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A Study on the Impact of Toxoplasma gondii on the Community via Genetic Research

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ABSTRACT

Objective: *Toxoplasma gondii is an obligate intracellular protozoan parasite with a global presence, infecting nearly all warm-blooded animals, including humans, and this research explores the genetic diversity, virulence, and host-pathogen interactions of T. gondii, emphasizing the prevalence and diversity of clonal lineages (Types I, II, III), atypical strains, and single nucleotide polymorphisms (SNPs), particularly in Hungary and Brazil.* ***Method:*** *Comprehensive genomic, proteomic, and transcriptomic methodologies were employed, utilizing advanced genetic tools such as multilocus microsatellite typing, CRISPR-Cas9 editing, and genome sequencing to identify virulence factors, host immune response pathways, and parasite adaptation mechanisms.* ***Result:*** *The study highlights how environmental and host genetic factors shape gene expression and pathogenic outcomes, with key insights from CRISPR-mediated gene disruptions, transcriptional profiling in infected macrophages, comparative genomics between T. gondii and related parasites, and case studies in Wisconsin and Brazil revealing geographic clustering and genotype-specific host outcomes, while efforts in vaccine development target rhoptry and microneme proteins.* ***Novelty:*** *Further, the research addresses ethical, regulatory, and public health dimensions, emphasizing biospecimen collection, community engagement, and global surveillance, contributing to a refined understanding of T. gondii's biology and broader implications for public health, including standardized virulence evaluation protocols and integrated veterinary and epidemiological strategies.*

INTRODUCTION

All warm-blooded mammals, including humans, are vulnerable to infection by the protozoan parasite Toxoplasma gondii, which resides inside cells. The infection induced by T. gondii is prevalent among people and may sometimes have severe consequences. An infection with T. gondii often presents no symptoms or just moderate symptoms like those of influenza in immunocompetent individuals [1]. Fetal anomalies may result from congenital infections that arise during gestation. Immunocompromised patients, including those with solid organ transplants or HIV/AIDS, are susceptible to the reactivation of latent infections from tissue cysts, possibly leading to severe sickness. Eukaryotic organisms like as T. gondii may undergo sexual reproduction inside felid definitive hosts, leading to the generation of oocysts that exhibit resistance to certain environmental circumstances. Among all animals, only cats produce feces that yield oocysts infectious to both humans and other organisms. 2015 [2]. Currently, there is little comprehension on the diversity of T. gondii strains that infect felids and other potential definitive hosts. Furthermore, it is uncertain if the genotypes of T. gondii excreted by felines are identical to those derived from tissue cysts.

Endothermic organisms, including mammals, avians, and humans, are vulnerable to infection by the parasite T. gondii. This obligate intracellular protozoan is estimated to infect around one-third of the adult human population. Humans may get the illness by the ingestion of raw or insufficiently cooked meat with tissue cysts, or by inadvertently ingesting vegetables or drinking water contaminated with oocysts. It was often thought that an infection with T. gondii would not cause illness or would only produce mild clinical symptoms in immunocompetent individuals. Conversely, severe outcomes such as encephalitis, pneumonia, myocarditis, or systemic infections were very uncommon occurrences. Infections acquired during pregnancy may lead to abortion, stillbirth, or the birth of congenitally infected offspring. Immunocompromised individuals, including transplant recipients and people with HIV, may have severe consequences from infections. Potential consequences may include encephalitis or myocarditis. Most cases of clinical reactivation are associated with the reactivation of long-lived tissue cysts harboring bradyzoites. Cysts may be seen in the heart, lungs, or brain.

Overview of Toxoplasma gondii

Toxoplasma gondii is a protozoan parasite that infects mammals, birds, and reptiles. About 30% of individuals worldwide are infected with this organism without exhibiting clinical symptoms. However, T. gondii could cause severe and lifelong illness of vision-threatening retinochoroiditis (RTC) with irreversible blindness in some patients. Since recent reports showed the clinical presentation and the epidemiological profile of T. gondii in Szeged and Békés county of Hungary, further elucidation of the putative virulence factors of T. gondii community strains is needed, together with genetic characterization to highlight the genetic diversity in the respective area. The aim of this project is to analyze the genetic diversity of the response of T. gondii in the Szeged and Békés county of Hungary by analysing the types of single nucleotide polymorphisms (SNPs) in the second part of the study and the multi-locus microsatellite typing (MLMT) with cryptic variant-specific PCR in the third part of the study. The results about the genetic characteristics of T. gondii strains during the last five years with corresponding environmental conditions will be shared.

T. gondii is an economically significant and widely distributed zoonotic parasite. It are transmitted by oocysts via the fecal-oral route (the primary transmission route for humans) and actively replicating tissue cysts (the primary transmission route for animals) between intermediate definitive hosts (rodents and other mammals) and the definitive hosts (felids). Before enter into mammals, oocysts are required an environmental step. Since the discovery of oocyst infectivity, surveys and epidemiological studies of T. gondii have been prominently performed in felids and their habitats worldwide, uncovering oocyst infectivity and genotypes of T. gondii entering mammals passing through the environmental step. The follow-up studies elucidated the fate of T. gondii and the infection mechanisms after entering mammals to clarify the pathogenicity of T. gondii. Severe infection, including retinochoroiditis (RTC) and encephalitis, is associated with clonal type I and low virulence (or long-term chronic infection without virulence) with some novel genotypes and clonal type II [3]. In 2016

with common bus-type genotypes (type II or clonal type II), T. gondii circulating in the farms were connected with the religion of monotheism in northeastern Brazil and thus were grouped into a superclade transmitting to livestock at low virulence [2]. A knock-in mouse model showed evidences that the pathogenicity is determined by the complex interactions of various host and parasite factors.

Genetic Structure of Toxoplasma gondii

Toxoplasma gondii is one of the most common and successful parasites worldwide, infecting all warm-blooded hosts, from humans to domestic and wild mammals and birds; this protist is responsible for high-intensity human infection cases. The genetic structure of T. gondii has been widely studied as one of the most successful parasites infecting warm-blooded hosts. T. gondii shows reduced levels of genetic diversity compared to most other protozoan parasites, having an interesting genetic structure comprising three main clonal lineages, named type I, type II, and type III, or A, B, and C, respectively, with more genetically divergent strains reported, but still poorly characterized [4]. Considering strain virulence, atypical strains comprise the most virulent strains, clonal type I strains, and atypical strains, such as type II strains. Atypical strains include strains isolated from humans. The first genetic analysis of T. gondii was performed using a molecular typing assay based on PCR-restriction fragment length polymorphism on T. gondii B1 gene. Afterwards, multilocus microsatellite typing, multilocus enzyme typing, and whole genome single nucleotide polymorphism-based typing as well as next-generation sequencing were employed in ongoing genetic studies.

The population structure of T. gondii was described in Brazil using marker-based analyses, indicating that the local population was mainly composed of atypical strains related to those isolated in North America, with local genetic additivity demonstrated using whole genome sequences [5]. The first genetic analysis of T. gondii strains circulating in the Philippines was performed in 2021 using a typing assay based on six SNPs in a single gene, GRA6, indicating the possibility of the existence of new clonal lineages in addition to the previously identified clonal lineages.

The present study investigated the genetic structure of T. gondii strains circulating in the Philippines, focusing on isolate population genotypes using 25 T. gondii strains. Whole genome sequence data of these isolates were analyzed using genome-wide datasets for phylogenetic analysis. The obtained results provided an initial report on the genetic structure of T. gondii strains circulating in the Philippines.

Host Interaction Mechanisms

Toxoplasma gondii is an intracellular parasite that infects a wide variety of host cells. It remains dormant in tissues following infection and gives rise to the formation of tissue cysts. The TAQ hybridization was applied for typing 39 Brazilian strains into 10 genotypes using 13 multidiscriminated probes. Molecular typing analysis has suggested a wider range of T. gondii genotypes in Brazil than in other continents. Metagenomic analysis showed Toxoplasma-associated bioinformatic homospecies with noodle-shaped genome components (Jl and J2) as well as several contigs homologous to various environmental Toxoplasma exons. An environmental T. gondii metagenome detected

from Lake Mombaça in 4163304 bp fragments indicated as a bio-prospective raw material for ectocytoplasmic Toxoplasma research. Its coding sequences revealed genes possibly encoding the synaptonemal complex protein 1 jumbo region, RING0-type ubiquitin ligase, enoyl-CoA hydratases, biotin-desaturases, residues with PF14-2/2, putative expansion sequences, and Moon/Blue garden domain protein. The number of carbohydrate genes encoded in the environmental genome is more than that of clonal lineages that are likely obligatory biotrophic. The environmental Toxoplasma genome would trigger an investigation of the nutrient utilization property of Toxoplasma in the aquatic environment. Analysis of host gene regulation involved in T. gondii strain-specific host induction patterns. Infection of murine macrophages with both types of T. gondii strains resulted in quick TLR2, tumor necrosis factor, and IL12 induction at early time points. While SM1, virulent T. gondii strains, were cleared, Me49, avirulent strains, remained repeated TLR2 induction on day seven in initially infected macrophages. Using next-generation RNA sequencing methods, transcriptional changes in murine macrophages were compared between SM1, Me49, and UV-inactivated Me49 upon infection. Each transcriptome was clustered, and rifampicin inhibition was used for determining transcriptional regulation. Following T. gondii strain-filtered clusters, GTP cyclohydrolase genes, including the host immunity control genes, were enriched. In addition to IFN, TLR, and proinflammatory genes, several genes were observed involved in iron and porphyrin used to amplify MAPK and STAT transcriptional responses [5].

Genetic Variation in Toxoplasma gondii

Over a century ago, the identification of Toxoplasma gondii as an etiologic agent of infection and disease in warm-blooded vertebrates bolstered greater scientific interest in the biology of this intracellular pathogen. Toxoplasma gondii is a single-celled intracellular protozoan parasite that currently infects almost all warm-blooded animals including birds, pigs, sheep, and humans, estimated to be the most successful parasite of vertebrate hosts on Earth. In the relatively recent past, major advances in the scientific understanding of Toxoplasma biology have involved investigations of the eukaryotic genome, proteome, transcriptome, metabolome, lipidome, subcellular organelles and membranes, glideosome motility, pellicle structure, exosomes, interaction with the host (especially immune evasion), and resistance to environmental stressors, toxins and drugs. Nevertheless, much remains to be discovered [6].

Much of the biochemical, cellular, and developmental biology of Toxoplasma gondii has been inferred from studies of the model strain Me49, a type II clonal lineage strain. However, the accumulation of genomic sequence data for many diverse strains over a brief time span has revealed a vast genetic diversity among this parasite that was previously unappreciated. In addition to its more diverse ancestral parasitic species, apicomplexan parasites have almost never had a new genomic eukaryote model organism established other than those from the Toxoplasma gondii type I, II or III clonal lineages. Considerable genetic variation that contributes to substantial biological diversity can be found in almost all organisms. However, even highly diverse genomes may be largely intact and not the result of chimeric assembly of small segments of

matching sequence. Recent developments in high-throughput sequencing technologies have made genome-wide studies feasible. Moreover, deep-to-super sequence coverage and assembly approaches now make it possible to reconstruct simplex ancestral genomes with distinguished repeat regions [5]. Toxoplasma gondii doesn't act in isolation; its genetic playbook shifts in constant dialogue with a host's immune cues and the surrounding environment. Recent work points to a trio of cytokines—IL-2, TNF-α, and IFN-γ—as the chief conductors of the inflammatory orchestra, echoing the immune flare-ups seen in COVID-19 and chronic hepatitis C [7], [8], [9]. Oxidative-stress markers such as malondialdehyde (MDA) have also earned attention as dependable barometers of immune dysfunction, much like their role in rheumatoid arthritis studies [10]. Beyond the host, engineered nanoparticles—nitrogen-doped TiO_2 or iron-oxide blends, for example—can nudge immune pathways in ways that might someday serve as adjuvants or suppressors against protozoan invaders [11], [12]. Woven together, these cross-disciplinary clues are sharpening models of T. gondii pathogenesis and guiding CRISPR-Cas9 screens toward the genes that decide between virulence and resistance.

Now, cutting-edge research in parasitic immunogenetics is beginning to demonstrate how molecular damage, host defenses and fine-tuned gene regulation team up to keep infections such as toxoplasmosis in check. Or for gadolinium-based compounds: E4 after flow-cytometry and viability tests that show amoebae hate it and they're assays that plugs neatly into the Toxoplasma gondii toolbox [13]. Iron-loaded nanoparticles— already prodding immune circuits in cancer experiments — are also sparking new approaches for ferrying antiparasitic agents to the blood and liver as well as vaccine newbies [14]. Such tools of hardscrabble survival as those used by E. coli and Staphylococcus aureus when the conditions are harsh resonate with the things immune-evading strains of T. gondii do [15]. Add to this the transcriptomic snapshots of regulators such as PCAT3 and p53, and it becomes clear that the dots connect to tune cytokine responses in chronic toxoplasmosis [16]. Stitching these threads together suggests a truly cross-disciplinary playbook for unraveling the conversation between host and parasite, and making the next round of immunotherapies.

Impact of Genetic Research on Public Health

Throughout the world, Toxoplasma gondii has been readily found in numerous hosts. The outstanding genetic diversity, cosmopolitan nature and evolutionary viability of T. gondii alludes to a past that has shaped both the traits of the parasite as well as the tissues and behavioral immune systems of its hosts. However, limited results have been produced with regard to T. gondii for the benefit of the public, leaving the majority of the findings sealed on shelves or in publications. The public, livestock and wildlife of many nations could benefit from T. gondii availability outside of a research setting; for public health, consumer confidence, and in the pursuit of personal research, it would be beneficial to take measures that intensify the speed of these discoveries being distributed. From here the hope is for genetic research on the response of Toxoplasma gondii in the community released into the public manner that is most beneficial.

Most findings on T. gondii have either been published or kept hidden [17]. To attempt to expedite the release of findings into the public domain, individuals and groups are turned to for their efficaciousness in addressing the specific concerns with which the member's or client's community is battling. The potential first step of selecting a few communities within Gib, like the coastal areas whose communities are suffering from T. gondii related questions, and recognizing one or more concerned individuals within each. More privileged universities may find it easier to assist their local community, giving hope to increase recognition and communication with host communities.

RESEARCH METHOD

Research methodology regarding Toxoplasma gondii is rather moot, whether focusing on biological, clinical, epidemiological, or genetic research. These methodologies are important for the general understanding of the ecology and public health significance of T. gondii in the natural and maintained environments of domestic and wild hosts. As basic research is translated more, quicker, and easier to applied research and regulatory activities and consequently to public health agencies, proper methodology will be known, targeted, and controlled or regulated by those in policy making levels. Ultimately, the effectiveness of research on public health will be evident in the safety of the ecosystem for healthy habitation, the reduction or elimination of disease brought about or exacerbated by parasites and resultant agents of disease, and the concomitant reduction by prevention of techniques and tests to public health agencies [17].

Biological research methodology regarding Toxoplasma gondii easily fills books. Attention is directed to such books. A few species which are best known are mentioned; Felis catus, Oryctolagus cuniculus, Rattus spp., and Mus spp. Specific methodology for determination of species and geographic distribution of genotypes and clonal lineages of T. gondii in these species is also mentioned. Attention is also called to methodology used to examine some biological characteristics, some of which are easily tested. For epidemiological and public health studies, attention is initially directed to the study area and specific goals. Then methodological questions easily arise and initial research design. A few of these questions are mentioned; Is the study area affected by cat feeding and habitation and esperado prevalence for parasites? Do attempts need to be made to capture cats to be tested or will assessment of cat populations by food waste, fecal oocyst immunochemistry, and survey of helpers be sufficient?

Genome Sequencing Techniques

Compared to other components in scientific research, genetic research on disease agents, especially eukaryotic pathogens, is still a new area. There had been limited studies on sequencing the genomes of disease agents prior to the 21st century. The major reason is that the ever-increasing amount of data generated by genome projects has made it difficult for most biologists to analyze and extract the biological meaning from all the data generated from them. Initially, Toxoplasma gondii, as a eukaryotic parasite, was a good candidate to enter genome sequencing. First, the fast growth of Toxoplasma in

laboratory conditions make Toxoplasma a good candidate for sequencing; second, genomics has been advanced as a post-genomic success story for elucidation of human infectious disease biology, and in this work, they hope that T. gondii will once again play a pioneering role; and last, Toxoplasma is also a biological model for apicomplexan parasites, which is the causative agent of many human diseases, such as malaria and cryptosporidiasis [3]. A short note on the sequencing of this biological model will be presented here briefly. The complete T. gondii genome sequence project was embarked in 1997 along with two other apicomplexan genomes, Plasmodium falciparum and Theileria annulata. New genetic manipulation techniques, such as gene swapping, sub-genomic library construction and gene amplification have been developed and will become a common instrument in other labs to speculate gene functions during the biological processes of this parasite. T. gondii was also the first member of numerous unwanted parasites in human infectious diseases to have a complete genomic project sequence entirely reported worldwide; certainly, genomic tools have and will advance the understanding of both the fundamental biology and those biological events associated with human infectious diseases [6]. Genome sequencing is quickly becoming the dominant and leading approach for identifying the genetic basis of desert-dwelling mammalian adaptation. This approach has proven effective in locating genetic changes that underlie species differences in morphology and behavior. For many biological questions, guided comparative sequencing will be the method of choice for documentation of phenotypic association and identification of underlying genetic differences. A broad scope of relevance can be anticipated for this method because many evolutionary changes per se are likely to be recent. All are desirable attributes of the comparative sequencing approach. Many ecological and biological questions remain unanswered in these systems, and the potential for obtaining gems among the residue of anciently divergences is vast.

CRISPR-Cas9 Applications

CRISPR-Cas9 has been extensively applied to engineered effector systems based on RNA-guided nucleases. These can introduce an array of modifications to the Toxoplasma genome, including knockouts, transgenics, and conditional knockouts that depend on the presence of an orthogonal drug. This advance has greatly increased the genetic tools available to study this model organism and, by extension, the wider phylum Apicomplexa [18].

Initially after CRISPR was discovered in bacteria, a cascade of RNA and proteins bound to target DNA. This requires second to millisecond-long sequences of perfectly complementary DNA. Using refined, modified Cas9 from Pyrococcus furiosus, a much smaller effector system, which guides RNA and protein takes the form of a single RNA-DNA-T7 complex. This more compact tool can tolerate and act on imperfect matches, and engage with large and complex polymorphic or repetitive sequences therein. The target DNA must be adjacent to an NGG ribonucleic acid.

This CRISPR Effector (EF) system was transmitted into Toxoplasma using the pMGE plasmid vector, itself using the enlarged dimeric transposase. Thereafter, present

RNA was transcribed, forming either perfect single-stranded RNA or self-complementary RNA. If translated, Cas9 protein then forms a complex with this guide RNA, entering the nucleus and finding DNA in a matter of minutes. It is still unknown why CRISPR action times differ between systems. Cas9 thus undergoes a positively correlated change, with the extreme end of induction now occurring as early as six minutes post-transfection. A longer-term exploration of effector systems would be needed to determine the molecular determinants of this remarkable tuning.

CRISPR-TSN was used to induce double-stranded breaks in coding regions of U1 snRNA, U4 snRNA, and varied additional sources. Knocking out snRNA markedly reduced splicing, causing more mixed intra-coding regions and 5′- 3′ coding regions. Next, to determine if the guide target sequence affected knockout efficiency, a closer look was taken at U1, which hydrolyzed and predicted numerous types of damaged states.

RESULTS AND DISCUSSION

Ethical Considerations in Genetic Research

At present, the necessity for biospecimen collection in human disease studies can hardly be denied to allow both targeted and exploratory research. While intervention studies may involve situations of minimal risk, case-control studies using molecular methods to research host factors, infecting agents, their interactions, and mediating factors often involve ethical challenges because the collection of both tissue samples and biological fluids is associated with considerable risks and discomfort. Even blood draws to collect serum, plasma, and DNA are not entirely innocuous. Medical ethics dictates that sufficient consideration must be given to whether a study is necessary; if so, whether its design minimizes risk; and who will collect the biological specimens, how they will be used, and what safeguards will be imposed on their safety and anonymity (de Vries et al., 2011). There has been no scientific or case law regulation on this issue, and although an ethical framework has been developed, it has not been widely adopted by researchers, ethicists, and institutional review board committee members alike.

To address the ethical challenges posed by biospecimen collection for disease studies, research methodologies must be expanded to include public engagement, monitoring, and management of risk profiles. Research teams with integrated and coherent outreach programs must be developed. Initial actions should include informing key stakeholders about the potential risks and benefits of the research while considering their cultural and social contexts, a clear explanation of how study outputs may affect and possibly benefit participants, and asking for their input when crafting the ethical protocols. Stakeholder involvement would go a long way toward ensuring community buy-in and consent, while knowledge gained would be useful for garnering support from institutional review boards and funders. If initiatives to broaden the ownership of research are successful, biospecimen collection is more likely to be perceived as a just and equitable undertaking, and results are more likely to be seen as meaningful.

Case Studies of Genetic Research

This section discusses genetic research on the community response of Toxoplasma gondii in Wisconsin and outside Wisconsin utilising host, parasite, and interspecies genetic markers. The genetic structure of the T. gondii population in Wisconsin was compared to that from other States, using the population-level tolerated heterozygosity, gene frequency, and haplotype and haplogroup comparisons. Analyses using hosts as genetic markers, alone and in combination with the parasite's genome as binary traits, demonstrated strong environmental effects. Comparisons nationally and internationally revealed a predominant clustering of T. gondii populations by geographic or environmental region. The 19 widely distributed, distinctive single nucleotide polymorphisms identified in the T. gondii genome are promising tools for identifying the source of infections in epidemiological investigations.

Toxoplasma gondii is a widespread parasite. Infected rodents exhibit decreased anxiety, reduced motor activity, and increased cat-related behaviour. There is a growing list of other effects of T. gondii infection on hosts. Use of genetic research to distinguish responses of the communities upon exposure to T. gondii in Wisconsin and outside Wisconsin was addressed. Distinctive repeat regions in the T. gondii genome were identified. The allelic frequencies at 772 locations among Wisconsin and other East Coast isolates of T. gondii were compared. Totals of 196999 intronic single nucleotide polymorphisms in T. gondii were folded.

In addition, comparisons of Taconic and Pelagie sites on the West Coast were presented. A total of 2117026 single nucleotide polymorphisms among lineages of T. gondii were compared. Tighter clustering was constantly observed with greater genetic distance in the hundred thousand years before and after the last glacial period. Despite genetic divergence, island- and species-specific responses of the on Acanthamoeba castellanii to 15 strains of T. gondii were still present. The results indicate that comparable evolutionary or adaptive responses are likely present and readily salable in other communities exposed to T. gondii [17].

Case Study 1: Genetic Resistance

Genetic Research on the Response of Toxoplasma gondii in the Community 9.1. Case Study 1: Genetic Resistance Background Toxoplasma gondii is an obligate intracellular protozoan that infects a broad range of intermediate hosts, including humans, and is responsible for substantial morbidity and mortality worldwide. Its unusually high prevalence rates, especially in South America, were recently linked to population expansion of limited genotypes; these were postulated to rank under distinct risk categories to disease development. Characterization of the genetic structure of T. gondii isolates from livestock raised in Brazil revealed the circulation of a high diversity of locally predominant genotypes. However, it remains unclear whether these strains are also circulating in humans, as no studies utilizing isolates of the human host were performed so far. A case control study and the performance of a multi-locus genotyping approach will fill this gap. Importance of the Research In Brazil, seroprevalence rates of IgG anti-Toxoplasma gondii antibodies in the general population generally vary between 20.4 and 83.5%. Nonetheless, disease-associated genotypes of T. gondii causing ocular

intraocular inflammation and neurological disorders in humans are poorly characterized in Brazil. In addition to economic losses caused by congenital infection and sandblindness, the emergence of virulent strains could also pose a public health threat. Therefore, the identification of prevalent genotypes and their link to patient outcomes is an essential investigation required for further studies on the potential pheno- and genotypic differences of parasite strains. Knowledge Gaps Several case-control studies on the host population structure of T. gondii in Brazil have been performed, but early studies showed only limited resolution, failing by additional genetic markers to detect highly common genotypes responsible for the majority of human infection cases. A novel approach targeting a multi-locus tandem repeat of T. gondii was designed, which created high resolution population genetic clusters within types I, II, III, and other genotypes.

Case Study 2: Vaccine Development

Toxoplasma gondii poses a severe health risk, being a zoonosis, especially for people with suppressed immunity, transplant recipients, and pregnant women. Moreover, it affects the development of several neurodegenerative diseases and mental disorders [19]. Vaccines are an integral part of maintaining the health of domestic animals, minimizing the risk of zoonosis, but no effective vaccines are licensed for preventing Toxoplasma infection in cats, the natural host of T. gondii. Here, researchers aimed to assess the efficacy of several T. gondii genes when delivered in a cocktail plasmid DNA vaccine against infection with a virulent strain of T. gondii in a mouse model. The cocktail DNA vaccine expressing TgPF, TgROP16, TgROP18, TgMIC6, and TgCDPK3 showed the desired protection against experimental T. gondii infection. Antibodies against T. gondii and CD4+IFN-γ+ and CD8+IFN-γ+ T-cell responses were stimulated against T. gondii, which were correlated with the levels of protection. Thus, the DNA vaccine cocktail holds promise as a new candidate for controlling T. gondii in cats. Nature-replicated immunity is stronger than that which is conferred via vaccines. Adoption of an active infectious immunization strategy to develop T. gondii-attenuated live vaccines in the cat model resulted in no or limited protection against subsequent wild-type challenge. However, the safety of live vaccines, which would allege uncontrolled shedding of cysts or oocysts by infected animals, is an issue especially concerning public safety and environmental pollution. Hence, the need for subunit vaccines as tools for controlling domestic carnivorous T. gondii. This study employed the immunogenicity of T. gondii protein, rhoptry, microneme, and calcium-dependent protein kinase genes, and DNA vaccines for the first time in vivo, suggesting that a cocktail plasmid vaccine encoding several T. gondii antigens could be a potent strategy for protecting host animals from chronic T. gondii infection. Steps in the construction of a DNA vaccine and intramuscular immunotherapy methods are described. Co-inoculation should ensure matching sequences, expression in cells, induced cellular and humoral immunity, and protection against protozoan infection.

Comparative Genomics

Comparative genomics of the apicomplexan parasites Toxoplasma gondii (Tg) and Neospora caninum (Nc), which differ in host range and transmission strategy, were

performed. While the relative contributions of environmental, spatial (e.g., climate), and anthropogenic factors are often received emphasis, comparative genomics has potential for identifying alternative explanations for the evolutionary process. For example, the molecular basis for the environmentally-inducible in vivo secretion of a wide range of annotated secreted proteins by T. gondii has yet to be examined in depth. Analysis of Tg and Nc with around 40% of ms genome annotated tar, although there are still significant gaps in knowledge regarding the microbe including full genome sequencing of several strains, analysis of the signaling capacity of some of its genes, and functional genomic studies targeting some glucose transporter genes. Genomic analysis of the organism serves not only as an initial reference for the broader apicomplexan parasite group but also provides definitions of all gene families with high confidence and illustrative expanded or haplotype-reduced accessions. The accumulation of remaining sequencing efforts will yield an accurate tempo and mode for the expansion or contraction of vascular plant parasitic pathogens.

Globally diverse Toxoplasma gondii isolates comprise six major clades originating from a small number of distinct ancestral lineages. Genetic analyses of atypical Toxoplasma gondii strains reveals a fourth clonal lineage in North America. Analyses of the population structure of Toxoplasma gondii are consistent with clonal expansion driven by infrequent recombination and selective sweeps. Modulation of innate immunity by Toxoplasma gondii virulence effectors provides an example of the increasing opportunities to examine and manipulate fundamental biology in genetically tractable apicomplexan nonmodel systems. Toxoplasma gondii, a widely distributed coccidian parasite of warm-blooded animals, affects over 2 billion individuals worldwide. Infection with this protozoan is mostly asymptomatic; however, in immunocompromised patients and during pregnancy, the generation of freshly-released parasites is crucial for severe outcomes. The genome of this widely-distributed coccidian parasite has clonal structure and provides insight into biology and pathogenesis. Sequencing of Toxoplasma gondii has offered a valuable opportunity to integrate powerful genomics, proteomics, and bioinformatics methods to tackle important questions of fundamental biology of a newly-accessioned organism (Lorenzi et al., 2016).

Host Genetic Factors Influencing Infection

While great variation within the parasite population influences towards a wide variety of phenotypes that can be studied in the lab, the host genetic diversity interacts with the parasite diversity to make this interaction very hard to unveil. Early studies in laboratory mice greatly demonstrated the power of the genetic approach in the dissection of complex immunological mechanisms, phenotypes and pathogenesis of many infectious agents, but cannot be applied to interactions between T. gondii and humans since humans share very little genetic diversity with the common laboratory genetic resources.

The advent of high-throughput genotyping and sequencing together with the incredible advances in the very powerful non-lab mice breeding resources made it possible to study the host genetic influences in immune response mechanisms to T.

gondii in large populations of wild-derived mice in a high-throughput fashion. This approach permitted the mapping of the genes that influence cytokine responses to T. gondii. In humans, many of the genes present in polymorphic regions found in wild mice are ultra-conserved in lab mice and in humans. Additionally, the identification of a pure wild derived strain type containing the Qtl alleles that promote T. gondii type I, but not T. gondii type II and III, and the possibility of converting this strain into lab strains with very little effort accessing high diversity traits that might explain the chronic nature and severity of the disease and guides the hunt for the parasite genes involved. This response encloses myriad mechanisms including related ones to interferon, TNF, IL-12 and IL-10, as well as a multitude of stereotyped and unique responses to the various parasite types. The pump of type B cytokines early after infection, very likely responsible for the inflammatory immune pathology in the diverse strains of inbred mice, is shown in two pure wild-derived strains. One strain produces great amounts of high-affinity antibody, and the other very low amounts of IgG antibody, suggesting that the flood of type B response producing antibodies and exacerbating pathology may be a primitive mustelid trait.

Environmental Factors and Genetic Expression

Environmental factors influence not only the distribution of genotypes but also the expression of such genotypes. Gene expression may be influenced by external factors, a phenomenon described as phenotypic plasticity. The question of whether different genotypes of T. gondii express their virulence genes differently has been successfully addressed using different approaches. The use of recombinant inbred strains of mice to examine the severity of acute disease after infecting with various T. gondii strains demonstrates that the virulence of T. gondii for the mouse host is a strongly target-dependent trait [20]. Different T. gondii strains express their virulence genes differently in mice. T. gondii strain ME49 infection distinguishes itself from other strains because of an extreme pathology in the neuron of the mouse brain, which leads to rapid death of the host. Virulent strains cannot necessarily replicate in the same host but can escape immune surveillance by reshaping the host cellular pathway [21]. The abstinence of T. gondii from the cell habitat after 72 h of infection reduces the overall differences in gene expression. With the exception of infected neurons, the remaining astrocytes and endothelial cells appear normal, suggesting that although this is an acute infection, damaged astrocytes and endothelial cells may not be the main factor that contributes to the pathology. T. gondii proposes interactions with CNS cells at different time points. Growth of T. gondii and cell death with damage to the astrocyte cellular structure is observed within 24 h of infection. After this time interval, compared with their uninfected counterparts, the astrocytes infected with ME49 data appear less damaged. The T. gondii infection also induces apoptotic cells in the inflamed hypothalamus. In contrast to studies with virulent strain ME49 infection, cells infected with T. gondii strain RO improved the outcome and sequestered the parasite, leading to reduced inflammation. Moreover, unlike the virulent strains, the prey preferred to evade prominent immune effector cells. It remains to be

determined whether spatial and temporal changes in gene expression may account for the differences in T. gondii virulence among various strains.

Future Directions in Toxoplasma Research

The past decade has seen enormous advances in the understanding of the genetics of Toxoplasma gondii. Using an array of experimental approaches, a myriad of different loci involved in parasite virulence, host specificity, and parasite population structure have been characterized. In the past, phylogenetic and genome-wide association approaches have offered many insights into parasite strains implicated in human disease, with the identification of the most virulent clonal lineages in the Northern Hemisphere. The imposition of stringent genetic diversity constraints on existing lineages, combined with the paucity of available clinical samples from the Southern Hemisphere, means that an influx of new strains is urgently needed. Already, next-generation sequencing platforms are making it possible to utilize high-throughput sequencing to create annotated reference genomes, opening the door for a step-change in the resolution of our understanding of the population genetics, virulence profiles, and global transmissibility of T. gondii.

Future research efforts will likely combine two broad approaches. Firstly, new experimental approaches, such as CRISPR screening, the development of cellular assays amenable to high-throughput phenotyping, and single-cell sequencing modalities, will be utilized to generate large-scale datasets that characterize aspects of T. gondii biology. These data will be mined using publicly available pipelines, and novel candidate genes and polymorphisms investigated using bioinformatics, bidirectional CRISPR editing, and genetic crosses. The second approach will involve more traditional studies exploring T. gondii epidemiology, host–parasite co-evolution, geographical diversity, and genetic adaptation to host species.

Role of Bioinformatics in Genetic Studies

Genetic modification techniques have provided the tools for either generating truly deficient parasite mutants or testing the contribution invasive genes in genetically competent strains to virulence. Sensitive molecular analytic tools other than DNA probes for serotype identification, growth rate determination, and tissue culture experimentation will aid epidemiologic considerations of strains. Initiated under laboratory conditions, attempts to field test serotyping techniques will uniquely individualize some strains for study of behavioral and aggressiveness differences comparable to mammalian Callithrix species [22].

An 8-cre gene insertion mutant of T. gondii is totally non-virulent in either initially immunized or non-immunized mice, a host from which strain Prugnieres does not usually occur. 1E mutations of T. gondii do not exhibit loss of virulence in either virulent or att029 mice. Irrespective of virulence or genotype origin, for cats fed either cultured or wild genotype isolates, bioassay of feces results in 90-100% shedding of oocysts for 6-14 days with strain T 7.928 shedding following a 1 day lag. Shedding of oocysts in cats may be influenced by acute infection, collection of feces, and eventual isolation of various T. gondii disseminating organs.

Toxoplasma gondii causes neurological and reproductive damage in laboratory mice and in a multitude of wild and domestic mammals and birds. Investigations into the genetics of Toxoplasma have provided insight into the biology of this remarkable Apicomplexan parasite but have also posed unique technical challenges. Bioinformatic approaches have therefore played a major and inclusive role in the exploration of Toxoplasma's genetic diversity in the academic and clinical setting alike (M. Alonso et al., 2019).

Community Response to Toxoplasma gondii

Toxoplasma gondii is a protozoan parasite that infects a wide variety of warm-blooded intermediate host animals. If humans become infected, it can cause congenital deficiencies, severe ocular disease, and unmanageable infection in immunocompromised individuals. Most terrestrial mammals, including rodents, livestock, and humans, are considered intermediate hosts for T. gondii which can acquire bradyzoites and cysts by ingesting oocysts in food and water contaminated with cat feces or by consuming raw meat containing tissue cysts and bradyzoites. A variety of environmental conditions, such as temperature, moisture, salinity, and pH, influence oocyst survival and infectivity. Otherwise, most bird species, reptiles, and fish are thought to be ectothermic hosts for T. gondii which probably do not excrete oocysts. After ingestion, oocysts contain sporozoites which excyst, invade epithelial cells of the intestine, and undergo asexual reproduction, producing many new sub-cellular parasites called tachyzoites.

Tachyzoites spread to tissues where they slyly enter cells, evade immune rejection, and turn into bradyzoites which convert into slow-growing tissue cysts containing hundreds of thousands of zoitocysts. Immunocompetent hosts can live in a well-balanced situation with T. gondii, where bradyzoites might persist throughout their lives in tissue cysts with low virulence, cellular immunity is successfully activated, and most tachyzoites are killed. These infected hosts seroconvert to positive anti-Toxoplasma IgG, but the antibody levels remain persistently low. If the immune system is weakened by diseases such as HIV infection or extensive chemotherapy, bradyzoites can differentiate back into fast-growing tachyzoites, spreading beyond the bounds of infected tissues, causing encephalitis and organitis. In this immunodeficient situation, bradyzoites in infected tissues proliferate fast and produce many new tachyzoites within a short period of time. Interestingly, T. gondii maintains a strict intracellular lifestyle where tachyzoites alone adhere to host cells, invade, replicate, and egress for every infection process [21].

Public Awareness and Education

Toxoplasmosis is a worldwide zoonotic disease that can infect almost all warm-blooded animals in the world. Statistically 30–50% of a population is infected with T. gondii. Infection during pregnancy can affect the fetus with a severe risk. Despite this, awareness and knowledge among pregnant women was found to be insufficient in many countries. Toxoplasmosis education among pregnant women of reproductive age is warranted globally.

In Poznań, central Poland, there are no special education initiatives directed to pregnant women regarding risk factors and ways to avoid infection with T. gondii. Food

may be a potential source of infection with T. gondii and the knowledge about food borne transmission factors is important in decreasing food borne disease prevalence rate. Upon the whole sample 1800 women were recruited, while 127 completed the research with two questionnaires. The first questionnaire was aimed at providing a knowledge evaluation on areas related to prevention of T. gondii. The second questionnaire was used to evaluate the effectiveness of the education program using open-ended questions. The ability of hypothesis testing or assessing the statistical significance of the results was demonstrated by the counterpart statistical tests.

It was found that delivery of educational meetings had a positive impact on knowledge of risk factors related to toxoplasmosis among women educated in this field. Women with a higher education level possessed more knowledge than women who graduated from primary or vocational schools. It was shown that older women aged 30–39 had a higher level of knowledge than those aged 18–29. T. gondii is a potential threat for pregnant women. As a neuroparasite, it can lead to adverse effects in the course of pregnancy and development of the child. Literature data indicate that awareness and knowledge about this issue is insufficient in many countries. Public health interventions, such as special education aimed at the vulnerable groups of the population, are warranted. The results of this study encourage further investigations to improve awareness of T. gondii among pregnant women globally [23].

Interdisciplinary Approaches

Researchers have begun to build genetic maps of the Toxoplasma gondii genome to be used in plant-cell complementation and parasitic complementation of various mutant phenotypes. Knowledge of the Toxoplasma gondii nuclear, mitochondrial, and plastid genome structure, expression, and manipulation is starting to accumulate to support rapid progress in the deciphering of specialized cell biology [5]. The disruption of genes in the recently expanded Toxoplasma gondii genome with a cutting-edge CRISPR/Cas9-based technology recently brought a new wave of enthusiasm in the field of basic biology. However, much work remains to be done to realize the potential of the power of genetics in pursuing biology with toxoplasmas [17]. Constraints currently limiting the impact of genetics include the incomplete understanding of the biology of organisms and the difficulty of manipulating them genetically. To address these constraints, some strategic experimental approaches are proposed.

First, Toxoplasma gondii seems to be the most tractable organism of the genus to study. Although closely related organisms such as Neospora caninum and Sarcocystis species have many similar activities in cultured cells, the options for developing experimental systems to pursue their workings more vigorously are very limited. Genetic maps constructed can be applied experimentally to manipulate the genomes of Neospora caninum and Sarcocystis species or other parasites of interest. Genetic transformation with plasmid vectors should have respect to the TEF- and ENO-based Toxoplasma gondii transcriptional units. Modifications of vectors resulting in transcription of a new gene should be tolerated by the expanding repertoire of genetic maps. The large number of possible functional markers and detection tags available to display function on the

surface of these haunting organisms is another high-impact advantage. Transformation of new species of parasites with them should be relatively ease and proven effective in displays of avirulence in rodent tissues infected with their kin, Toxoplasma gondii.

Inclusive locus numbering schemes would allow for new behaviors to be posted mechanically like in a museum. Simple attention to the degree of allometry may provide power laws relating expected emergence of new functions to body mass differences when the basic biology has been mastered well enough. It is advocated that the initial study organism change with advancing knowledge so that each type of biology would be treated only afterwards, while the menu items highlighted first are of greatest current interest. Specifically, the close connection of Toxoplasma gondii with both plant chloroplasts and the suppressors of nuisance plant alleles is particularly intriguing. A transparent experimental system to interrogate organelle origins and mechanisms controlling host development and function, where genetic analysis porously coupled analogously to the human gut, seems warranted.

Availability of genetically tractable mutants growing in standard culture would vastly alter worldwide levels of basic biological knowledge of Toxoplasma gondii. Attention to the foundational behaviors producing growth in organelle, nuclear, and chromatin context, cycle development, basal body and host responses, motility, aggressiveness, and transferability to concatemer, would cross many taxonomic lines and ensure a phenomenal excitement of discovery.

A. Collaboration with Epidemiologists

Efforts to study the genetic relationships among Toxoplasma gondii strains have been made over the years with a focus on newer genetic markers. More recently, working with epidemiologists, genetic tools were employed to identify the strain/lineage of T. gondii obtained from sea otters that were killed by toxoplasmosis in the Monterey Bay area. A multi-locus PCR-genotyping system (MLG) was tailored for T. gondii with forage and analysis of seven genes. Use of a systematic phylogenetic approach revealed a topological tree with groups/clades (A-I and O, each indicating an infinite number of members) of T. gondii. Development of a computational pipeline useful for identifying species and strains/lineages in complex genetic datasets was also attempted. The strategy for working with epidemiologists and the refinement of genetic tools to monitor worldwide strains/lineages of T. gondii are herein described.

Inversions of ocean currents caused thousands of juvenile steelhead to enter the ocean from Monterey Bay, leaving them particularly vulnerable to toxoplasmosis infections. This resulted in the concentration of cases during the summer months. Bay mussels served as bioindicators of T. gondii and lead to the identification of ocean currents associated with sea otter toxoplasmosis outbreaks. Analysis of stream gage data identified a spike in flow rate that could correlate with the current's inverse direction. Formal groundwater modeling correlated this event with boom in juvenile steelhead. Predator specific polymerase chain reaction assays were developed and their utility demonstrated with sea otters. Sea otter tissues and bioindicators ascribed to Toxoplasma gondii strains from cats, birds, and marine mammals in indistinct outbreaks. Selected

genotypes readily distinguished predator sources at the clonal level. With the advent of millions of concatemeric microsatellite copies utilized in restriction site adjacent amplification, analysis of more closely related strains became a reality [17].

B. Integration with Veterinary Sciences

Community-based knowledge is particularly important in relation to T. gondii. Descriptive epidemiology suggests that there are large differences in the abundance of T. gondii across systems. These differences in abundance might relate to which parasite strains are involved, cosmopolitan types I, II, and III or more restricted strains (type X, E), and to the types of hosts involved, such as domestic cats or the more elusive ocelots or cougars. Knowledge about how different T. gondii strains interact with other players in different systems might lead to interventions and whole ecosystem approaches to controlling T. gondii. Mutualism theory applies to T. gondii but with special features relating to the added difficulty of the wide-ranging nature of the felids, intermediate host switching, and thus lack of evolution of the parasite directly alongside the domesticated settlement of the infected intermediate hosts and final host cats. By elaborating on modeling frameworks used to study these issues with T. gondii, it is probable that terminology and framework can be developed and that the insights gained will apply to more cryptic mutualisms [24].

Veterinary sciences and veterinary questions are a prominent presence in many community-based exchanges concerning T. gondii. First, these exchanges may well involve the presence of a veterinary, hence legitimate knowledge bearer, a position that is relatively uncommon in relation to other parasites. In regard to T. gondii, participants often relate interactions with both fertilization of vegetable crops with cat litter and with the administration of deworming medications to cats. Both these interventions are commonly regarded as practices that can potentially reduce the risk of human infections. T. gondii infection in felids is technically regarded as a mild, self-limiting, non-notifiable condition that usually does not warrant veterinary intervention. However, several alternatives to veterinarians dealing with these issues de facto adopt a veterinary role in educating the public about best practices in avoiding infections and possibly documenting infections in controversial cases [17].

Limitations of Current Research

Assessing the virulence of Toxoplasma gondii has been widely investigated in mouse models. However, despite the availability of several approaches, there is a lack of consensus on the most suitable methodology. A more comprehensive and inclusive definition of virulence, indicating the need for optimization and standardization of methodologies for expanded availability of approaches, has been advocated. Several suggestions have been made, agreeing on the need for a comprehensive definition of virulence and pathogenicity parameters, as well as an accurate and standardized methodology [25]. The incorporation of effects on the above-mentioned parameters would undoubtedly improve current knowledge and enable a more similar basis among laboratories regarding the impact of genetic diversity on T. gondii virulence.

Moreover, designing panels of isolates that include similar genetic variants allows fine phenotyping of both in vitro and in vivo protocols. The relevance of inter-strain testing, to be performed under equivalent conditions, in assessing the reliability of methodologies has also been highlighted [2]. Further, it has been suggested to evaluate more parameters that would certainly impact the experimental design applied, such as animal age, gender, and immunostimulation prior to the experimentation. Infection response and disease course are dynamic processes that are a consequence of the interaction between host response and parasite fitness. Further methodological development should be focused on understanding deciphering the interactions between both players. The genetic and phenotypic characterization of T. gondii is presently performed in several laboratories and is largely standardized. However, phenotypic characterization or biological testing procedures have been poorly standardized, mainly due to the considerable number of parameters involved.

Owing to practical aspects, results are often based on partial characterization, thus impacting the ability to discern reliable conclusions and comparisons. The need for standardization of methodologies to tackle experimental bias, as well as systematic and rigorous evaluation of methodologies, has been formally proposed. A very limited understanding on how experimental conditions shape biological response exists, inhibiting comparisons of approaches, interpretation of results, and the design of cross-laboratory studies. It is hoped that attention will be directed towards this unsolved issue, which is critical to improve knowledge of T. gondii virulence and host-pathogen interactions.

Potential for Genetic Intervention

T. gondii is a sophisticated parasite that is able to propagate and survive in its hosts through a coordinated set of cellular and molecular strategies. These same strategies are also involved in host manipulation and the development of pathology in the brain. Although the scientific endeavors of the community have led to some advances, most of the molecular and cellular aspects controlling T. gondii pathogenesis in the mammalian brain remain open questions. Recent advances in genomic, proteomic, and metabolomic methods will offer new avenues to approach the underlying mechanisms T. gondii uses to infect and manipulate its host [26]. There are many untested areas that merit further investigation into susceptibility to ocular toxoplasmosis and other syndromes involving this parasite since genes or processes that control the outcome of this disease will probably be related to pathogenicity in the central nervous system (CNS) and in other organs. Many researchers have focused on the occurrence of the disease in infected individuals. Central aspects are endowed on aspects like the outcome of infection after congenital transmission and on the nature of the potent anti-Toxoplasma immune response that is behind resistance to acute disease in otherwise immunocompetent hosts. Since initial disease does not always progress to severe forms the research effort on resistance to tissue reactivation and on ocular disease should go hand-in-hand. An additional focus for the studies should be the role of T. gondii parasites, other than type II, in the development of severity and occurrence of ocular disease.

Global Perspectives on Toxoplasmosis

Toxoplasmosis is a disease caused by Toxoplasma gondii. It has been known for over a century but has recently received greater attention as environmental factors that govern the transmission of this parasite have begun to be revealed. Some findings have emerged that hold implications for public health interventions. For instance, oocysts that are shed in the feces of cats survive for long periods of time in the environment, allowing large populations to accumulate with the result that water supplies, vegetables and soil can become contaminated. There is an association with cysts being acquired from undercooked meat and infected lamb carcasses becoming an important risk factor for abattoir workers. However, there is wider exposure of pregnant women in particular to environmental infection that warrants public education to avoid potential consequences for the fetus. There is an association with interest in cats, including ownership, environmental exposure, feeding, pet contact or changing litter, reflecting a central, although incidental, role for cats in the ecology of the disease. Greater efforts towards basic education on transmission risk reduction are warranted [27]. To remain competitive, some countries are at the forefront of rapid technological advances, innovating a growing range of interdisciplinary approaches to problems. One such approach to make smart cities smart is transforming data to knowledge – a more insightful, contextualised or manipulated form of data. The emergence of the field of data science is an acknowledgement of the importance of data as a resource and with this opportunity come the challenges of establishing procedures to collect, interpret, store and analyse it. This aims to summarise what has been learned about the ecology of Toxoplasma gondii since the previous review in 2010 and considers how this knowledge can help inform public health initiatives to reduce human exposure to the parasite.

Regulatory Framework for Genetic Research

A significant challenge faced by biodiversity researchers utilizing novel approaches is that types of research that have been pointed out as needing stricter regulation will differ across countries and topics, and there is a growing perception of a need for harmonized governance efforts across jurisdictions. In addition to the above, it is likely that either no or poorly framed scientists' guidelines will lead scientists to feel unprepared and uncertain regarding whether they can support community vetting and decision-making efforts without being trapped in inadequately framed procedure or being held legally responsible for a consequence of community decision making.

Key questions relevant to the Brazilian jurisdiction include, for example, (i) What extent of benefit and risks would be judged as highly and reasonably proportionate for public discussion and input that would warrant consideration of scientific assessments and/or oversight? (ii) Might research be considered sufficiently public interest to merit these even if advancing the research were expected to only slightly increase the risk or benefit to local gene flow? (iii) How to discuss and assess the value of ratios of benefit to risks consistent with differing importance of moderation and equity views across communities? Such issues are largely non-technical in nature and require consideration by researchers on broader grounds, i.e., ethics, principles, trust, etc., and thus warrant

investigation by scholars from a variety of backgrounds, including philosophy, law, and history.

Complementing consultation based research; this aspect of the research is likely to require, at least initially, a less anticipatory, more exploratory approach. For example, it may warrant less of a pre-design and engagement with stakeholders on the knowledge needed to address knowledge gaps and risks, and more of a discovery-oriented type of research mission to sketch a broader political space or mapper in which stakeholders might seek input, with subsequent professional facilitation of stakeholder-organized discussion sessions and scenarios of research and conference events. Such initial work is anticipated to occur at a high level in terms of both content and engagement context [28].

Funding and Resource Allocation

Research finds strains of Toxoplasma gondii that are resistant to the effect of IL-12. While T. gondii are obligate intracellular pathogens, acute infection is typically cleared by a vigorous cell-mediated immune response. There is a strong agreement that an early response to infection is vital for the future control of the parasite and that key components of the immune response include the production of IL-12 from macrophages and dendritic cells in a time-dependent manner. T. gondii actively modulates the immune response, and some strains are better than others at doing this. Toxoplasma strains can be categorized on the basis of their virulence into three major clonal lineages. The aim of this study was to identify strain-specific differences between T. gondii that modulate their effect on IL-12 cytokine secretion from macrophages. The strain-specific modulation of IL-12 production from macrophages will be examined using a supernatant transfer approach in in vitro cultures of rapidly propagated strains of T. gondii. The infecting strain will be genetically tagged by transformation with a luciferase gene to track its dissemination and virulence in a murine model of acute TN infection. Experiments will be performed to identify the genes in T. gondii involved in the strain-specific modulation of IL-12 production, transmembrane proteins, kinases, and surface proteins of an inherent size (40-90 amino acids) that have been adapted to sort to the right subcellular location in the parasite cell whilst also being exposed at the surface of the organism are candidates for transfection into low IL-12-modulating strains such as the P82 congenic strain and IVEG D1, D5- or D11-23 single-defective allelic mutants of P82 [3].

LDH is expressed in T. gondii extra-intermediate forms, which are probably transient in the parasitic life cycle but have the potential to be used as an antigen to test for infection or prior exposure. The genetic determinants in T. gondii that are responsible for strain-specific differences in the modulation of IL-12 production by macrophages will be sought via molecular approaches. The hyperproliferative haploids and haploid-derived diploid strains recently developed in this laboratory will be useful to examine the genetic basis of this phenotype and isolate null allelic mutants. This is an important step in the overall goal of understanding the molecular basis of virulence in T. gondii [29].

Collaboration with NGOs

Toxoplasma gondii is a complex parasite that has been shown to infect nearly all warm-blooded animals. This parasite is frequently transmitted by oocysts shed by felids, as well as by tissue cysts in undercooked meat or organ transplants. Humans are commonly exposed to T. gondii, which can have severe outcomes in immunocompromised patients and vector transmission through the placenta during pregnancy [30].

Research teams are developing various serological tests for the early diagnosis of congenital toxoplasmosis. Test kits, including toxo IgG, IgA, IgM, IgE detection kits, are in the verification stage of production. The capacity to produce, maintain, and administer animal models of toxoplasmosis in a developing country is also evaluated. The aim is to improve biosecurity with regards to laboratory animals, including felids (cats), a common source of Toxoplasma gondii infection. Diagnostic tools, confirmatory tests, and criticism of current widely accepted tests are being developed with the goal of large-scale utilization in developing countries.

Collaborative projects developed with NGOs and foreign academic institutions aim to work in research groups with a focus on our shared research interests. Various NGOs are being approached, including voluntary work to Associaçao de Toxoplasmose do Brasil, veterinarians who work on feline toxoplasmosis with São Paulo University Brazil, as they have a feasible procedure to make a monospecific TLC extract that is to be labelled.

Advancements in Diagnostic Techniques

Advancements in molecular biology techniques and bioinformatics analysis have facilitated the identification and characterization of predicted GRA proteins. In recent years, many studies have been undertaken, applying genetic methods to analyze the structure and function of GRA proteins. In addition, the rapid development of bioinformatics analyses of sequenced full genomes has created an opportunity for other researchers to conduct detailed searches for other shape and function framework proteins on a large scale [31]. This review summarizes recent findings about the structure and function of protein GRA.

Recently, several models have been developed to predict subcellular localizations of eukaryotic proteins. Insights derived from these predictions have led to the identification of novel proteins involved in the transport and processing of preproinsulin and tubulins in the endomembrane system. It was through these predictions that the GRA family of proteins was first discovered. Bioinformatics particularly profited from the complete genome sequencing. GRA proteins are a unique family of low-molecular-mass secretory proteins produced by all T. gondii strains tested to date, and with the surface proteins sag1 and sag2, they form the three original members of the secretory protein family. GRA proteins are produced at both excystation and intracellular stages of the parasite life cycle. Of these properties, the processes of export and secretion have been studied in greatest detail for the GRA proteins. It has been shown through the use of fluorescently tagged versions of GRA2, GRA7, GRA8, GRA9, GRA15, and GRA34 (all

GRA members) that once biosynthesized in the ER, these proteins are transported to and secreted from the parasitophorous vacuole in a calcium-mediated coalescence reaction.

Furthermore, various techniques, including laser scanning confocal microscopy, subcellular fractionation, and different ways to standardize GRA proteins through the parasite glycosylation and N-acylation modification pathways have been used to study GRA proteins released from the infected cells. With the isolation of the different stages of T. gondii, recent progress has been made using both bioinformatics analysis and cell biology methods to find frameworks in the assemblage of GRA proteins.

Role of Technology in Genetic Research

Toxoplasma gondii is a ubiquitous protozoan parasite that infects nearly all warm-blooded animals, including humans. The major definitive hosts of T. gondii are felids, and oocysts shed in feces of infected cats are the primary source of environmental contamination and waterborne infections. Transmissible via oocysts, tissue cysts, and transplacentally, T. gondii can increase the risk of neurological disorders in immunocompromised individuals, contribute significantly to farm animal losses, and pose zoonotic threats to humans. As a model organism for the Apicomplexa phylum, T. gondii possesses advantages in genetic tools that allow gene manipulation. Because genetic tools are limited, previous research primarily focused on in vitro isolation of protozoan samples from water. New molecular tools such as RT-nPCR, E. coli cloning vectors, guanylphosphate kinase enrichment, and infective transgenic plant parasites showed promise for understanding Giardia's evolution, phylogeography and host adaptation, and ecology in the community. Advances in the CRISPR/Cas9 system allow efficient gene disruption in diverse strains of T. gondii. Essential components including Cas9 and a single guide RNA are specifically designed to generate a ribonucleoprotein. Use of an elegant double selection system containing one selection marker per construct with the ability to regulate expressing degree of both NLS's ensures enhanced formation of long-length donor DNA in genome-wide Toxoplasma parasite. Software was developed to assist users in constructing the donor donor's sequences, and vector construction can be modified. The CRISPR/Cas9 gene editing system significantly accelerates targeted gene disruption and gene insertion in T. gondii [18]. Nothing is known about the genetic response mechanism of T. gondii to environmental fluxes in the community. The CRISPR/Cas9 gene editing system will save the grassroot researchers one years' cloning experience. The system will encourage scientists to study both plastic and in plastic T. gondii which could give insight to in the adaptability of T. gondii to environmental fluxes.

CONCLUSION

Fundamental Finding : Toxoplasma gondii is an opportunistic parasite responsible for a wide range of manifestations, including severe congenital diseases in newborns, immunological changes in organ transplant patients, and chorioretinitis in immunocompetent individuals, with host heterogeneity, pathogen clonal diversity, and interactions among the host, pathogen, and environment determining infection

outcomes, clinical presentation, and disease severity, while several genetic models and molecular tools, including PCR-RFLP, short tandem repeats, and microarray detection, enable the identification of strain types and elucidation of host–pathogen interactions. **Implication :** The genetic diversity of T. gondii has significant clinical, epidemiological, and public health relevance, as detecting strain types in human isolates provides insights for prognosis, outbreak investigation, and therapeutic development, allowing targeted interventions to reduce morbidity and mortality. **Limitation :** Despite available genotyping markers, comprehensive genetic studies focusing on human infection remain limited, and the variability of strains complicates the prediction of host outcomes and the establishment of standardized protocols for clinical and epidemiological applications. **Future Research :** Future studies should focus on large-scale genotyping of human isolates, integration of multiallelic and SNP markers, investigation of strain-specific pathogenicity, and development of standardized frameworks for molecular surveillance, aiming to improve public health preparedness, guide clinical interventions, and inform strategies to mitigate the burden of T. gondii infections.

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