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The early years of *Toxoplasma* research: What's past is prologue

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Abstract

In the century since the first description of *Toxoplasma gondii*, history and circumstance have led scientists to define this organism in diverse contexts. From its discovery by researchers shaped by early 20th century versions of the germ theory to its more recent roles as an important globally distributed pathogen and a model apicomplexan, our definitions of *Toxoplasma* are as much a reflection of our frame of reference as they are an absolute definition of this organism. Although these transformations act as portals for new avenues of investigation, the essential questions that inform current research are founded in the work of early investigators who studied *Toxoplasma*.

Keywords

Toxoplasma gondii; Anton van Leeuwenhoek; Charles Nicolle; Alfonso Splendore

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Infectious disease is one of the few genuine adventures left in the world. The dragons are all dead and the lance grows rusty in the chimney corner.... About the only sporting proposition that remains unimpaired by the relentless domestication of a once free-living human species is the war against those ferocious little fellow creatures, which lurk in dark corners and stalk us in the bodies of rats, mice and all kinds of domestic animals; which fly and crawl with the insects, and waylay us in our food and drink and even in our love.

-- Hans Zinsser, *Rats, Lice and History*, 1935

Over 200 years before *Toxoplasma gondii* was first described, the backdrop was set for this discovery by the pioneering microscopist Anton van Leeuwenhoek (1632–1723). In addition to the earliest description of bacteria, Leeuwenhoek is credited with being the first to observe a number of protozoa, including both free-living (*Tetrahymena*) and pathogenic (*Giardia*) organisms (Fred, 1933; Ford, 1982; Corliss, 2002). It is also likely that Leeuwenhoek was the first scientist to observe an apicomplexan, although his account is far less charming than his later depiction of *Giardia*. “I examined the bile from three old rabbits. The first had a very few small globules, but very many oval corpuscles... In the bile of the two other rabbits there was nought but globule...” Leeuwenhoek’s description of the “oval corpuscles” occurs in a 1674 letter to the Royal Society of Great Britain. Clifford Dobell (1886–1949), a parasitologist at the Imperial College, London and later at the Medical Research Council who was an expert on

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intestinal parasites of man and biographer of Leeuwenhoek (Hoare and Mackinnon, 1950; Ford, 1986) pointed out that these inclusions are consistent with an infection of one of the rabbits by the coccidian *Eimeria stiedae*. Dobell concluded that “I think that there can be no doubt that the ‘oval corpuscles’ – called *eijronde deeltgens* in the original – which Leeuwenhoek discovered in the gall-bladder of one of his ‘three old rabbits,’ were the oocysts of the coccidian *Eimeria stiedae*...” (Dobell, 1932). Although many *Eimeria* species infect the intestine, *E. stiedae* replicates in the bile duct epithelia; zygotes lyse from host cells into the bile and oocysts are expelled into the feces, consistent with the location of the Leeuwenhoek’s corpuscles.

The tangled chronology of the discovery of apicomplexans is extensively described by Norman Levine in chapters of the books ‘The Coccidia: *Eimeria*, *Isospora*, *Toxoplasma* and Related Genera’ and ‘The Biology of the Coccidia’ (Hammond and Long, 1973; Long, 1982). After Leeuwenhoek’s chance observation of *E. stiedae*, the Gregarines were the next apicomplexans to be discovered in 1787 by Cavolini and in 1828 by Dufour. Dufour described both gamont and cyst stages but did not recognize the connection between these forms, a problem that reoccurred with many other members of this group. Coccidia were described by many scientists throughout the 1800s, with the term “coccidia” originally being used to name a species (*Coccidium oviforme*) based on observations of oocysts. Since the schizonts of the same organism had previously been called both *Monocystis stiedae* and *E. stiedae*, the term coccidia is now only used to describe the group of parasites in the suborders Adeleorina and Eimeriorina..

Toxoplasma was discovered by scientists in the early 20th century working in the intellectual context of the studies of Robert Koch (1843–1910) and Louis Pasteur (1822–1895). Koch’s work to isolate defined pathogens associated with specific infectious disease syndromes and Pasteur’s studies to promote immunity with convalescent sera or vaccination with attenuated strains informed many of the approaches to infectious disease research at the time. It was an era in which physician-scientists were frequently deployed overseas to colonial territories or other destinations of economic interest to their British, European or North American home countries to study diverse infectious agents endemic to these areas. The scientists recognized as first describing *Toxoplasma* are Charles Jules Henry Nicolle (1866–1936) and Louis Herbert Manceaux (1865–1934) who were based in Tunisia and Alfonso Splendore (1871–1953) who worked in Brazil. However, some historians have also credited two other researchers with the discovery of *Toxoplasma* (Binazzi, 1986; Cox, 2002). In 1908, the same year as the papers by Splendore and Nicolle and Manceaux were published, Samuel Taylor Darling (1872–1925) observed what may have been *Toxoplasma* in a human muscle biopsy, but identified it as *Sarcosporidia* (Darling, 1909). Alphonse Laveran (1845–1922) who identified *Plasmodium* as the agent of malaria may also have observed *Toxoplasma* prior to its description by Splendore, Nicolle and Manceaux (Laveran, 1900; Bruce-Chuvatt, 1981; Nye, 2002). The organisms he detailed in 1900 were in the nucleated red blood cells of the paddy bird *Padda oryzivore*. (This attribution may not be correct since although *Toxoplasma* will invade the organelle-free red cells of mammals, it doesn’t develop. Since bird red cells are nucleated, but lack organelles, it is unlikely that the parasites Laveran observed were *Toxoplasma*, but are likely to be another apicomplexan such as a *Hepatozoon* species.)

It is instructive to consider the lives of these scientists (Nicolle, Manceaux, Splendore, Laveran and Darling), because their common experiences illustrate the nature of infectious disease research at this time. All five men were physician-scientists working overseas. Nicolle, Manceaux and Laveran were French, working in North Africa (Tunisia and Algeria) (Kean et al., 1978; Bruce-Chuvatt, 1981; Nye, 2002; Pelis, 2006). Samuel Darling was an American working in Panama during construction of the canal and Alfonso Splendore was an Italian who spent the bulk of his scientific career in Brazil (Chaves-Carballo, 1970; Kean et al., 1978;

Chaves-Carballo, 1980; <http://www.area.cs.cnr.it/imseb/malaria/grassi/splend/>). Splendore, Darling, Laveran and Nicolle are better known for their work on other infectious agents than for their discovery of *T. gondii*. Splendore's work on South American blastomycosis (also known as Lutz-Splendore-Almeida disease) demonstrated that the causal pathogen was a fungus, *Zimonema brasiliense* (now known as *Paracoccidioides brasiliense*). Splendore worked on a number of infectious disease conditions including leishmaniasis, sporothricosis and yaws (*Treponema pertenue*) in addition to his studies on *Toxoplasma*. During his service in Panama, Darling described *Histoplasma capsulatum* infection in humans and worked on endemic *Anopheles* species which served as vectors for malaria (Riley and Watson, 1926; Chaves-Carballo, 1970, 1980). Laveran discovered the agent of malaria and was awarded the 1907 Nobel Prize in Physiology or Medicine for his work on parasitic agents of disease (Bruce-Chuvatt, 1981; Nye, 2002). Similarly, Nicolle was awarded the 1928 Nobel Prize in Physiology or Medicine for his 1909 elucidation of the louse as the vector for transmission of epidemic typhus (*Rickettsia prowazeki*) and Hans Zinsser's epic book on typhus, *Rats, Lice and History*, was dedicated to Nicolle in acknowledgement of these studies (Gross, 1996). Nicolle carried out research on a number of endemic infectious diseases: relapsing fever (*Borrelia*), trachoma (*Chlamydia trachomatis*), kala azar (*Leishmania*) and epidemic typhus (Pelis, 2006). He was also one of the scientists to develop the concept of the asymptomatic infection (symptomless individuals can transmit disease) (Garnham, 1977). The life and work of Louis Herbert Manceaux is less well documented but it is known that after medical training, he was a military physician on active duty in medical corps during World War I. He retired from the army to join the Pasteur Institute and to practice medicine in Paris (Kean et al., 1978).

Charles Nicolle was born September 21, 1866 in Rouen, France, the son of a physician while Alfonso Splendore was born in Fagnano Castello, Italy on April 25, 1871 (Pelis, 2006; <http://www.area.cs.cnr.it/imseb/malaria/grassi/splend/>). There are many parallel aspects to the lives of Splendore and Nicolle. Both men studied medicine with an emphasis on microbiological research. Splendore studied at the University of Rome where he then continued to work at the Institute of Hygiene with the noted malariologists Angelo Celli (1857–1914), Claudio Fermi (1862–1952) and Giovanni Battista Grassi (1854–1925). Nicolle's path to research was a deliberate reorientation after he discovered (while learning to auscultate patients) that he was losing his hearing (Pelis, 2006). He compensated for this by focusing his studies on laboratory medicine at the Pasteur Institute Paris where he worked with Emile Roux (1853–1933) and Elie Metchnikoff (1845–1916). At age 36, Nicolle was appointed as director of the Pasteur Institute in Tunis where he remained for his scientific career. Alfonso Splendore moved to Sao Paulo in 1899 where he worked as a physician and carried out tropical disease research with Professor Adolfo Lutz (1855–1940) of the Bacteriological Institute. He founded the laboratory of the Umberto I Hospital and later became director of the laboratory at the Portuguese Charity Hospital.

Splendore returned to Italy in 1912 where he was Professor of Bacteriology at the Universities of Rome and Parma for 8 years. During World War I, he was able to exploit a form of biological control to avert an invasion of voles which had nearly destroyed the harvest in southern Italy (<http://www.area.cs.cnr.it/imseb/malaria/grassi/splend/>). He observed high mortality among these rodents and hypothesized that the deaths were due to a pathogen which he named *Bacterium pitymys*. By releasing animals that were captured and infected in the laboratory he facilitated disease spread to eliminate the voles. Curiously, this pathogen may have been another coccidian parasite: there is no organism known as "*Bacterium pitymys*" but Norman Levine attributes discovery of an apicomplexan (*Sarcocystis pitymys*) to Splendore in 1918 (Splendore, 1918; Levine, 1986, 1988).

Alfonso Splendore moved back to Brazil in 1920 where he resumed his medical practice and research. In the autumn of 1925, Charles Nicolle traveled to Argentina, Brazil and Uruguay

(Pelis, 2006). His travels were inspired by an invitation to Buenos Aires from Carlos Chagas (1879–1934), newly appointed director of the Oswaldo Cruz Institute. Nicolle traveled first class and brought the agents of typhus and canine kala azar with him in infected guinea pigs and dogs as a gift to the scientists in South America. During this visit, Nicolle visited numerous research institutes and hospitals and, intriguingly, it is possible that in these travels he encountered Splendore in Brazil. No such meeting is documented, but scientific circles were very small at this time, favoring the notion of such an encounter.

After his return to Brazil in 1920, Splendore lived in the Italian community of Sao Paulo until his death in 1953. He is remembered in his birthplace, Fagnano Castello, where the main square is named in his honor and in his adopted country with a street (Rua Dr. Alfonso Splendore) in Sao Paulo (<http://www.area.cs.cnr.it/imseb/malaria/grassi/splend/>). Charles Nicolle became an influential proponent of research done at the Pasteur Institute in Paris but was a harsh critic of its leadership. He remained director of the Pasteur Institute in Tunis until his death in 1936 and was buried behind the entrance to the Institute in Tunis (Pelis, 2006).

Both Splendore and Nicolle observed *Toxoplasma* as a lethal infection in captive rabbits and gundis used for experimental infections. These laboratory animals may have acquired a particularly virulent strain of *Toxoplasma* from cysts shed by cats that were frequently employed to eliminate escaping laboratory animals. Studies by Eduard Chatton and Georges Blanc indicated that gundis in the wild were not detectably infected with *Toxoplasma*, and it is well-established that many investigators, including Nicolle, kept cats as vermin hunters in animal quarters, suggesting that food or bedding might have been contaminated with oocysts (Chatton and Blanc, 1917). An alternative explanation might be that the lethal infections were caused by a sudden increase in parasite load in caged (potentially overcrowded and stressed) research animals. Norman Levine makes the argument that visible coccidial infection is an artificial phenomenon, “the result of abnormal overcrowding of a single host species in a limited area. Only under these circumstances do animals become infected with enough oocysts to become ill.” (in Hammond and Long, 1973). Since *Toxoplasma* can cause a symptomless illness, the acute infection of rabbits and gundis in captivity may have contributed to the discovery of *Toxoplasma*. It is also interesting to note that although *Toxoplasma* is distributed throughout the world, it was discovered by researchers who were sensitized to the existence of novel pathogens in their search for the agents of exotic diseases. One explanation might be that in the United States, the United Kingdom and Europe, scientists who observed *Toxoplasma* in animal tissues misidentified it as other, previously characterized, coccidian species. Pasteur’s oft-quoted observation that *dans les champs de l’observation le hasard ne favorise que les esprits préparés* (in the field of observation, chance favors the prepared mind) truly reflects the receptivity of these transplanted investigators to the diagnosis of new-found rather than previously characterized pathogens.

It is important to remember that the coccidian nature of *Toxoplasma* was not revealed for another 50 years (see articles by Dubey (2009) and by Ferguson (2009)), so the initial classification of the parasite was based on comparisons with other well-described protozoans. At the turn of the 20th century, the Cambridge parasitologist George Henry Faulkner Nuttall (1862–1937) was the leading researcher on piroplasms and founder of the Molteno Institute of Biology and Parasitology at Cambridge University. Nuttall’s published descriptions of the replication cycle of piroplasms were sufficiently similar to what Nicolle observed with the parasites obtained from the gundis (*Ctenodactylus gundi*), that Nicolle provided a preliminary classification as *Piroplasma quadrigeminum* (Nicolle, 1907). However, it is clear from one of Nuttall’s retrospective articles that he had disagreed with Nicolle’s classification (Nuttall, 1913), which probably influenced Nicolle to pursue another line of investigation. Upon further investigations of the parasites in liver tissue preparations, Nicolle observed “Les éléments parasités sont toujours des mononucléaires” (the cells that parasites invade are always in

mononuclear) and “la présence fréquente de deux corps chromatiques: grand et petit karyosomes” (frequently observed presence of two chromatin bodies: large and small karyosomes) (Nicolle and Manceaux, 1908). The association of the parasite with monocytes and the presence of two chromatin-staining bodies are certainly reminiscent of *Leishmania* species, leading to the suggestion that the organisms be identified as *Leishmania gondii*. The “petit karyosome” (currently known as the apicoplast) would not be definitively described for another 90 years and it is now abundantly evident that the apicoplast genome is unrelated to the kinetoplast DNA of *Leishmania*. Splendore also initially concluded that the parasites he isolated from a rabbit were *Leishmania* (Splendore, 1908). Nicolle, Manceaux and Splendore continued to pursue more extensive microscopic analyses of the parasite from experimental infections in a variety of tissues and concluded that their parasite was indeed something completely different, “et nous proposons, pour désigner ce genre nouveau, le nom de *Toxoplasma* (de τοξον, arc). Le nom du parasite du Gondi sera donc *T. gondii*.” (and we propose to name this new type *Toxoplasma* (arc). Thus we will call the parasite of gundis *T. gondii*.) (Nicolle and Manceaux, 1909). Splendore agreed with Nicolle’s and Manceaux’s assessment that his rabbit-derived parasite was “morphologiquement identique” to the newly named *T. gondii* (Nicolle and Manceaux, 1909) and used the establishment of a new genus to name his parasite *Toxoplasma cuniculi* (Splendore, 1910). After these fundamental studies, *Toxoplasma* was isolated from a variety of host species including humans and the striking similarity of parasites reported led to the consensus that all the isolates should be grouped as a single genus-species, *T. gondii*.

The phylogenetic placement of *Toxoplasma* remained a thorny question for nearly 60 years. Habegger’s comment of 1953 concerning phylogeny as “le probleme le plus ancien et le plus obscur dans l’etude de la toxoplasmose” (the oldest and most obscure problem in the study of toxoplasmosis) is cited in a 1956 article that discusses evidence for and against *Toxoplasma* to be classified with *Mastigophora*, with *Trypanosomidae*, with unclassified *Sporozoa*, with the Babesiidae, between the orders Coccidiida and Adeleidea, near the Coccidia, and between *Babesiidae* and *Haemogregarinidae* (van Thiel, 1956). Elucidation of the relationship of *Toxoplasma* to other coccidia required discovery of the sexual cycle stages (see articles by Dubey (2009), by Ferguson (2009) and by Dubremetz and Ferguson (2009)). The initial investigations of phylogenetic relationships have been transformed into diverse current areas of investigation that include *Toxoplasma* population structures, evolution of the tissue cyst stage and acquisition of the apicoplast (see articles by Grigg and Sundar (2009) and by Boothroyd (2009)).

Although the elucidation of the *Toxoplasma* sexual cycle only occurred in the late 1960’s, earlier cell biological studies, particularly ultrastructural studies carried out in the 1950s and 1960s suggested that *Toxoplasma* was related to coccidian parasites. These studies also revealed a wealth of information about novel organelles of the Apicomplexa and defined the novel process of parasite replication termed endodyogeny (see article by Dubremetz and Ferguson (2009)). Advances in cell biological techniques have powered the transition from histological samples to electron microscopy samples, immunofluorescence microscopy and ultimately living fluorescent protein-tagged parasite lines that can be viewed in real time. Although *Toxoplasma* was passaged in animals by the earliest investigators, later technical advances made it possible to culture the parasite in embryonated chicken eggs (1929) and in tissue culture (1937) (Levaditi et al., 1929; Sabin and Olitsky, 1937). The ability to pass *Toxoplasma* through animals and to grow it in cell culture have led to cell biological, biochemical and genetic studies and the exploitation of *Toxoplasma* as an experimentally tractable model organism to study common aspects of apicomplexan biology (see articles by Sibley (2009), Boothroyd (2009), and by Boyle and Radke (2009)).

Toxoplasma was first recognized to be a human pathogen 20 or so years after its initial description in animal infections. In 1923, the Czechoslovakian ophthalmologist Josef Jank (1886–1963) described treating an 11 month old child who was suffering from hydrocephalus at the Czech Children's Hospital in Prague. The boy's eyes contained striking retinal lesions and post-mortem histology of the eyes showed evidence of inflammation and "numerous small, dark brown spots, droplike and ringlike small shapes, which represented sporozoites..." (translation in Kean et al., 1978). Although Janku could not identify the parasites, when similar clinical findings were observed in 1939 by Abner Wolf, David Cowin and Beryl Page at Columbia University, (USA), they were able to transfer the infection from post-mortem tissues to rabbits, mice, chicks and guinea pigs. Many other critically important medical studies followed (see article by Weiss and Dubey (2009)), which demonstrated that *Toxoplasma* infection could cause serious birth defects in children when acquired in utero and that adults could acquire an infection that caused ocular damage. With the advent of the AIDS epidemic nearly 30 years ago, *Toxoplasma* became an often diagnosed opportunistic infection in immuno-compromised adults and in many circumstances was the first medical indicator that the patient had a weakened immune system. The discovery that *Toxoplasma* is a human pathogen made research on pathogenesis, on virulence and on how the immune system controls infection essential areas of current research.

Since many coccidian species are agricultural pathogens, early investigations were directed towards identifying inhibitory agents that could be used in agricultural settings to treat infections in cattle, sheep and chickens. In 1939, sulfonamides were found to inhibit *Eimeria tenella* growth in chickens (Levine, 1939). Sulfonamides had been identified as antibacterial agents a few years earlier by scientists following up on Paul Ehrlich's concept of dye-based drugs at Bayer AG in Germany. Due to the high cost of sulfonamides during World War II (all American soldiers were given a first aid kit containing sulfa powder and told to apply it to any open wound), these compounds were not exploited by the poultry industry until after the war ended (Long, 1982; Hager, 2006). The utility of sulfonamides to treat experimental *Toxoplasma* infection of mice was established by Sabin and Warren in 1942 (Sabin and Warren, 1942). Eyles and Coleman demonstrated the synergism of sulfonamides with pyrimethamine in 1953, which has proven to be the most effective treatment to date for human infections (Eyles and Coleman, 1953). The increased incidence of *Toxoplasma* infection in AIDS patients (particularly prior to highly active antiretroviral therapy) and the exploitation of *Toxoplasma* as a model organism for other apicomplexan pathogens has led to greatly increased research to identify novel parasite-specific drug targets for new therapeutics (see article by Boothroyd (2009)).

Present research on *Toxoplasma* is embedded in a variety of conceptual frameworks, ranging from its role as a frank human pathogen to its exploitation as a model organism. In addition, *Toxoplasma* has gained unforeseen popular currency, ranging from attention-catching studies suggesting that infection changes the behavior of humans to its central role in the tragic Irvine Welsh novel "Trainspotting" where an HIV-infected heroin addict acquires a lethal *Toxoplasma* infection from a kitten he adopts. Despite the inaccuracy in Welsh's book (in the vast majority of immunocompromised patients, toxoplasmosis is the result of reactivation of latent parasites rather than a new infection) this example might have intrigued Charles Nicolle, who wrote seven novels and a number of short stories (Pelis, 2006). A website devoted to the parasite lists eight "references in fiction" for *Toxoplasma* ranging from television medical drama to science fiction (<http://alltoxoplasma.blogspot.com/>). Whether the parasite serves as a fictional device, or as an experimental organism, during the past century it has captured the attention and imagination of an increasing number of diverse thinkers and has occupied an expanding number of roles that serve to increase our understanding of evolution, virulence, pathogenesis and cell biology. Given its multifaceted identity, the next century of *Toxoplasma* research promises to be even more informative.

References

- Binazzi M. Historical aspects of cutaneous toxoplasmosis. *Int J Dermatol* 1986;25:401–404. [PubMed: 3531045]
- Boothroyd JC. *Toxoplasma gondii*: 25 years and 25 major advances for the field. *Int J Parasitol* 2009;39.[in this issue]
- Boyle JP, Radke JR. A history of studies that examine the interactions of *Toxoplasma* with its host cell: Emphasis on in vitro models. *Int J Parasitol* 2009;39.[in this issue]
- Bruce-Chuvatt LJ. Alphonse Laveran's discovery 100 years ago and today's global fight against malaria. *J R Soc Med* 1981;74:531–536. [PubMed: 7021827]
- Chatton E, Blanc G. Notes et reflexions sur le *Toxoplasme* et la toxoplasmose du gondi (*Toxoplasma gondii* ch Nicolle and Manceaux, 1909). *Arch Inst Pasteur Tunis* 1917;10:1–41.
- Chaves-Carballo E, Samuel T. Darling and human sarcosporidiosis or toxoplasmosis in Panama. *Jama* 1970;211:1687–1689. [PubMed: 4984269]
- Chaves-Carballo E, Samuel T. Darling: studies on malaria and the Panama Canal. *Bull Hist Med* 1980;54:95–100. [PubMed: 6991038]
- Corliss JO. A salute to Antony van Leeuwenhoek of Delft, most versatile 17th century founding father of protistology. *Protist* 2002;153:177–190. [PubMed: 12125759]
- Cox FE. History of human parasitology. *Clin Microbiol Rev* 2002;15:595–612. [PubMed: 12364371]
- Darling ST. Sarcosporidiosis: with report of a case in man. *Proc Canal Zone Med Assoc* 1909;1:141–152.
- Dobell, C. Anthony van Leeuwenhoek and His Little Animals: Being Some Account of the Father of Protozoology and Bacteriology and His Multifarious Discoveries in these Disciplines. John Bale; London: 1932.
- Dubey JP. History of the discovery of the life cycle of *Toxoplasma gondii*. *Int J Parasitol* 2009;39.[in this issue]
- Dubremetz JF, Ferguson DJP. The role played by electron microscopy in advancing our understanding of *Toxoplasma gondii* and other apicomplexans. *Int J Parasitol* 2009;39.[in this issue]
- Eyles DE, Coleman N. Antibiotics in the treatment of toxoplasmosis. *Am J Trop Med Hyg* 1953;2:64–69. [PubMed: 13007923]
- Ferguson DJP. Identification of faecal transmission of *Toxoplasma gondii*: Small science, large characters. *Int J Parasitol* 2009;39.[in this issue]
- Ford BJ. Bacteria and cells of human origin on van Leeuwenhoek's sections of 16741,2. *Trans Am Microsc Soc* 1982;101:1–9. [PubMed: 7043861]
- Ford BJ. The Leeuwenhoekiana of Clifford Dobell (1886–1949). *Notes Rec R Soc Lond* 1986;41:95–105. [PubMed: 11616094]
- Fred EB. Antony van Leeuwenhoek: On the Three-hundredth Anniversary of his Birth. *J Bacteriol* 1933;25(iv):2–18. [PubMed: 16559605]
- Garnham PCC. Charles Nicolle and Inapparent Infections. *Am J Trop Med Hyg* 1977;26:1101–1104.
- Grigg ME, Sundar N. Sexual recombination punctuated by outbreaks and clonal expansions predicts *Toxoplasma gondii* population genetics. *Int J Parasitol*. 2009[in this issue]
- Gross L. How Charles Nicolle of the Pasteur Institute discovered that epidemic typhus is transmitted by lice: reminiscences from my years at the Pasteur Institute in Paris. *Proc Natl Acad Sci U S A* 1996;93:10539–10540. [PubMed: 8855211]
- Hager, T. *The Demon Under the Microscope: From Battlefield Hospitals to Nazi Labs, One Doctor's Heroic Search for the World's First Miracle Drug*. Harmony Books (Random House, Inc.); New York: 2006.
- Hammond, D.; Long, P. *The Coccidia: Eimeria, Isospora, Toxoplasma, and related genera*. University Park Press; Baltimore: 1973.
- Hoare CA, Mackinnon DL. Clifford Dobell. 1886–1949. *Obituary Notices of Fellows of the Royal Society* 1950;7:35–61.
- Kean, BH.; Mott, KE.; Russell, AJ. *Tropical Medicine and Parasitology: Classic Investigations*. Cornell University Press; Ithaca, New York, USA: 1978.

- Laveran A. Au sujet de l'hématozoaire endoglobulaire de *Padda oryzivora*. C R Soc Biol (Paris) 1900;52:19–20.
- Levaditi C, Sanchis-Bayarri V, Lapine P, Schoen R. Etude sur l'encephalomyelite provoquee par le *Toxoplasma cuniculi*. Ann Inst Pasteur, Paris 1929;43:673–736.
- Levine ND. Taxonomy of *Toxoplasma*. J Protozool 1977;24:36–41. [PubMed: 325203]
- Levine ND. The Taxonomy of *Sarcocystis* (Protozoa, Apicomplexa) Species. The Journal of Parasitology 1986;72:372–382. [PubMed: 3091802]
- Levine, ND. The protozoan phylum Apicomplexa. CRC Press; Boca Raton, Florida, USA: 1988.
- Levine PP. The effect of sulfanilamide on the course of experimental avian coccidiosis. Cornell Vet 1939;29:309–320.
- Long, PL. The Biology of the Coccidia. University Park Press; Baltimore, Maryland, USA: 1982.
- Nicolle C. Sur une piroplasmose nouvelle d'un rongeur. C R Soc Biol (Paris) 1907;63:213–216.
- Nicolle C, Manceaux L. Sur une infection á corps de Leishman (ou organismes voisins) du gondi. Sur une piroplasmose nouvelle d'un rongeur 1908;147:763–766.
- Nicolle C, Manceaux L. Sur un protozoaire nouveau du gondi. C R Soc Biol (Paris) 1909;148:369–372.
- Nuttall GHF. Piroplasmosis. Parasitology 1913;6:302–320.
- Nye ER. Alphonse Laveran (1845–1922): discoverer of the malarial parasite and Nobel laureate, 1907. J Med Biogr 2002;10:81–87. [PubMed: 11956550]
- Pelis, K. Charles Nicolle, Pasteur's Imperial Missionary: Typhus and Tunisia. University of Rochester Press; Rochester, New York, USA: 2006.
- Riley WA, Watson CJ. Histoplasmosis of Darling: With Report of a Case Originating in Minnesota. Am J Trop Med 1926;s1–6:271–282.
- Sabin AB, Olitsky PK. *Toxoplasma* and Obligate Intracellular Parasitism. Science 1937;85:336–338. [PubMed: 17815128]
- Sabin AB, Warren J. Therapeutic effectiveness of certain sulfonamides on infection by an intracellular protozoon (*Toxoplasma*). Proc Soc Exptl Biol Med 1942;51:192–123.
- Sibley D. Development of forward genetics in *Toxoplasma gondii*. Int J Parasitol 2009;39.[in this issue]
- Splendore A. Nota sopra il Kalaazar, ibidem, idem, seduta del 7 settembre. Sopra un nuovo protozoo parassita de' conigli, *Toxoplasma cuniculi*, (III nota preliminare). Rev Soc Scient Sao Paulo 1910;5:167.
- Splendore A. Studi nell' interesse di una lotta biologica contro le arvicole. Bollettino dello Ministerio d'Agricoltura, Roma, Seria B 1918;2:1–10.
- van Thiel PH. The taxonomic status of *Toxoplasma gondii*. Antonie van Leeuwenhoek 1956;22:248–256. [PubMed: 13382130]
- Weiss LM, Dubey JP. Toxoplasmosis: a history of clinical observations. Int J Parasitol. 2009[in this issue]
- Welsh, I. Trainspotting. W. W. Norton & Company; New York, New York: 2002.
- Zinsser, H. Rats, Lice and History. Pocket Books Incorporated; New York, New York: 1935.