Exploration of Immunology

Open Exploration

Open Access Original Article

Elevation of cytokines and antibodies in guinea pigs experimentally infected with *Tunga penetrans*

Janet Tarus[1,2], John Muoma[1], Dennis M.W. Ochieno[1,2], Maurice Omolo[2,3], Kelvin Kiprotich[1,2], Nicholas Kitungulu[1,4], Jackson Cheruiyot[1*]

[1]Department of Biological Sciences, School of Natural Sciences, Masinde Muliro University of Science and Technology, Kakamega 50100, Kenya

[2]Center for African Medicinal and Nutritional Flora and Fauna, Masinde Muliro University of Science and Technology, Kakamega 50100, Kenya

[3]Department of Pure and Applied Chemistry, School of Natural Sciences, Masinde Muliro University of Science and Technology, Kakamega 50100, Kenya

[4]Department of Agriculture, Livestock and Fisheries, County Government of Vihiga, Mbale 50300, Kenya

***Correspondence:** Jackson Cheruiyot, Department of Biological Sciences, School of Natural Sciences, Masinde Muliro University of Science and Technology, Kakamega P.O. Box 190-50100, Kenya. jcheruiyot@mmust.ac.ke

Academic Editor: Calogero Caruso, University of Palermo, Italy

Received: August 30, 2024 **Accepted:** April 27, 2025 **Published:** May 26, 2025

Cite this article: Tarus J, Muoma J, Ochieno DMW, Omolo M, Kiprotich K, Kitungulu N, et al. Elevation of cytokines and antibodies in guinea pigs experimentally infected with *Tunga penetrans*. Explor Immunol. 2025;5:1003198. https://doi.org/10.37349/ei.2025.1003198

Abstract

Aim: Tungiasis caused by *Tunga penetrans* is a neglected tropical disease that majorly affects children, the elderly and persons living with disabilities in rural homes in sub-Saharan Africa. The disease is characterized by swelling and inflammation symptoms, especially on the hands and feet. However, it is unclear whether inflammatory responses induced by *T. penetrans* may be associated with alterations of cytokine and antibody profiles. The study evaluated the immunological changes: cytokine and antibody profiles of experimentally raised guinea pigs exposed to *T. penetrans*.

Methods: A total of 24 guinea pigs were experimented on; 16 were exposed to *T. penetrans* while 8 were controls. Blood samples were collected before and after exposure. Enzyme-linked immunosorbent assay (ELISA) technique was used to quantify cytokines and antibodies. Data analysis was performed using GraphPad Prism 10.4.

Results: At day 10 of post-infection, guinea pigs showed significant elevation ($p < 0.05$) of pro-inflammatory cytokines, tumor necrosis factor alpha (TNF-α) (235 pg/mL), and interferon gamma (IFN-γ) (425 pg/mL) in the serum. Anti-inflammatory cytokine had a delayed elevation, with interleukin-4 (IL-4) peaking to 357 pg/mL by day 15, while IL-10 rose to 367 pg/mL by day 15 of post-infection. Total systemic circulating levels of antibodies in serum were significantly elevated ($p < 0.05$), with immunoglobulin E (IgE) elevating to 232 ng/mL while IgG peaking at 272 ng/mL on day 15 post-infection.

Conclusions: Pro-inflammatory cytokines elevated during the early stages of infection may serve as early markers for the infection, and their potential role in the pathogenesis of tungiasis needs to be explored further. The study has established that IgE and IgG are important antibodies that are produced in response

to tungiasis, and their efficacy in controlling the infection needs to be further explored for potential alleviation of severe forms of the infection.

Keywords

Tungiasis, neglected tropical disease, cytokine, antibodies, *Tunga penetrans*, guinea pigs

Introduction

Tungiasis is a parasitic skin disease caused by the penetration of the gravid female flea *Tunga penetrans* into the epidermis [1–4]. The disease is endemic in the tropics of Sub-Saharan Africa and the Americas [5–8]. The epidemiology and clinical manifestations of the disease have been well documented, while the host-parasite interaction on immune response is still limited.

The life-cycle of the flea begins when only mated female flea burrows into the stratum corneum of the skin, anchoring itself in the dermis. After a span of one to two days, the embedded flea begins to feed on host blood and tissue, rapidly increasing in size to accommodate egg maturation. The rapid growth creates a characteristic lesion often visible as a white nodule. When eggs are released from the embedded female and deposited on the ground in a favorable environment, larvae hatch 3–4 days later and undergo numerous larval stages before pupating 10–14 days later. The pupae stage lasts for another 10–15 days before adults emerge, ready to infect their next host [9, 10].

Pathogenesis starts when the flea embeds itself beneath the host's skin [11]. The flea feeds on blood and grows to as large as 2,000 times, triggering a localized inflammatory response marked by mast cell degranulation, infiltration of macrophages and lymphocytes, causing pain, swelling, and itching as a result of inflammation [12]. In humans, lesions are observed to have secondary bacterial infections, which later lead to ulceration, necrosis, and hyperkeratosis [13]. Infestations left untreated may continue to cause chronic inflammation and complications such as sepsis or tetanus [14].

Previous research indicated the presence of macrophages, lymphocytes, eosinophils, and mast cells in inflammatory infiltrates. Tungiasis infection has been reported to considerably increase the levels of tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) cytokines in humans [12, 14]. Studies on Wistar rats have revealed that during tungiasis infections, anti-inflammatory IL-10 cytokines are abundantly produced, which is associated with reduced blood TNF levels [15]. It is unclear whether the effect is similar in other animal models. However, it is still unclear exactly when the cytokines are generated and released locally shortly after flea penetration.

Antibody studies done in other ectoparasites, such as *Sarcoptes scabiei* (scabies) and *Dermatobia hominis* (myiasis), indicate that these infections result in immunoglobulin E (IgE) and IgG release. IgE causes histamine release, which causes itching and inflammation [16]. Scabies and myiasis have been reported to cause significant local inflammation and discomfort [17, 18], a pathology that is similar to tungiasis. IgG is essential for both long-term immunity and pathogen clearance. Recurring exposure to tungiasis raises IgG levels, which facilitate the identification and elimination of parasites [19].

The immunopathogenesis of tungiasis with regard to the interplay of cytokines and antibodies remains unclear. This study sought to determine the concentration of pro-inflammatory cytokines known for activation of Th1 (TNF-α and IFN-γ), Th2 interleukin-4 (IL-4), and immunomodulatory cytokine IL-10, and the levels of IgE and IgG antibodies in an experimental guinea pig model with an aim of providing an understanding of the host-parasite relationship. The findings aim to inform strategies for better management and control of tungiasis, ultimately contributing to the reduction of its burden in endemic regions.

Materials and methods

Experimental animals

Twenty-four (24), eight (8) week old American calico guinea pigs were used in the study. The animals were sourced from a commercial breeder specializing in pureline breeding and confirmed by a veterinary doctor to be free of infections and parasites. The animals were transferred to a research animal house at Masinde Muliro University of Science and Technology. The animals were split into groups of four animals per stainless free wire-mesh cage measuring 45 cm × 30 cm × 30 cm with a well-fitted water bottle in a clean and well-ventilated animal house. The animals were maintained on a standardized diet of commercial feed pellets from Royal Feeds Company (composition: 18% fiber, 13% protein, 3.5% oil, 5% ash, 0.7% calcium, 0.5% phosphorus, 2.6% fat, and 1,000 IU/kg of vitamin D_3). The animals were kept for two weeks to acclimatize prior to the commencement of the experimental procedures.

Experimental design and natural infection

The guinea pigs were divided into two groups and marked. Sixteen animals were exposed to sand fleas while eight animals were used as controls (un-infected). Baseline measurements of cytokines and antibodies were obtained from all animals on day zero, as shown in Figure 1. The exposed animals were confirmed to be infected by jiggers using a magnifying lens (JMH magnifying glass with 30× handheld large 18 LEDs). Infected animals were confirmed by the presence of small white protrusions, accompanied by redness, swelling, and signs of discomfort in the animal. Upon detection of infestation, the animals were taken back to the animal house for monitoring and experimental procedures. In the infected group, a minimum of three fleas were observed to have burrowed into the paw pads and ear lobes of each animal.

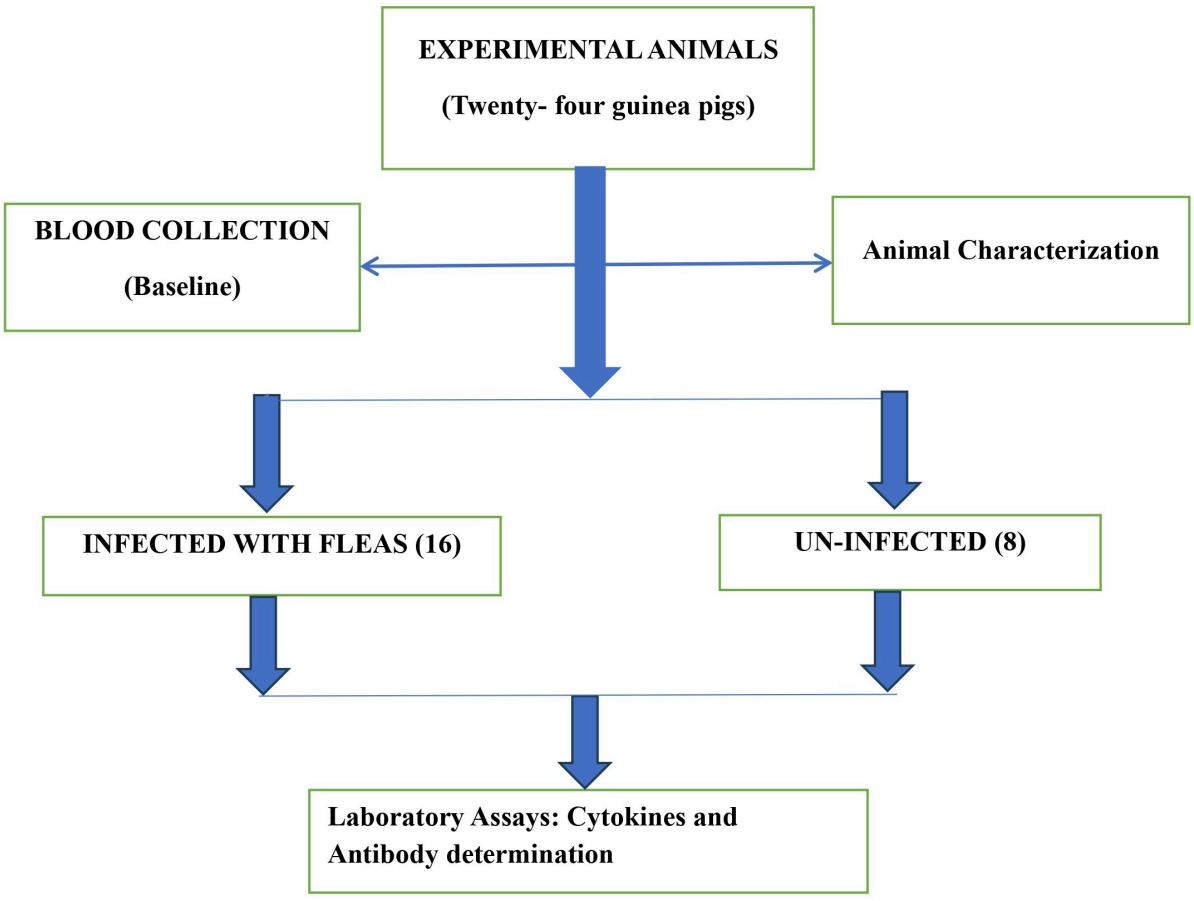

Figure 1. Schematic diagram of experimental design illustrating the key workflow of the study

Blood collection and serum preparation

Peripheral blood sample (1 mL) was collected in a sodium heparin tube at 5-day intervals (0, 5, 10, 15, and 20 days post-infection) from the lateral saphenous vein using a 25-gauge needle on a 2 mL syringe. The sample was left to coagulate at room temperature for 30 minutes and then centrifuged in a refrigerated centrifuge (Benchmark Scientific Inc., Sayreville, NJ, USA) at 4°C for 15 minutes at 2,000 × g. After centrifugation, serum was aliquoted into two tubes, each containing 200 µL, for cytokine and antibody assays.

Determination of cytokine concentration

TNF-α, IL-10, and IL-4 were determined using the guinea pig interleukins enzyme-linked immunosorbent assay (ELISA) kit (96 wells) (Wuhan Fine Biotech Co. Ltd, China). All samples, standards, and blanks were assayed in duplicates. The serum sample with the highest positive outcome and a negative control sample were used to optimize the ELISA procedure following checkerboard titrations. The samples, standards, and reagents were prepared according to the manufacturer's instructions. Briefly, the samples (100 µL each), 2-fold diluted, were pipetted into pre-coated sample test wells followed by incubation for 90 minutes at 37°C. The plate contents were discarded and washed two times with the wash buffer provided with the kit. 100 µL of biotin-labeled antibody at a dilution of 1:100 was pipetted into the bottom wells containing test sample, standards, and blank wells, followed by incubation for 60 minutes at 37°C. The cover was removed and the plate was washed three times with wash buffer and allowing the buffer to stay for two minutes in every wash. 100 µL of horseradish peroxidase (HRP)-streptavidin conjugate was added into each well, covered, and incubated at 37°C for 30 minutes. The plate was then washed five times, allowing the wash buffer to stay for 2 minutes for every wash. 90 µL of tetramethylbenzidine (TMB) substrate was pipetted into each well and incubated in a dark room for 20 minutes, followed by the addition of 50 µL of stop solution 2M H_2SO_4 into each well. The optical density was measured at absorbance 450 nm in a microplate reader (RT-9600) (Ray to Life and Analytical Sciences Co., Ltd) immediately after the addition of the stop solution.

IFN concentration was determined using a guinea pig IFN-γ ELISA kit 96T (Wuxi Donglin Sci and Tech Develop Co., Ltd, China) as outlined above.

Determination of antibody concentration

IgG and IgE antibody detections were performed using guinea pig-specific IgG and IgE pre-coated ELISA assay kit (Eagle Biosciences, USA). Standards were prepared according to the manufacturer's instructions. 100 µL samples in duplicate were pipetted into pre-coated wells and incubated at room temperature for 20 minutes. After incubation, the contents were aspirated. The wells were then completely filled with wash buffer and washed three times. 100 µL of enzyme-antibody conjugated with HRP at a dilution of 1:1,000 was pipetted to each well and incubated at room temperature for 20 minutes. After incubation, the wells were washed with washing buffer three times. 100 µL of TMB substrate solution at 1:100 dilutions was pipetted into each well and incubated in the dark at room temperature for 10 minutes. 100 µL of 1:100 dilution stop solution 2M H_2SO_4 was then added to each well, followed by optical density measurement in a plate reader at absorbance at 450 nm.

Data analysis

Statistical analyses were performed using GraphPad Prism software (version 10.4). Data entry was conducted in Microsoft Excel 2019. Descriptive statistics were expressed as mean ± standard deviation. Kruskal-Wallis was used to analyze mean difference across infected animals, while a post-hoc test was performed using Dunnett's multiple comparison test to check the difference between controls and post-infection days. Correlations between cytokine and antibody levels were assessed using Spearman's rank correlation coefficient. Statistical significance was established at $p < 0.05$ for all analyses.

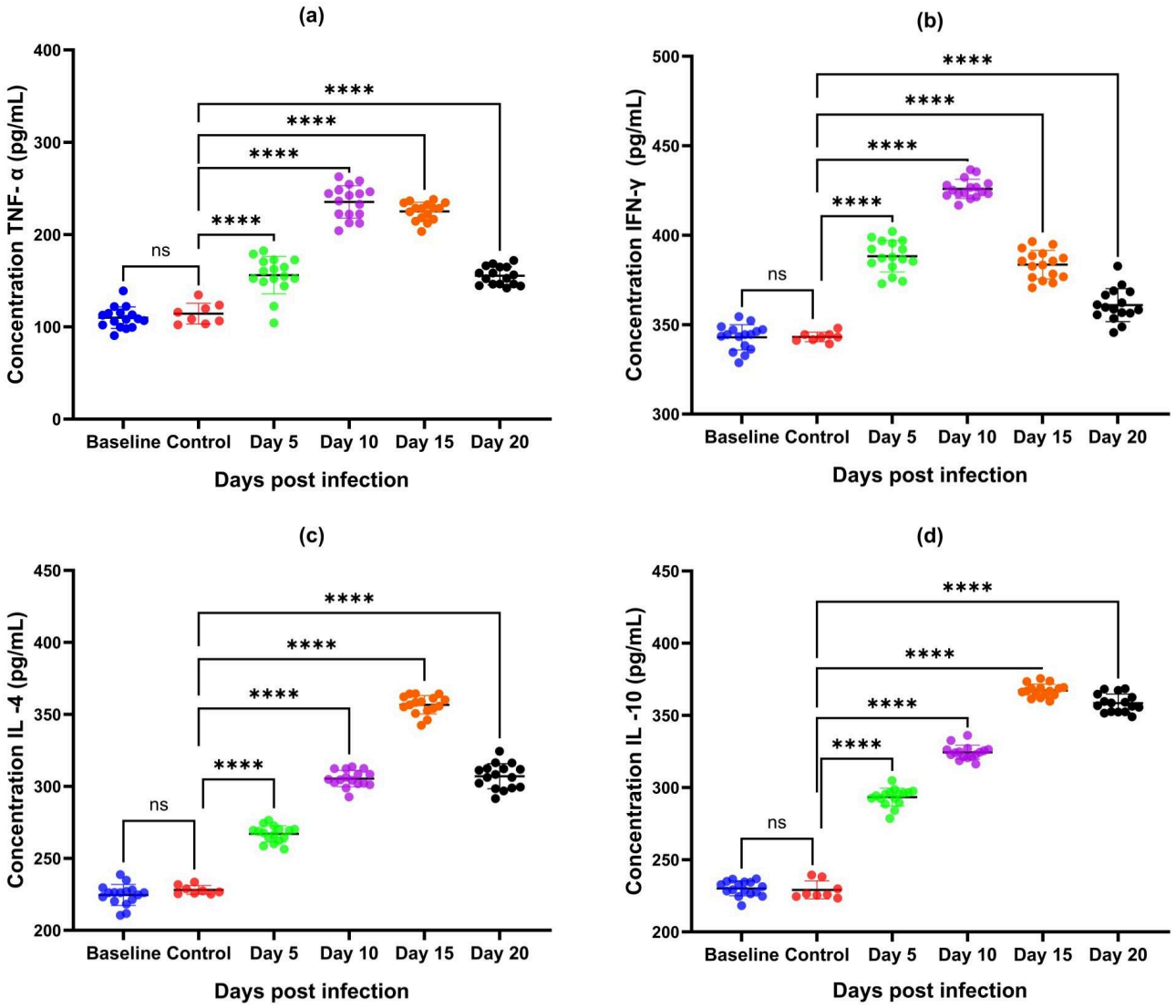

Figure 2. Concentrations of pro- and anti-inflammatory cytokines in serum following *T. penetrans* infection. (**a**) TNF-α, (**b**) IFN-γ, (**c**) IL-4, and (**d**) IL-10. Asterisk shows the significant effect, **** shows $p < 0.0001$. ANOVA analysis was used to calculate the *p*-value between control and infected, while Dunnett's multiple tests for comparisons between the control ($n = 8$) and infected ($n = 16$). ns: not significant

Ethical consideration

Experimental animals were taken care of to ensure that the procedures were appropriate and humane. During blood collection, animals were sedated to minimize pain and stress, in accordance with approved laboratory animal care protocols [20–22]. In order to reduce distress and pain to the animals, no more than three attempts were made, continuous sampling was avoided, and collecting more than three samples per day was avoided. Aseptic techniques were used in laboratory animals whenever possible. All surgical procedures and anesthetization were conducted under the direct supervision of a person competent in the use of the phlebotomy procedures. The vein was punctured using a 25G needle, and enough volume of blood was collected with a syringe.

Results

Systemic levels of pro-inflammatory and anti-inflammatory cytokines

The blood levels of pro-inflammatory cytokines TNF-α and IFN-γ increased significantly during the first two weeks of infection as compared to the controls. TNF-α level increased at day 5 (155.96 ± 20 pg/mL) and peaked at day 10 (235.39 ± 17 pg/mL) and declined steadily from day 15 (225.1 ± 9 pg/mL) and day 20 (155.263 ± 9 pg/mL) respectively, at $p < 0.0001$ as shown in Figure 2a. IFN-γ was elevated at day 5 (388.23

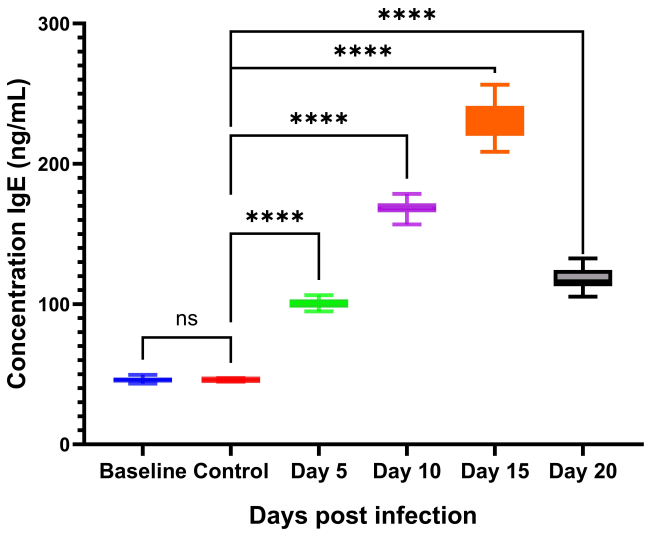

Figure 3. IgE concentrations following infection with *T. penetrans*. Asterisk shows the significant effect, **** shows $p < 0.0001$. ANOVA analysis was used to calculate the *p*-value between control and infected, while Dunnett's multiple tests were used for comparisons between the control ($n = 8$) and infected ($n = 16$). ns: not significant

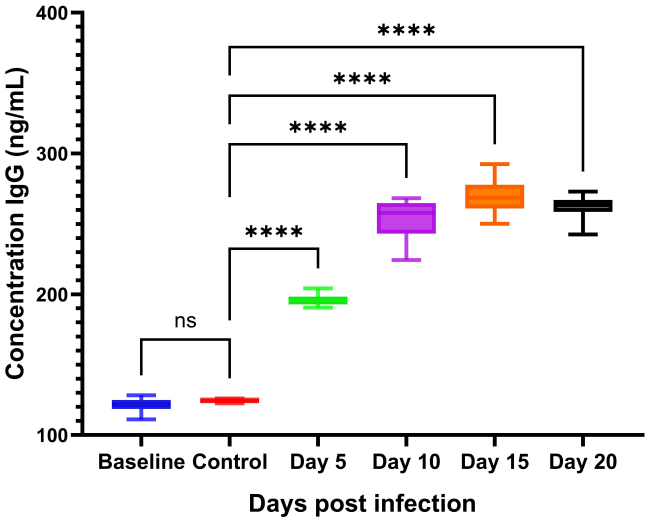

Figure 4. IgG concentrations following infection with *T. penetrans*. Asterisk shows the significant effect, **** shows $p < 0.0001$. ANOVA analysis was used to calculate the *p*-value between control and infected, while Dunnett's multiple tests for comparisons between the control ($n = 8$) and infected ($n = 16$). ns: not significant

± 8 pg/mL) and peaked at day 10 (425 ± 5 pg/mL). The levels declined steadily on day 15 (383.53 ± 7 pg/mL) and day 20 (360.94 ± 9 pg/mL) at $p < 0.0001$, as shown in Figure 2b.

Anti-inflammatory cytokines IL-4 and IL-10 increased during the course of infection. The levels of IL-4 elevated significantly at day 5 (267.03 ± 7 pg/mL) increased considerably at day 10, peaked at day 15 (305.45 ± 5 pg/mL), (356.7 ± 6 pg/mL) respectively and decreased significantly at day 20 (307.08 ± 8 pg/mL) at $p < 0.0001$ as shown in Figure 2c. IL-10 elevated considerably at day 5 to day 15 (293.41 ± 6 pg/mL), (324.41 ± 4 pg/mL), (367.17 ± 4 pg/mL) respectively, and decreased significantly at day 20 (358.48 ± 6 pg/mL) at $p < 0.0001$ as shown in Figure 2d.

Systemic levels of IgE and IgG

IgE showed a significant increase in levels of serum concentrations at day 5 (100.70 ± 3 ng/mL), 10 (167.81 ± 5 ng/mL), 15 (231.9 ± 13 ng/mL) and decreased significantly at day 20 (117.66 ± 7 ng/mL) at $p < 0.0001$ as compared with control as shown in Figure 3. IgG increased significantly from day 5 (196.1 ± 9 ng/mL), 10 (253.91 ± 13 ng/mL), 15 (271.61 ± 15 ng/mL) and decreased considerably at day 20 (261.95 ± 7 ng/mL), respectively, at $p < 0.0001$ after flea penetration as compared with controls as shown in Figure 4.

Determining the relationship between cytokines and antibodies

Positive correlation was observed between TNF-α and IgE; IFN-γ and TNF-α; IgE and IL-4; IgG and IL-4 (r = 0.774, r = 0.636, r = 0.896, r = 0.791) (Table 1). There was moderate negative correlation between IFN-γ vs. IL-10 (r = –0.518, $p < 0.01$) and weak negative correlation between TNF-α vs. IL-10 (r = –0.369, $p > 0.01$).

Table 1. Spearman's rank correlation of cytokines and antibodies at post-infestation days

Cytokines/Antibodies	*N*	Mean	TNF-α	IFN-γ	IL-4	IL-10	IgE	IgG
TNF-α	16	192.931	1					
IFN-γ	16	389.6611	0.636**	1				
IL-4	16	309.0694	0.548**	0.100	1			
IL-10	16	340.2647	–0.369	–0.518**	0.739**	1		
IgE	16	154.469	0.774**	0.246	0.896**	0.444**	1	
IgG	16	245.295	0.479**	0.094	0.791**	0.241	0.626**	1

** Correlation is significant at the 0.01 level (2-tailed)

Discussions

Tungiasis is an ectoparasitic disease caused by sand fleas penetrating into the skin, causing an inflammatory reaction with pain and itching, leading to limited mobility [4, 23]. The selection of guinea pigs as an experimental model was due to its similarities in immunological response to that of humans both in pathogenesis and symptomatology [24] making it an ideal model.

During post-infection, on day five, the guinea pigs showed early clinical manifestations of the disease, that is localized swelling, redness, and pain at the infection site. These symptoms are characteristic of acute inflammatory response commonly observed in both infected Wistar rats and humans at the initial stages of infection [25, 26]. The penetration of *T. penetrans* into the skin triggered immune response that involved both Th1 and Th2 pathways. This early phase of infection is characterized by a pronounced increase in Th1 cytokines such as TNF-α and IFN-γ [24, 25]. TNF-α is essential for recruiting immune cells such as neutrophils and macrophages to the infection site as it activates macrophages, which play an important role in controlling and eliminating the parasite [12, 27]. IFN-γ enhances the microbicidal activity of macrophages and promotes the differentiation of Th1 cells, both of which are crucial for initiating a strong immunological response against the parasite [1]. This early-stage immune profile, which includes the recruitment of lymphocytes, macrophages, eosinophils, and mast cells, mirrors what has been observed in human tissues infected with *T. penetrans* [27]. These findings imply that there is a relationship between the immunological presentation of the disease-infected guinea pig and that of the Wistar rat model, indicating the latter's suitability as a model animal.

The infected guinea pigs continued to show more pronounced signs of inflammation by day 10, increase in swelling and pain was observed. At this stage, Th1 cytokines were at its peak due to engorgement and growth of the flea causing more tissue damage and intense swelling while Th2 IL-4 started to elevate in order to induce humoral immune response by promoting B cell activation and class switching, which leads to the production of IgE which is observed to elevate by day 10 [28]. Also, IL-10 increased significantly to inhibit inflammatory and immune reactions mounted against the parasite by invasive TNF-α and IFN-γ, as seen in scabies [12]. The shift from Th1 to Th2 is critical for balancing the immune response, preventing chronic inflammation, and facilitating parasite control.

It was observed that at day 15, the flea attains maximum hypertrophy, eggs are released in addition to fecal material and watery secretion. This is consistence with the Wistar rat study conducted in Brazil [25]. IL-4 and IL-10 levels were observed to be at their peak level. Th2 cytokine IL-4 could be activated due to antigenic secretions, which induce a humoral response by inducing B-cell activation and class switching to produce IgE and IgG, which is observed to peak on the same day [29]. IL-10 could also be peaking at day 15 to inhibit excessive hypersensitivity mediated by IgE as a result of mast cells and eosinophils in combating the parasite [30]. Previous studies done on scabies, an ectoparasitic disease, reported increased circulating

IgE levels in humans [31, 32]. However, the timing of the antibody-mediated immune responses, as well as their relative importance in establishing protective immunity in tungiasis and other ectoparasites, are still largely unknown.

After the reproductive cycle, the flea dies, and the carcass of the parasite is expelled from the epidermis by the host's repair mechanism [27]. After the dislodgement of *T. penetrans* from the skin, guinea pigs showed minimal localized inflammation, pain, and sensitivity due to tissue damage. This clinical outcome was supported by the continued reduction in cytokine levels and an increase in IgG antibodies, as also seen in patients with myiasis [31]. IgG is a key antibody for long-term immunity and plays a critical role in neutralizing the parasite, preventing re-infection, and providing sustained immune protection [19, 32]. Increased levels of IgG during this phase are an indication that guinea pigs have developed a long-lasting immunity against *T. penetrans*, while the shift from the initial IgE-mediated response to a more sustained IgG response, a typical immune resolution in parasitic infections. The pattern of early IgE production followed by sustained IgG production has also been reported in other studies in ectoparasitic infections, such as scabies and myiasis [23, 33].

The strong relationship between the TNF-α and IgE in the present study could be linked to the protective mechanism of guinea pigs against the sand fleas during disease progression, since they both play the role of parasite clearing [33]. Negative correlation between TNF-α, IFN-γ levels, and IL-10, the mechanism of inverse correlation indicates that IL-10 regulates the immune response to protect the host from tissue damage due to inflammatory reactions, which has also been reported in humans with *Sarcoptes scabies* infections [33]. There was a significant positive correlation between IL-4 and IgE, which is because IL-4 induces the class switching of B-cells and co-expression of IgE to fight extracellular parasites [31].

The study had a limited sample size; therefore, future studies to consider enhancing the number of animals and focusing on understanding the mechanistic interplay of cytokines and antibodies both in pathogenesis and parasite clearance. Although our study found low variance in cytokine and antibody levels across the post-infection days, regardless of minor variations in sex and age of the animals, further studies need to consider using a highly homogenous population to check whether the immunological response would be similar or lower.

The findings reveal a dynamic interplay between Th1 and Th2 immune pathways during tungiasis infection in American calico guinea pigs. The early phase of infection is defined by a strong inflammatory response, with elevated levels of TNF-α and IFN-γ, which gradually transition to a Th2-dominated phase marked by increased IL-4 and IL-10. The immune shift aided in IgE and IgG production, demonstrating a coordinated mechanism for immune balance. This observation provides an understanding of host-parasite interactions and immune regulation in ectoparasitic infections. This study lays a foundation for future research in pathophysiology and management strategies of tungiasis infection.

Abbreviations

ELISA: enzyme-linked immunosorbent assay

HRP: horseradish peroxidase

IFN-γ: interferon gamma

IgE: immunoglobulin E

IL-4: interleukin-4

TMB: tetramethylbenzidine

TNF-α: tumor necrosis factor alpha

Declarations

Acknowledgments

We are grateful to Simon Masinde Kasili and Maurice Ndiema for the phlebotomy expertise in the withdrawal of blood from the animal. Linet Kim, Elisha Kimutai, Willy Aganyanya, and Lynn Omari for the technical assistance. We are also indebted to Evelyn Angariuki, Florence Avugwi, and Joram Omuoma for coordinating and providing their homes as the site of infestation.

Author contributions

JT: Conceptualization, Methodology, Validation, Data curation, Investigation, Writing—original draft, Writing—review & editing. JM: Conceptualization, Investigation, Resources, Writing—review & editing, Supervision, Funding acquisition. DMWO and MO: Funding acquisition. KK: Investigation, Methodology, Validation, Data curation. NK: Investigation, Writing—review & editing. JC: Conceptualization, Investigation, Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

The experimental procedure was done in accordance with regulations of the Institutional Scientific and Ethical Review Committee of Masinde Muliro University of Science and Technology (MMUST/IERC/172/2023), and the National Commission for Science Technology and Innovation granted the licence for the study (NACOSTI/P/23/26624).

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

All relevant data are available from the corresponding author on request [jcheruiyot@mmust.ac.ke].

Funding

The study was partially supported by Kenya National Research Fund Grants (NRF), reference number: [NRF/GRANT/VOL III/55]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Publisher's note

References

1. Abrha S, Heukelbach J, Peterson GM, Christenson JK, Carroll S, Kosari S, et al. Clinical interventions for tungiasis (sand flea disease): a systematic review. Lancet Infect Dis. 2021;21:e234–45. [DOI] [PubMed]

2. Mutebi F, Krücken J, Feldmeier H, von Samsom-Himmelstjerna G. Clinical implications and treatment options of tungiasis in domestic animals. Parasitol Res. 2021;120:4113–23. [DOI] [PubMed]
3. Linardi PM. Fleas and Diseases. In: Arthropod Borne Diseases. Cham: Springer; 2017. pp. 517–36. [DOI]
4. Silvestri V, Mushi V, Ngasala B. Tungiasis. In: Vascular Damage in Neglected Tropical Diseases. Cham: Springer; 2024. pp. 81–9. [DOI]
5. Deka MA. Mapping the Geographic Distribution of Tungiasis in Sub-Saharan Africa. Trop Med Infect Dis. 2020;5:122. [DOI] [PubMed] [PMC]
6. Deka MA, Morshed N. Geospatial Analysis of Tungiasis Disease Transmission Risk in East Africa. In: Braimah JA, Bisung E, Kuuire V, editors. Health Geography in Sub-Saharan Africa. Cham: Springer; 2023. pp. 177–93. [DOI]
7. Heukelbach J, Harvey TV, Calheiros CML. *Tunga* Spp. and Tungiasis in Latin America. In: Infectious Tropical Diseases and One Health in Latin America. Cham: Springer; 2022. pp. 151–68. [DOI]
8. Obebe OO, Aluko OO. Epidemiology of tungiasis in sub-saharan Africa: a systematic review and meta-analysis. Pathog Glob Health. 2020;114:360–9. [DOI] [PubMed] [PMC]
9. Nagy N, Abari E, D'Haese J, Calheiros C, Heukelbach J, Mencke N, et al. Investigations on the life cycle and morphology of *Tunga penetrans* in Brazil. Parasitol Res. 2007;101:233–42. [DOI] [PubMed]
10. Nwalozie R, Ezenwaka CO. Tungiasis: Biology, Life Cycle, Epidemiology, Diagnosis, Prevention, and Treatment. South Asian J Parasitol. 2023;6:83–93.
11. Mphande FA. Skin Disorders in Vulnerable Populations: Causes, Impacts and Challenges. Singapore: Springer; 2020. [DOI]
12. Leiferman KM, Peters MS. Eosinophil-Related Disease and the Skin. J Allergy Clin Immunol Pract. 2018;6:1462–82.e6. [DOI] [PubMed]
13. Feldmeier H, Heukelbach J, Eisele M, Sousa AQ, Barbosa LM, Carvalho CB. Bacterial superinfection in human tungiasis. Trop Med Int Health. 2002;7:559–64. [DOI] [PubMed]
14. Feldmeier H, Heukelbach J, Eisele M, Ribeiro R, Harms G, Mehlhorn H, et al. Investigations on the biology, epidemiology, pathology and control of *Tunga penetrans* in Brazil: III. Cytokine levels in peripheral blood of infected humans. Parasitol Res. 2003;91:298–303. [DOI] [PubMed]
15. Feldmeier H, Witt LH, Schwalfenberg S, Albuquerque Ribeiro R, Queiroz Cunha F, Harms G, et al. Investigations on the biology, epidemiology, pathology and control of *Tunga* penetrans in Brazil. V. Cytokine concentrations in experimentally infected Wistar rats. Parasitol Res. 2004;94:371–6. [DOI] [PubMed]
16. Galli SJ, Metz M, Starkl P, Marichal T, Tsai M. Mast cells and IgE in defense against lethality of venoms: Possible "benefit" of allergy. Allergo J Int. 2020;29:46–62. [DOI] [PubMed] [PMC]
17. Heukelbach J, Walton SF, Feldmeier H. Ectoparasitic infestations. Curr Infect Dis Rep. 2005;7:373–80. [DOI] [PubMed]
18. Vega-López F, Ritchie S. Skin Tropical Infections and Dermatology in Travellers. In: Principles and Practice of Travel Medicine. John Wiley & Sons, Inc.; 2013. pp. 165–96. [DOI]
19. Wells B, Burgess ST, McNeilly TN, Huntley JF, Nisbet AJ. Recent developments in the diagnosis of ectoparasite infections and disease through a better understanding of parasite biology and host responses. Mol Cell Probes. 2012;26:47–53. [DOI] [PubMed]
20. Birck MM, Tveden-Nyborg P, Lindblad MM, Lykkesfeldt J. Non-Terminal Blood Sampling Techniques in Guinea Pigs. J Vis Exp. 2014:e51982. [DOI] [PubMed] [PMC]
21. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, et al.; European Federation of Pharmaceutical Industries Association and European Centre for the Validation of Alternative Methods. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol. 2001;21:15–23. [DOI] [PubMed]
22. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother. 2010;1:87–93. [DOI] [PubMed] [PMC]

23. Osorio-Pinzon J, Palencia A, Cruz-Calderon S, Rodriguez-Morales AJ. Myiasis and Tungiasis. Curr Trop Med Rep. 2021;8:112–20. [DOI]
24. Padilla-Carlin DJ, McMurray DN, Hickey AJ. The guinea pig as a model of infectious diseases. Comp Med. 2008;58:324–40. [PubMed] [PMC]
25. Feldmeier H, Eisele M, Van Marck E, Mehlhorn H, Ribeiro R, Heukelbach J. Investigations on the biology, epidemiology, pathology and control of *Tunga penetrans* in Brazil: IV. Clinical and histopathology. Parasitol Res. 2004;94:275–82. [DOI] [PubMed]
26. Liu X, Walton SF, Murray HC, King M, Kelly A, Holt DC, et al. Crusted scabies is associated with increased IL-17 secretion by skin T cells. Parasite Immunol. 2014;36:594–604. [DOI] [PubMed]
27. Mishra PK, Palma M, Bleich D, Loke P, Gause WC. Systemic impact of intestinal helminth infections. Mucosal Immunol. 2014;7:753–62. [DOI] [PubMed]
28. Bhat SA, Mounsey KE, Liu X, Walton SF. Host immune responses to the itch mite, *Sarcoptes scabiei*, in humans. Parasit Vectors. 2017;10:385. [DOI] [PubMed] [PMC]
29. Walton SF, Pizzutto S, Slender A, Viberg L, Holt D, Hales BJ, et al. Increased Allergic Immune Response to *Sarcoptes scabiei* Antigens in Crusted versus Ordinary Scabies. Clin Vaccine Immunol. 2010;17:1428–38. [DOI] [PubMed] [PMC]
30. Athanasiou LV, Spanou VM, Katsogiannou EG, Katsoulos PD. Hematological Features in Sheep with IgG and IgM Antibodies against *Borrelia burgdorferi sensu lato*. Pathogens. 2021;10:164. [DOI] [PubMed] [PMC]
31. Sharaf MS. Scabies: Immunopathogenesis and pathological changes. Parasitol Res. 2024;123:149. [DOI] [PubMed] [PMC]
32. Kupper TS, Fuhlbrigge RC. Immune surveillance in the skin: mechanisms and clinical consequences. Nat Rev Immunol. 2004;4:211–22. [DOI] [PubMed] [PMC]
33. Eberle JU, Voehringer D. Role of basophils in protective immunity to parasitic infections. Semin Immunopathol. 2016;38:605–13. [DOI] [PubMed]