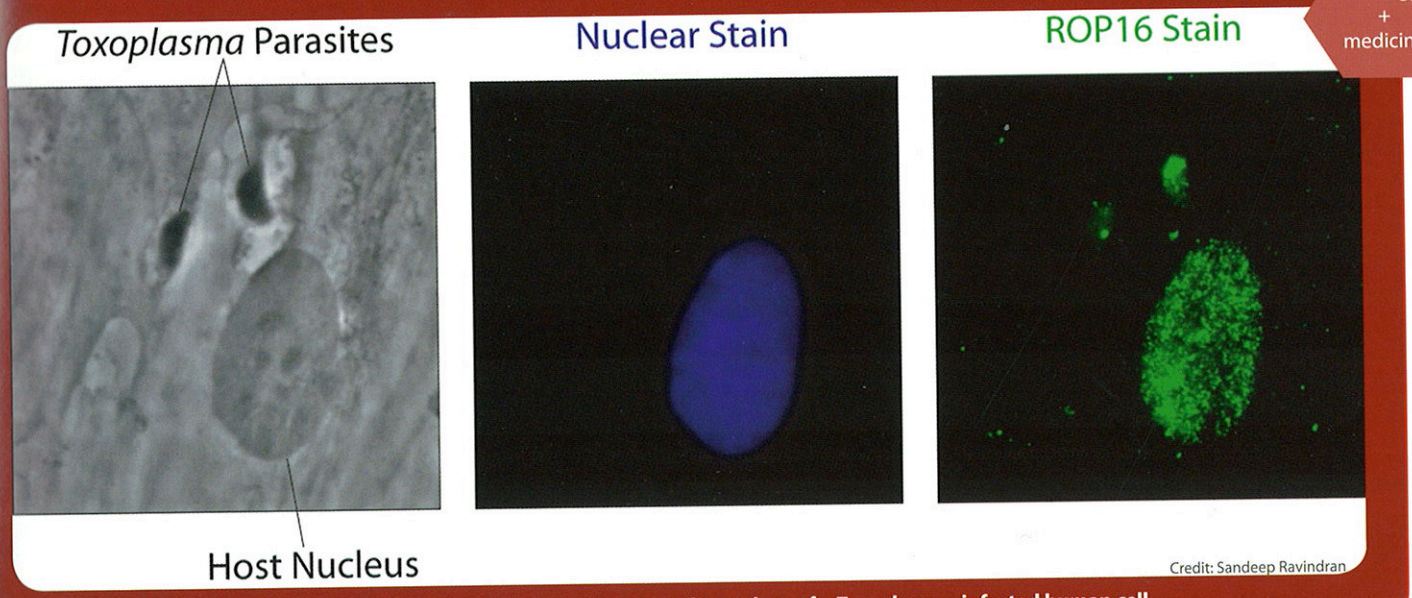


Figure 4: Fluorescence microscopy images showing ROP16 staining the nucleus of a *Toxoplasma* –infected human cell

the parasite end up producing very different diseases." The group's results appeared in the December issue of *Science* and the January issue of *Nature*.

Research Implications

According to Dr. Jeroen Saeij, the first author of the paper published in Science, the importance of the research lies in the fact that "*Toxoplasma* is able to modulate the host cell by injecting proteins into the host cell." Dr. Jon Boyle, the first author of the Nature publication, also noted, "Differences in the virulence of *Toxoplasma* strains have been well described, but we had no idea what kinds of genes were responsible for these differences. This finally gives us a gene-by-gene context for understanding how virulence works."

"Knowing with which strain someone is infected is important because different therapies might be needed depending on the strain a person is infected with," Saeij noted. Boyle also pointed out, "This may lead to better predictions about the severity of human infections, especially in cases where the developing fetus is at risk." If someone is found to be infected with a highly virulent strain, they can immediately be given more aggressive treatment before the disease becomes more severe.

Another important application for the lab's findings are that these proteins are potential targets for more specific and effective anti-toxoplasmosis drugs. Additionally, the fact that just two of these proteins are able to dramatically influence the host provides clues to the virulence mechanisms of *Toxoplasma*. The lab plans to continue using these techniques to identify other proteins that allow *Toxoplasma* to cause disease. By identifying all the genes involved in *Toxoplasma* virulence, there is hope that this could potentially lead to therapeutic advances against both toxoplasmosis and other parasitic diseases like malaria. S

> "What gets me really excited is that we now know how different strains of the parasite end up producing very different diseases." – *Boothroyd*

Both researchers pointed out an immediate application of their work, which is to use ROP18 to identify which strain a person has contracted. Currently, physicians check for *Toxoplasma* infection by looking for antibodies generated by the human body in response to the parasite. However, they look for general *Toxoplasma* antibodies which are common to all three strains. As a result, they can identify whether a person is infected with *Toxoplasma*, but they can not identify which strain it is. Boyle explained how ROP18 may be used to identify which strain a person has: "Other proteins have been used for this, but ROP18 has a marked advantage because in non-virulent strains (like Type III) this gene is expressed at very low levels (if at all). Therefore, the presence of antibodies to this protein would be a clear indication that a virulent strain was responsible for the infection."

SANDEEP RAVINDRAN *is a fourth year graduate student in Microbiology & Immunology. In addition to science writing, he enjoys visiting new places and hanging out with friends.*

To Learn More

-- Please visit the departmental website of Dr. John Bothroyd: **http://cmgm.stanford.edu/micro/boothroyd/boothroydlabdesc.html**

-- Please read: Saeij; JP. Boyle, JP. "Polymorphic secreted kinases are key virulence factors in toxoplasmosis." *Science*. 2006 Dec 15;314(5806):1780-3

-- Read: Saeij, JP. Coller, S. et al "Toxoplasma co-opts host gene expression by injection of a polymorphic kinase homologue." *Nature*. 2007 Jan 18;445(7125): 324-7

layout design: Annie Lee