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EXPERIMENTAL RAT MODELS IN INDUCED PARASITIC INFECTION: INOCULATION ROUTES, IMMUNE SYSTEM RESPONSES, AND SEASONAL INFLUENCES: A REVIEW

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ABSTRACT
Experimental parasitology in laboratory rats has a long tradition, owing to the physiological proximity of the rat to humans and to the ease with which infection can be established under laboratory conditions. This review draws on work carried out over the past several decades and brings together three themes that the literature usually treats in isolation, although in practice they are deeply intertwined: the routes by which a parasite is introduced into the animal host; the immune mechanisms, early and late, that follow, comprising the rapid innate responses and the slower but more specific adaptive responses they instruct; and the influence that ambient temperature and seasonal variation exert on the strength and the direction of those responses. The parasites considered include *Toxoplasma gondii*, *Leishmania* species, *Cryptosporidium parvum*, *Schistosoma japonicum*, *Fasciola hepatica*, *Strongyloidesratti* and *Echinococcus granulosus*. A theme common to these organisms is that the mode of delivery, and therefore the anatomical setting of the first immunological encounter, can alter the character of the reaction: from a predominantly cellular to a predominantly humoral response, and from an acutely inflammatory to a more regulatory profile. The evidence likewise indicates a substantial seasonal variation in immune parameters, demonstrated experimentally in the published literature yet seldom reported in primary studies. The review closes with a call for greater attention to standardization, and to the explicit documentation of environmental conditions, in parasitological research that uses the rat as a model.

Keywords: *rat model; innate immunity; adaptive immunity; experimental parasitology; inoculation routes.*

1. INTRODUCTION

Parasitic diseases remain among the most significant infectious diseases of humans and animals alike, and more than a billion people are thought to carry one or more parasites at any given time [1]. The veterinary picture is much the same. In many developing and middle-income countries, Iraq among them, the loss of livestock to helminth, protozoan and ectoparasitic infection places a real limit on food production. Against this background, experimental animal models occupy an essential place, because they allow the biology of a given host–parasite relationship to be examined under defined conditions that are, for the most part, reproducible. Among laboratory mammals the rat holds a particular position. *Rattus norvegicus* shares a high degree of physiological and immunological similarity with humans, is relatively affordable to maintain, and can be infected with a wide range of parasites of medical and veterinary importance [2]. The outbred Wistar and Sprague–Dawley strains have become the workhorses of experimental parasitology, while inbred lines such as Fischer 344 and Lewis are reserved for studies that require greater genetic uniformity. The immunological literature on the rat is now substantial enough to serve as a meaningful reference against which new observations in experimentally parasitised animals can be interpreted.Even so, two variables that exert a large

influence on experimental outcome are handled inconsistently in the published work. The first is the route of inoculation. It is generally accepted that intraperitoneal, oral, subcutaneous and intracerebral delivery give somewhat different results, yet the mechanistic basis for those differences, and their practical consequences for the interpretation of immune data, are rarely discussed in sufficient depth [3]. The second is the influence of season and ambient temperature. Immune function in mammals is not static across the year: melatonin rhythms, corticosteroid tone and lymphocyte activity all shift with photoperiod and thermal environment, and those shifts are large enough to alter infection outcomes in ways that could easily be mistaken for true biological differences between experimental groups [4].The parasites established in rat models fall into three broad categoriesprotozoa, helminths and cestodes and each category presents its own biological challenge, so that an effective and stable infection depends both on the inoculation method chosen and on the way the organism engages the host immune system. The present review therefore takes these strands together: the rat strains commonly employed and the parasites established in them; the routes of inoculation and their immunological consequences; the innate and adaptive responses that follow; and the seasonal and thermal context in which all of this takes place. The aim is a practical one, namely to help researchers align experimental design with the biological question being asked, and to report the conditions under which their results were obtained.

2. RAT STRAINS COMMONLY EMPLOYED IN PARASITOLOGY RESEARCH

Choosing the proper rat strain is a critical decision. Strains display different immune tendencies which can affect how the parasite interacts with the strain and as a result, the outcome can be unique to that particular strain. This is particularly relevant for studies where authors provide general immunological findings based on a study involving a single strain of rat and fail to mention that other strains could have provided different findings. Table 01 summarises the strains most frequently used in rat parasitology, together with their characteristic immune profiles and the settings for which each is best suited.

Table 01. Rat strains and immunosuppression models commonly employed in experimental parasitology, with their characteristic immune profiles and typical applications.

Strain / model	Genetic background	Characteristic immune profile	Representative parasite models	Practical considerations
Wistar	Outbred	Vigorous and predictable responses; Th2-biased to helminths (raised IgE, eosinophilia, IL-4 and IL-5) but Th1-biased, with high IFN-γ, to intracellular protozoa	Toxoplasma gondii; Cryptosporidium parvum; Echinococcus granulosus	The most widely used strain internationally, including most research institutes in the Arab world; outbred variability calls for adequate group sizes
Sprague–Dawley	Outbred	Heritable natural resistance to Schistosoma japonicum mediated by innate effectors — mast cells and platelets — rather than by T cells or antibody [6]	Schistosoma japonicum; T. gondii; Cryptosporidium spp.	The preferred choice when the question concerns natural resistance rather than susceptibility; general immune vigour is high
Fischer 344 and Lewis	Inbred	Genetically uniform, which narrows the between-animal variance of immune readouts	Studies in which genetic uniformity is a design requirement	Permits smaller groups for the same statistical power, but findings may prove strain-restricted
Dexamethasone	Drug-induced model	Broad suppression of	Cryptosporidium parvum — prolonged,	Models the immunocompromised

		both cellular and humoral immunity [7]	severe infection where immunocompetent rats produce only a mild, transient one	patient (HIV/AIDS, transplantation, oncology); the protocol itself imposes immune alterations that must be allowed for in interpretation [8]
Cyclophosphamide	Drug-induced model	Preferential depletion of proliferating lymphocytes [7]	Establishment of infections that an intact host would clear rapidly	Same interpretive caveat as above; the degree of suppression should be verified rather than assumed

2.1 Wistar Rats

Wistar rats are outbred rats that display immune responses that are quite strong and behave predictably in a lab setting. Because of these traits, Wistar rats are one of the most commonly used strains in parasitic research. They have widespread usage across the globe, including most research institutes in the Arab world. responses to helminth infections are characterized by the Th2 type and lead to increased IgE and eosinophils, as well as increased IL 4 and 5 . However, intracellular parasites like Toxoplasma gondii induce a Th1 type response with increased production of IFN-γ. In light of such context-boundedness of the Wistar rat immune response, this strain can be flexible, but response to experiments needs to be framed by the parasite as well as the inoculation method employed

2.2 Sprague-Dawley Rats

Sprague-Dawley (SD) rats are particularly interesting to the field of parasitology because they display an heritable natural resistance to Schistosoma japonicum, a finding which shows that T cells and antibodies are not playing the leading role in this resistance, but effector cells of the innate immune system (i.e. mast cells and platelets). This finding, which came from very discerning depletion studies, was originally a finding that was described as counter-intuitive because this type of resistance had previously been thought to be impossible. SD rats had been use in model of toxoplasmosis and cryptosporidiosis, and their general immune vigour makes them a reasonable choice when the studies wishes to read natural infections resistance rather than susceptibility activity.

2.3 Immunosuppressed Models

The parasitological studies are conducted on pharmacologically immunosuppressed rats before or duringthe infection. Dexamethasone, a corticosteroid that at high doses inhibits both cellular and humoralimmunity, and cyclophosphamide, which preferentially acts on proliferating lymphocytes, are among themost commonly used agents [7]. These treatments allow for a successful parasitological challenge onthose species that otherwise may be cleared quickly. For example, Cryptosporidium parvum wouldonly cause a mild and transient infection in healthy adult Wistar rats, yet would cause prolongedand severe infections in rats with dexamethasone-induced immunosuppression. The model withimmunosuppressed rats offers the opportunity to study the situation of other patients, for example,those infected with HIV/AIDS and patients who have undergone organ transplantation or arecurrently receiving oncological treatment. It is worth remembering that the immunosuppressionprotocol adds multiple immune alterations that may interfere with the parasitic immune controlmechanisms, which the researchers must take into consideration [8].

3. PARASITES USED IN EXPERIMENTAL RAT INOCULATION STUDIES

The parasites that have been most extensively studied in rat models fall into three broad biological categories: protozoa, helminths, and cestodes. Each category presents distinct biological challenges to the host immune system and requires different inoculation strategies to establish reliable infection.

3.1 Protozoan Parasites

Protozoan Parasites Toxoplasma gondii is probably the most intensively studied parasite in the rat immunology literature. An obligate intracellular organism, T. gondii can be inoculated in the form of free The rapidly dividing form (tachyzoite), tissue cysts containing bradyzoites, or sporulated oocysts obtained from cat faeces. Each form has a different infection kinetics, with the production of tachyzoites induces acute infection rapidly and oocyst ingestion more accurately mimics the natural oral route. Induces immune reactions that start in the gut-associated lymphoid tissue, which then spread. systemically [9]. In immunocompetent rats, infection with T.gondii is kept in check, but not eliminated. This had been eliminated, and tissue cysts had remained in the brain and muscle for the lifetime of the animal chronic State that is mediated mostly by continuous surveillance by the CD8+ T-cells. The leishmania species have been experimentally introduced in the rats by subcutaneous and intradermal route. model the natural sandfly transmission route. Rats are less susceptible than The immune responses that mice make to Leishmania are interestingly strain dependent and mice can be made susceptible to Leishmania; Leishmania (L. donovani) is more likely to

cause accumulation of the parasites in the spleen and liver. mixed immune response, whereas the cutaneous species (L. major) gives rise to localised lesions which are smaller in size. Producing Th2 cells.The ratio of IFN-γ-producing Th1 cells to IL-4-driven producing Th2 cells greatly influences and determines and resolution. Th2 activity [10]. Cryptosporidium parvum is notable, as it has been the subject of an increasing number of studies. In recent years a number of rat studies have been conducted, some of which have been stimulated by a renewed interest in waterborne disease. outbreaks. An immunocompetent intratracheal rat model was described even recently in 2024, showed that rats can be infected with Cryptosporidium in the lungs as well as in the gut. the proper experimental parameters [11]. This type of sophisticated model becomes available to expand the research toolkit considerably

3.2 Helminths

The tolerant of limited collateral damage to untovulable neural tissue Intracerebral inoculation of T. The CNS infection in rats produced by gondii or Echinococcus protoscoleces is a consistently reliable occurrence and is characterized by microglial activation, astrocytosis, localised blood-brain barrier disruption, at higher doses fatal encephalitis. The immunological laws in the brain are very different from Results from intracerebral models cannot be extrapolated to those in peripheral tissues. To generalise to IP or SC infection studies.

Intravenous Route Parasites are disseminated systemically immediately into the bloodstream by IV delivery, which bypasses all peripheral. immune barriers. This pathway is only applied in specific experimental instances modelling haematogenous. it is not common used in the diagnosis of high stage parasitic diseases, or in giving a defined dose for kinetic studies. It was used as a primary infection model due to its artificiality and the strong and fast immune responses. it can provoke

3.3 Cestodes

Echinococcus granulosus, which causes cystic echinococcosis (hydatid disease) in humans and many animal species, has been established experimentally in rats by intraperitoneal or intracerebral injection of protoscoleces. The organism grows slowly, forming hydatid cysts in the peritoneum, liver, or brain over a period of months [14]. The rat model has illuminated aspects of cyst biology and the immunological mechanisms by which the host attempts to contain but rarely eliminates the developing larval cestode. Studies using this model have also been central to evaluating the pharmacokinetics and efficacy of benzimidazole antiparasitic drugs.

4. ROUTES OF INOCULATION AND THEIR IMMUNOLOGICAL CONSEQUENCES

The question of how a parasite enters the host is far more than a logistical detail. The anatomical site of initial encounter determines which immune cell populations are first activated, what cytokines they produce, and through a cascade of intercellular signalling events how the adaptive response is subsequently shaped. The same parasite, delivered by different routes, can produce responses that differ in character as much as in magnitude. Figure 1 summarises, for each of the routes discussed below, the anatomical site of first encounter and the immune populations that are engaged there.

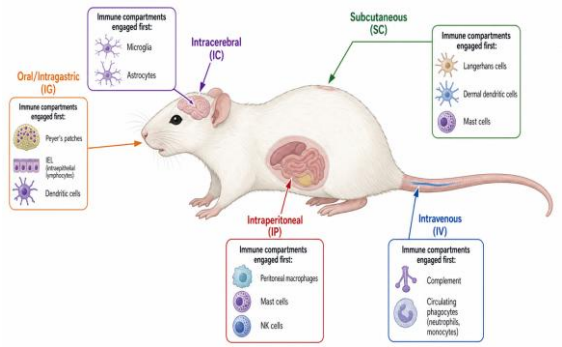

Figure 01: Routes of inoculation in the rat and the immune compartments they engage first. The anatomical site at which the parasite is introduced determines which resident populations sense it, and therefore the character of the early response that instructs adaptive polarisation.

4.1 Intraperitoneal Route

Intraperitoneal (IP) injection is the most commonly employed route in experimental rat parasitology, largely because it is technically straightforward and reliably delivers a defined inoculum without the losses associated with oral dosing. The peritoneal cavity contains resident macrophages, mast cells, natural killer cells, and B1 B-lymphocytes, all of which become activated rapidly following introduction of parasitic material [15]. IP inoculation with T. gondii tachyzoites produces an intense early peritonitis within 24–48 hours, followed by systemic dissemination and subsequent immune containment. The limitation of this route is its artificiality few parasites reach the peritoneum via natural infection and researchers should be cautious about assuming that immunological findings from IP models translate directly to naturally infected hosts.

4.2 Oral/Intragastric Route

Oral gavage or voluntary ingestion is the natural route for many of the most important parasites in both human and veterinary medicine. Ingestion of T. gondii oocysts, Fasciola metacercariae, Cryptosporidium oocysts, or Echinococcus eggs engages the gastrointestinal mucosal immune system Peyer's patches, intraepithelial lymphocytes, lamina propria

dendritic cells, and mesenteric lymph nodes before the parasite gains access to systemic compartments [16]. The mucosal immune response initiated at these sites has a characteristic profile: strong secretory IgA production, activation of mucosal mast cells, and a tendency toward Th1 polarisation in the case of intracellular organisms. Studies comparing oral and intraperitoneal delivery of T. gondii have shown that oral infection produces a more pronounced mucosal IgA response while IP infection leads to earlier systemic cytokine elevations, illustrating how the route shapes not just the magnitude but the geography of the immune response.

4.3 Subcutaneous Route

Subcutaneous (SC) injection deposits infective material into the dermis and underlying connective tissue, an environment populated by Langerhans cells, dermal dendritic cells, mast cells, and a network of lymphatic vessels draining to regional lymph nodes. This route best approximates natural infection for vector-borne parasites such as Leishmania species, where the sandfly's bite introduces promastigotes into the skin. Rat models using SC Leishmania inoculation have contributed substantially to understanding how cutaneous dendritic cells process and present leishmanial antigens, and how the balance of cytokines in the draining lymph node particularly the ratio of IL-12 to IL-10 determines whether the response polarises toward parasite-controlling Th1 or parasite-permissive Th2 [10].

4.4 Intracerebral Route

The brain and spinal cord have a place in the body. They are protected from the system.This is different from parts of the body.The brain and spinal cord have their rules for the immune system.The immune cells that are usually found in parts of the body are not as active in the brain and spinal cord.Instead microglia are the immune cells that help protect the brain and spinal cord.The brain and spinal cord try to avoid inflammation.This is to prevent damage to the brain and spinal cord.When we put T. Gondii or Echinococcus protoscoleces into the brain of rats it causes an infection in the brain.This infection has effects, including: Microglia become active Astrocytes become active The barrier between the brain and blood gets disrupted At doses it can cause fatal encephalitisThe rules of the immune system in the brain are different from those in other parts of the body.So we cannot assume that the results from brain infection studies will be the same as those from studies of infections, in parts of the body.The brain infection studies are used to understand how the central nervous system gets infected.These studies help us learn about brain and spinal cord infections.The brain and spinal cord are very important.So we need to study themIntracerebral route helps us study these infections.T. Gondii and Echinococcus protoscoleces are used in these studies.

4.5 Intravenous Route

Intravenous delivery produces immediate systemic parasite dissemination, bypassing all peripheral immune barriers. This route is used in specific experimental contexts modelling haematogenous spread in advanced parasitic disease, or delivering defined doses for kinetic studies but is rarely used as a primary infection model because of its artificiality and the rapid, severe immune reactions it can provoke [18]. IV inoculation triggers complement activation within minutes and can cause acute inflammatory responses that overwhelm the slower adaptive mechanisms the researcher may wish to study.

4.6 Comparative Considerations

When one surveys the literature comparing immune outcomes across inoculation routes, a few generalisations hold reasonably well. Oral infection tends to produce strong mucosal IgA and Th1 responses for intracellular organisms, and Th2-predominant responses for helminths. SC infection drives regional lymph node responses with a skin-associated dendritic cell contribution that shapes downstream T-cell polarisation. IP infection rapidly mobilises peritoneal innate immunity and produces early systemic cytokine elevations. IC infection engages a qualitatively distinct microglial-centred immune response with limited peripheral amplification. Researchers designing experiments should deliberately align route choice with biological question and should report the chosen route explicitly, with the reasoning behind it, in any publication [3]. These comparisons are set out in full in Table 02.

Table 02: Comparative summary of the principal inoculation routes used in experimental rat parasitology.

Route	Parasites and stages typically delivered	Immune compartment engaged first	Dominant early response	Principal advantages and limitations
Intraperitoneal (IP)	T. gondii tachyzoites; Echinococcus granulosusprotoscoleces	Peritoneal macrophages, mast cells, natural killer cells and B1 B-lymphocytes [15]	Intense peritonitis within 24–48 h, followed by systemic dissemination and early cytokine elevation	Technically straightforward and delivers a defined inoculum without the losses of oral dosing; but few parasites reach the peritoneum naturally, so translation to natural infection is uncertain
Oral / intragastric	T. gondii oocysts; Cryptosporidium oocysts; Fasciola	Gut-associated lymphoid tissue: Peyer's patches,	Strong secretory IgA, mucosal mast-cell	Reproduces the natural route for most food- and water-borne parasites; but

	metacercariae; Echinococcus eggs	intraepithelial lymphocytes, lamina propria dendritic cells, mesenteric lymph nodes [16]	activation, Th1 polarisation for intracellular organisms and Th2 for helminths	the delivered dose is less precise because of losses in gastric transit
Subcutaneous / intradermal (SC)	Leishmania promastigotes	Langerhans cells, dermal dendritic cells and mast cells, draining to the regional lymph node	Regional lymph-node priming in which the IL-12 : IL-10 balance decides Th1 versus Th2 polarisation [10]	The closest approximation to vector-borne transmission; but it is applicable only to parasites with a skin-entry stage, and lesion kinetics are slow
Intracerebral (IC)	T. gondii; E. granulosusprotoscoleces	Microglia and astrocytes within an immune-privileged compartment	Microglial activation, astrocytosis and focal blood–brain barrier disruption; fatal encephalitis at higher doses	The only direct way to model central nervous system infection; but the findings cannot be extrapolated to peripheral routes, and the procedure is demanding and costly in welfare terms
Intravenous (IV)	Defined parasite doses for kinetic studies; models of haematogenous spread	Complement and circulating phagocytes; all peripheral barriers are bypassed	Complement activation within minutes and immediate systemic dissemination, with severe acute inflammation [18]	Gives precise dosing and models advanced disseminated disease; but it is highly artificial, and the early reaction may overwhelm the slower adaptive events under study

5. INNATE IMMUNE RESPONSES IN RAT PARASITIC INFECTION

The very first responses to parasitic infection, developed in the first hours and days, are mediated by cells and molecules that do not require prior antigen exposure. This innate arm of immunity is rapid but not very specificrecognising broad molecular signatures of pathogen origin through pattern recognition receptors including Toll-like receptors, NOD-like receptors, and C-type lectins [19]. In rats, as in other mammals, the innate response to parasites determines not only the immediate inflammatory environment but also the instruction set that drives subsequent adaptive immunity.

5.1 Macrophages

Peritoneal and tissue macrophages are typically among the first cells to encounter an experimentally introduced parasite. heir response depends on the type of stimulus. Organisms that live inside cells like T. Gondii trigger a kind of macrophage activation called the M1 state. In this state cells make oxide, reactive oxygen and cytokines that fight inflammation such as TNF-α IL-1β and IL-6 . These help kill parasites or create an environment that's not friendly to them. Helminths on the other hand tend to activate macrophages in a different way called the M2 state. This leads to the production of IL-10 arginase and transforming growth factor beta. These molecules reduce inflammation. Help remodel tissue, rather than killing parasites. T. Gondii has developed proteins, like ROP and GRA proteins that are secreted to avoid being killed by macrophages. This shows how T. Gondii and the immune system have evolved together with the immune system exerting pressure, on T. Gondii to adapt.

5.2 Natural Killer Cells and Neutrophils

Natural killer cells are really important when it comes to controlling parasites. They do this by sending out something called IFN-γ. This helps get macrophages ready to fight off the guys. It also starts to create an environment that will help decide what kind of job T-cells will do later on. In studies with rats that have toxoplasmosis, when the Natural killer cells are taken away before the rats get infected the parasites spread fast. This shows how important Natural killer cells are in the beginning. Neutrophils are another type of cell that shows up at the scene of the infection quickly. Usually within a few hours. They are drawn in by signals from damaged tissue and special cells called mast cells. Once they arrive Neutrophils help kill the parasites by eating them up releasing chemicals and creating traps to catch them. We can see Neutrophils doing their job clearly when parasites infect the skin or the peritoneal area. Natural killer cells and Neutrophils are both crucial, in fighting off parasites.

5.3 Mast Cells and Eosinophils

Mast cells are found in places in our body like the skin, the mucosa in our gut and our respiratory tract. This means they are in a position to deal with parasites that enter our body through these places. For example, when SD rats get infected with S. Japonicum, the mast cells help fight the infection by releasing serotonin and special proteins that can kill the parasites. Eosinophils are another type of cell that helps fight parasites. When we get infected with kinds of worms our body produces a lot of eosinophils. These cells work with antibodies to kill the parasite larvae. They cover the larvae with proteins called IgG and IgE and then they release toxic substances that directly attack the parasites. This way of killing parasites has been studied a lot in rats infected with Strongyloides and Trichinella. Mast cells and eosinophils, like these play a role, in keeping us safe from parasites

6. ADAPTIVE IMMUNE RESPONSES

The adaptive immune system is what gives us the ability to fight off infections that our body would not be able to handle otherwise. This is different from immunity. When we look at rats that have parasites, we can see that the adaptive immune system starts to work within five to ten days of the infection. The adaptive immune system keeps changing over time. It gets stronger it changes how it works and it moves to parts of the body. This is what happens over weeks or months. The adaptive immune system is shaped by the parasite, how the infection got into the body and the genetic makeup of the rat. The adaptive immune. The parasite work together in a way that is unique, to each rat and each infection. Figure 2 places these adaptive events in sequence with the innate responses described in Section 5.

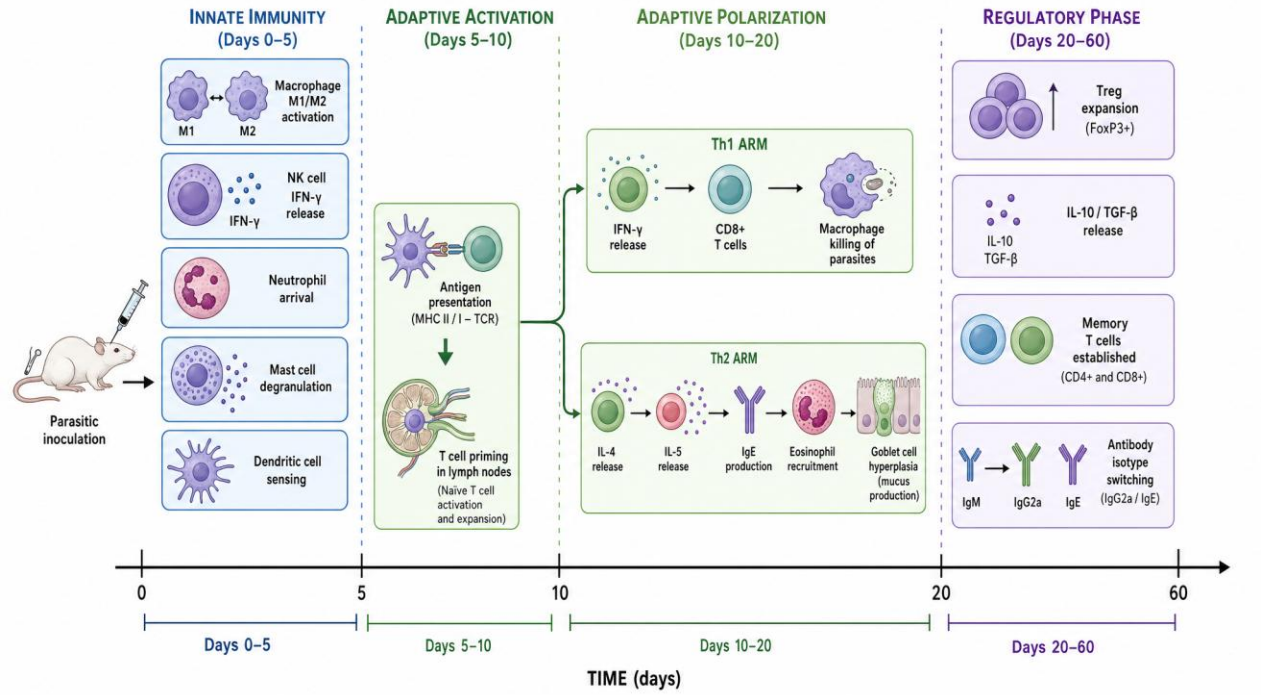

Figure 02: Temporal sequence of innate and adaptive immune events following experimental parasitic inoculation in the rat. Innate sensing instructs dendritic cells, which in turn direct the response toward the Th1 arm for intracellular protozoa or the Th2 arm for helminths; a regulatory layer restrains both arms from about day 10 onward. The timing shown is indicative and varies with parasite species, inoculum dose and route of delivery.

6.1 T Helper Cell Polarisation

The Th1/Th2 framework, first articulated by Mosmann and Coffman in the late 1980s [23], remains the most useful organising concept for understanding how rats respond immunologically to different classes of parasite. Intracellular parasites T. gondii, Leishmania donovani drive Th1 polarisation through IL-12 secretion by dendritic cells and macrophages, leading to IFN-γ-dominated responses that activate macrophage killing pathways. Extracellular helminths Fasciola hepatica, Strongyloidesratti, Schistosoma spp. drive Th2 polarisation via IL-4 and IL-13, producing eosinophilia, IgE class switching, goblet cell hyperplasia, and smooth muscle changes in the intestine that collectively accelerate worm expulsion [24]. This polarisation is not absolute regulatory IL-10 is produced in both types of infection, and Th17 responses have been documented in some helminthic models but the Th1/Th2 balance remains a reliable predictor of infection outcome.

6.2 Regulatory T Cells

Regulatory T cells (Tregs), defined by the expression of the transcription factor FoxP3 and secretion of IL-10 and TGF-β, control the magnitude of Th1 and Th2 responses in chronic parasitic infections. Their buildup in schistosome-

infected rats correlates with less hepatic immunopathology, particularly smaller granulomas around eggs, but also increased worm burdens, indicating the trade-off between parasite control and tissue protection [25]. A similar immune regulatory dynamic has been observed in chronic rat models of toxoplasmosis, where Treg expansion in the brain is thought to limit immunopathological damage to neural tissue while permitting parasite persistence as dormant cysts.

6.3 Antibody-Mediated Immunity

The humoral arm of the adaptive response is particularly prominent in helminthic infections, where parasite-specific IgE and IgG1 antibodies facilitate eosinophil- and mast cell-mediated damage to larvae through antibody-dependent cellular cytotoxicity (ADCC). In protozoal infections, IgG antibodies opsonise extracellular parasite stages for macrophage phagocytosis and activate the complement cascade. Measurement of parasite-specific antibody isotypes particularly the IgG/IgE ratio in experimentally infected rats provides a useful indirect index of Th1/Th2 balance, since IgG2a production is driven by IFN-γ while IgE is driven by IL-4 [26]. Leptospirosis models in inbred rats have demonstrated that robust IgG responses against bacterial outer membrane components become detectable by approximately eight weeks post-infection, coinciding with resolution of renal colonisation [27].

6.4 CD8+ T Lymphocytes

Cytotoxic CD8+ T cells are essential for killing host cells that harbour intracellular parasites. In rat models of toxoplasmosis, CD8+ T lymphocytes recognise parasite-derived peptides presented on MHC class I molecules and eliminate infected cells via perforin/granzyme-mediated cytolysis [28]. The importance of this population has been demonstrated through depletion experiments showing dramatically higher parasite burdens and mortality in rats lacking functional CD8+ T cells. During the chronic phase of T. gondii infection, CD8+ T-cell surveillance of the brain is considered the primary mechanism preventing reactivation of dormant bradyzoite-containing cysts a finding with direct relevance to understanding toxoplasmic encephalitis in immunocompromised patients.

7. SEASONAL AND ENVIRONMENTAL TEMPERATURE EFFECTS ON IMMUNE FUNCTION IN RAT PARASITIC MODELS

Of all the variables that affect experimental outcomes in rat parasitology, seasonal and temperature-related factors are arguably the least consistently controlled and reported. This is a significant problem, because the immune system of mammals rats included is not a fixed, season-independent machinery. It oscillates across the year in response to photoperiod and thermal signals that are transduced through neuroendocrine pathways into meaningful changes in immune cell number, activity, and cytokine production [29].

7.1 The Neuroendocrine–Immune Interface

The pineal gland figures out how long the days are by getting signals from the eyes. It responds by changing how much melatonin it makes. Melatonin is made for a time in the winter when the nights are long and it is made for a shorter time in the summer. Melatonin works directly on the cells that help us fight off sickness. It also works indirectly by reducing the amount of corticosteroids that the adrenal gland makes. When there is a lot of melatonin like in the winter it usually helps the system: it makes the cells that fight off sickness stronger it helps the lymphocytes work better it makes more IFN-γ and it slightly changes the balance of the immune system to Th1. This means that if you give the amount of T. Gondii tachyzoites to rats that live in the winter and, to rats that live in the summer the winter rats might be able to fight off the infection better and have stronger signs of a healthy immune system. And this is not because the T. Gondii tachyzoites are different it is because the winter rats have a different immune system setting. Figure 3 summarises this neuroendocrine pathway together with the parallel effects of ambient temperature on host immunity and on the viability of parasite stages outside the host.

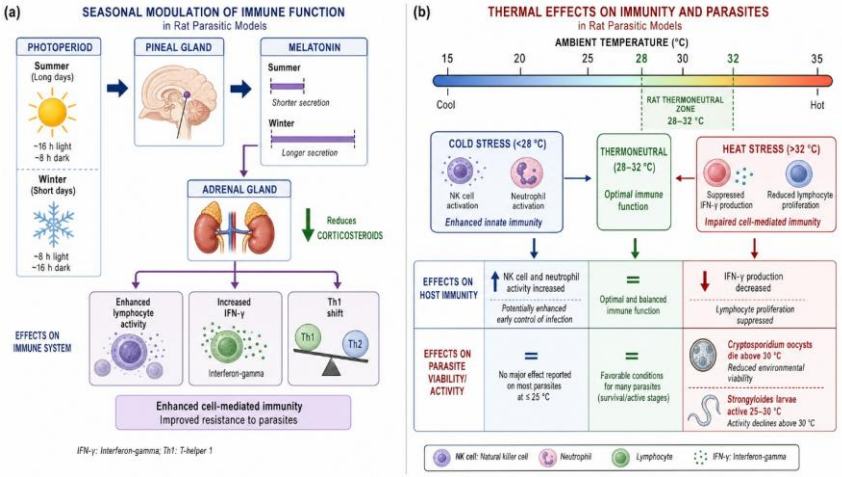

Figure 03: Seasonal and thermal modulation of immune function in rat parasitic models. (a) Photoperiod is transduced through pineal melatonin and adrenal corticosteroid tone into a measurable shift in effector immunity, so that an

identical inoculum meets a different host baseline in winter and in summer. (b) Ambient temperature relative to the rat's thermoneutral zone modifies host immune parameters in one direction and the survival and infectivity of parasite stages in another.

7.2 Effects of Ambient Temperature on Immune Parameters

Laboratory rats are typically housed at temperatures between 20 and 24°C, which is actually below the rat's thermoneutral zone of approximately 28–32°C [31]. Housing below thermoneutrality activates cold-stress pathways elevated corticosterone, adrenergic signalling, brown adipose thermogenesis that have complex and sometimes opposing effects on different immune compartments. Cold stress tends to enhance NK cell activity and neutrophil respiratory burst while suppressing macrophage phagocytic efficiency and reducing lymphocyte trafficking through secondary lymphoid organs. In tropical research settings where air conditioning may be unreliable, summer indoor temperatures can reach 35–40°C, producing a qualitatively different stress response: heat stress suppresses IFN-γ production, reduces lymphocyte proliferation, and can impair the Th1 responses that are critical for controlling intracellular parasites [32]. Any study comparing immune parameters across seasons conducted in such settings may be confounding true biological variation with thermally induced immune modulation.

7.3 Seasonal Variation in Parasite Infectivity

The host is not the thing that changes with the seasons. The survival and infectivity of parasites outside the host also depend on the temperature. For example Cryptosporidium oocysts can survive for months in cold water but they die quickly when the temperature is above 30°C.Strongyloides infective larvae are most active when the soil temperature is between 25°C and 30°C and they can easily penetrate the skin at this temperature. However when the temperature is below 15°C the larvae do not develop properly. Fasciola metacercariae that are on pasture can survive for a time when it is cool and moist. This means that when scientists study parasites in a controlled environment the parasites may not be as strong or infectious, at times of the year even if they use the same amount of parasites each time. This can make it harder to understand the results of these studies because the season can affect the outcome of the infection. [33].

7.4 Practical Recommendations for Researchers

The effects of the season on our system are really important: at the very least people who do research should write down and share the time of year and the average temperature where they did their experiments. If they have the money to do it they should keep the environment controlled. This means having a schedule with twelve hours of light and twelve hours of dark and keeping the temperature at around twenty two degrees Celsius. This is what big groups that care for laboratory animals say we should do. People who do research in places like Iraq, where it can get very hot in the summer and very cold in the winter should really pay attention to this. It would be great if future studies could test peoples systems at the start of each experiment. This means checking things like how well their immune system is working, how much cortisol and melatonin they have and doing a complete blood count. This would help us see if there are any differences in peoples systems, at different times of the year that could affect the results of the study. Seasonal immune effects and immune baseline differences are very important to consider when doing research and measuring baseline immune parameters can help us understand these seasonal immune effects better.

8. IMMUNOLOGICAL BIOMARKERS AND ASSESSMENT METHODS IN RAT PARASITIC MODELS

A detailed immunological characterization of infected rats incorporates many analysis modalities, spanning from whole blood cellular components characterization to molecular analyses for gene expression. Capturing the situation in its entirety is not possible with any single assay, and the selection of biomarkers should be consistent with the exercised hypotheses pertaining to the immunological aspects.

The evaluation of hematological parameters, total and differential white blood cell counts, remains the most straightforward and available indicator of systemic immune response. Eosinophilia is prominent in helminthic infection and is visible as early as in the second week post the challenge. Antigen dependent, clonal expansion of lymphocytes is evidenced by lymphocytosis, coupled with neutrophilia which is characteristic of either superinfection with bacteria of acute type, or high intensity of innate inflammation and its associated activities. These parameters can be evaluated in any resource-limited settings and must be included in any of the studies of experimental parasitology. The predominant method for quantifying particular cytokines (IFN-γ, IL-4, IL-5, IL-10, IL-12, TNF-α, IL-6, TGF-β) and parasite-specific antibodies of various isotypes and subclasses, in serum, bronchoalveolar lavage, peritoneal fluids and other tissue homogenates, is the enzyme-linked immunosorbent assay (ELISA). There has been a considerable increase in the last decade in the availability of rat specific, validated, research ELISA kits. Flow cytometry is a more advanced method for the analysis and identification of numerous leukocyte populations in peripheral blood to elucidate more on the molecular aspects, quantitative real-time PCR (RT-qPCR), with the Livak ($2^{-\Delta\Delta Ct}$) method, is a valid option for assessing the levels of expression of cytokine genes in tissues.

9. DISCUSSION

When you look at the evidence some things really stand out for researchers who work with rat models. First the way you give the rats the parasite is really important. A lot of studies do not talk about this enough they just mention it briefly in the methods section.. This is a big deal because it affects how the rats immune system responds. If a researcher changes the way they give the rats the parasite from injecting it into their belly to giving it to them they might get different results. This is not because they did anything it is just that the immune system responds differently to the two methods. Researchers should always explain why they chose a method and think about how the parasite normally infects rats in the wildThe Th1/Th2 framework is useful. It is too simple and can be misleading if you rely on it too much. These days we know a lot more about the system, including Th17 cells, innate lymphoid cells and how the immune system remembers things. Rat parasitic models are helping us learn more about these immune responses. The research on immunology, which we talked about in Section 7 raises a really important point: experiments done in the summer and winter can produce different results not because the parasite is different but because the rats immune system is different at different times of the year. This is especially important for laboratories in places like Iraq, where they might not be able to control the temperature and housing conditions, for the animals. The solution is not expensive researchers just need to start writing down and reporting the temperature and housing conditions.. They need to start thinking about these things as variables that can affect their results rather than just assuming they are always the same.

There are, of course, limitations in the body of evidence reviewed here. Many studies are conducted with small sample sizes (often six to ten animals per group) that provide limited statistical power for detecting modest immune differences. Positive and negative controls are not always included. And the overwhelming majority of published work uses either Wistar or SD rats, leaving other strains and potential strain-specific findings of broader interest underexplored. Greater attention to these design issues, guided by reporting frameworks such as the ARRIVE 2.0 guidelines [37], would substantially improve the value of rat parasitology research.

10. CONCLUSION

Rat models will continue to be a cornerstone of experimental parasitology in the foreseeable future. They are immunologically sophisticated, the depth of the existing literature allows new findings to be interpreted against it, and they are tractable to a range of experimental manipulations. All of these arguments are strongly in their favour. What this review has tried to demonstrate is that the utility of these models is maximised by attention to three interrelated sets of variables that are too often treated in isolation: (i) the route of delivery of the parasite; (ii) the cascade of innate and adaptive immune events that ensue; and (iii) the seasonal and thermal context in which those events take place.None of these variables is fully independent of the others. An oral infection in a winter-housed Wistar rat will produce a different immune signature from an intraperitoneal infection in a summer-housed Sprague-Dawley animal, even if the parasite and dose are identical. Understanding these interactions is not merely an academic exercise it is a prerequisite for designing experiments whose results will withstand independent replication and whose implications can be confidently extrapolated to real-world infection settings.Future work in this area should be focused on systematically characterising seasonal immune baselines in commonly used rat strains, developing and validating more physiologically faithful inoculation models better replicating natural infection routes and integrating emerging single-cell and transcriptomic approaches to generate higher resolution maps of the immune landscape during experimental parasitic infection. Advances in these areas will strengthen the bridge between laboratory rat models and the parasitic diseases for which they are used to understand.

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