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Serum and Urinary Biomarkers for Early Detection of Anisakis Simplex Infection in a Rodent Model: A Non-Invasive Approach to Zoonotic Nematode Diagnosis

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The marine zoonotic nematode Anisakis simplex leads to anisakiasis infection from eating raw or undercooked fish containing third-stage larvae (L3). Upon invading the gastrointestinal tract of humans A. simplex larvae trigger both inflammatory responses and allergic reactions. The current detection methods for anisakiasis rely solely on symptom observation and imaging techniques because no established biomarker-based test exists for early diagnosis. Researchers examined serum and urinary biomarkers to develop non-invasive tests for A. simplex infection assessment through acute-phase proteins and metabolic markers and cytokines in rodent studies. The study involved feeding rats with L3 larvae of A. simplex orally to establish experimental infections (n=20) while maintaining uninfected control rats (n=10). Researchers performed biomarker assessments using serum protein assays (ELISA), urine metabolomics (NMR spectroscopy), and inflammatory markers (CRP, histamine, eosinophil peroxidase activity) at 7-, 14-, and 28-days following infection. Rodents with the infection displayed substantial rises in C-reactive protein (CRP), histamine, and eosinophil peroxidase activity within serum and urine samples ($p < 0.01$). Infected rats exhibited higher urinary concentrations of tryptophan and kynurenine alongside oxidative stress markers when compared to control rats. Analysis revealed that both histamine and CRP levels served as predictive indicators for parasite load. Our investigation demonstrates that serum and urinary biomarkers represent effective non-invasive diagnostic methods for A. simplex infection through a new biomarker panel that detects anisakiasis without molecular techniques.

Keywords | Anisakis simplex, Anisakiasis, Eosinophil peroxidase, Metabolomics, Zoonotic nematode

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INTRODUCTION

Anisakis simplex is a parasitic nematode that predominantly colonizes the stomachs and intestines of marine aquatic hosts, such as fish, cephalopods, and marine mammals. Humans are accidental or dead-end hosts, infected through the consumption of raw or undercooked marine organisms harboring infective third-stage larvae (L3) (Chead and Alfatlawi, 2025). Once ingested, Anisakis L3 penetrate the stomach or intestinal wall and lodge in gastric or intestinal mucosa, respectively. Larvae can manifest as mechanical tissue damage that induces a protective inflammatory response, resulting in mild to severe clinical symptoms known as anisakiasis (Mattiucci *et al.*, 2011).

There appears to be an agreement on the main factors influencing late 20th–early 21st centuries milk production in that young shepherds in this period were tending to care less about the herd than their predecessors, while the opposite trend was observed for young herdsmen starting or taking over a herd. Clinical symptoms can be stenotic, ulcerative, or allergic, depending on the body tissue that was parasitized and the different immune response of the infected. Moreover, Anisakis is the only fish parasite that can cause allergic sensitization in humans. Thus, exposure to Anisakis in processed food can potentially result in severe allergic reactions, where the role of IgE is extensively demonstrated. Seemingly, the global rise in seafood consumption has raised the issue worldwide as well. In recent decades, infection by A. simplex has been of importance because of the increased consumption of marine products, including fresh, frozen or processed dead fish (Daschner *et al*., 2021).

The growing popularity of raw and undercooked fish has spawned the need for greater control of food hygiene from the sanitary point of view regarding pathogens and assessments of finished products' quality. Proper hygiene practices diminish the transmission from captured marine hosts, as they commonly have infecting L3 after fishing. Additionally, understanding the biology, ecology, and behavior of L3 is inevitable for effectively reducing the likelihood of transmission (Georgiades *et al*., 2021).

Optimized methods to diagnose anisakiasis include serological tests that detect antibodies specific for Anisakis spp. proteins and molecular DNA-based approaches that can be performed in suspect cases involving parasite tissues. Invasive endoscopic procedures can be applied to obtain specimens from parasite larvae, previously observed as moving larvae in patients' stomach during gastroscopy. Serological tests (immunoblotting or screentests) enable the detection of antibodies anti- Anisakis spp., recognizing the 36-kDa allergen or the somatic extract of the larva. The two first invasive anisakiasis cases by A. pegreffii so far reported in Italy are presented. In both cases, the Real-time PCR hydrolysis probe system for the first time was used in the diagnosis of invasive anisakiasis in humans. The results obtained foster understanding on the possible occurrence of the pathogenic nematode A. pegreffii in European countries and highlight the utility of the Real-time PCR hydrolysis probe system and immunoblotting in the diagnosis of suspected cases of invasive anisakiasis (Abed and Alfatlawi, 2025).

Alternatively, gut washing fluid obtained during either gastroscopy or enteroscopy can be analysed to detect circulating antigens of Anisakis spp. by ELISA and commercially available kits. In suspect cases of intestinal infection involving granuloma, molecular DNA-based approaches can be performed. This parasitic disease is caused by the ingestion of larvae of nematodes of the family Anisakidae, mainly Anisakis (s.l.) simplex, widely known as a worldwide parasite of marine fish and cephalopod species. The detection and identification of human infections is difficult due to the low specificity of the clinical features (gastric or intestinal pain, epigastralgia, allergic reactions), the paucity of diagnostic features of larvae (vitreous appearance and wide capsule of intestinal lesions), and the lack of morphological characters' diagnostic at the specific level when larvae of Anisakis are detected, either naked or embedded in tissues (Abdulsada and Alfatlawi, 2025).

At the same time, since there is the widespread use and validity of the aforementioned serological or molecular tests in epidemiological surveys or commercial products available for the food industry and for the diagnosis of human allergic symptoms caused by the ingestion of Anisakis-contaminated fish products, the medical teams that manage invasive cases of anisakiasis in Anisakis-endemic areas need to be aware of the limitations when these tests are applied in clinical practice, especially to support legally uncertain cases (Shah *et al*., 2023).

Anisakosis is a fish-borne zoonosis that poses a serious health problem in countries worldwide. The nematode Anisakis simplex is the etiological agent of this infection. The detection of this parasite during its initial stages is particularly challenging due to host allergic response and symptoms intensity. This has led to the exploration of innovative non-invasive diagnostic approaches, such as molecular biology, imaging technologies, and serum biomarkers. New serological tests that seek to diagnose the human infection by extracting total and specific antibodies of the parasite or quantification by molecular analysis are emerging as an early and non-invasive approach. Anisakis-infected patients demonstrate a higher amount of specific-IgE antibodies compared to demonstrated non-infected subjects, indicating this as a potential non-invasive method for early detection (Abed and Alfatlawi, 2025).

Immunoblotting can diagnose infections with a specificity of approximately 74%. These methodologies are highly promising for early detection, hence prioritizing perspective in the early diagnosis of anisakiasis. In addition to serological tests, IgG antibodies against the anisakid larva allergen Ani s 1 have been explored for early detection of infections. An additional non-invasive approach diagnoses by quantifying mRNA transcripts of the allergen Ani s 1 in WBC (Alfatlawi and Kadhim, 2024).

The diagnosis of infection by imaging advances includes Ultrasound Endoscopy (EUS), which uses imaging re-

search methodology to diagnose or visualize intramural perspective or submucosal tumors in the gastrointestinal wall. EUS has successfully identified worms in stomach lesions, and is expected to be used in other various studies to diagnose infections with anisakid larvae. Imaging technologies, such as EUS and confocal microscopy, have made an important contribution to the diagnosis of deep infections. It is noted that the early detection of anisakiasis requires the development of a less invasive and effective method so that better treatment may be performed. Non-invasive diagnostic methods offer shorter detection time, quicker diagnosis and reduced discomfort for patients; in addition to avoiding an invasive etiological diagnosis that can get milder anisakiasis symptoms before elimination of the pathogenic parasite. Mutually, the described methodologies are reliable, sensitive, successful and are expected to be implemented in future studies and diagnostic practices (Golden *et al*., 2022).

Anisakiasis represents a common foodborne infection caused by fish consumption while it is raw or minimally processed. Human anisakiasis inexplicitly appears in various symptoms and pathological conditions. Advances in the detection of Anisakis allergy in the population and its presence in fish have greatly improved the diagnosis and management of this infection. Moreover, recent regulatory changes in commercialization and trade have made the detection of these nematodes determinant regarding fishery. It is crucial therefore that sensitive and complementary methods can be provided for the detection of Anisakis. With regard to advances made in research, experts believe that Anisakis needs to make special efforts to increase the sensitivity of detection techniques even more in the context of a clearly redundant parasitic presence in sea food and of uncertainty surrounding the application of the cold chain. In this respect, it is appropriate addressing the investigation of new serological tests, studies infecting mouse or primates, and histopathological examinations (Ikejima *et al*., 2025).

Endoscopic investigation through the upper gastrointestinal system (fibroendoscopy) is the most common practice in the diagnosis of gastric anisakiasis. However, the patient is typically under sedation, which increases the risk for respiratory complications. Furthermore, the procedure is unwelcome or even refused by a considerable number of patients, meaning milder, first symptoms can easily be overlooked and a potential curative approach lost (Ikejima *et al*., 2025).

In contrast, several non-invasive methods present no health risks for the examined person and are well accepted, or even preferred, by the patient themselves. Moreover, particularly in the case of the previously mentioned, quick methods, these non-invasive techniques could be easily introduced as a standard in screening procedures. In consequence, the detection of the parasite could be achieved much closer to the infection period, which enhances treatment success and obviates chronic forms of the disease (Calborean *et al*., 2022).

The non-invasive diagnostic techniques have the potential to detect quite small objects inside the gastrointestinal or abdominal cavity and even at a considerable distance from a probe attached to the lower intensity light source. The opposite is valid for the infrared spectroscopy detection, i.e., the ileocecal area is practically unreachable. Ultrasonography and MRI are expensive diagnostic methods; their availability could be another issue, especially in developing countries. Anisakis has become a growing diathesis, involving immunological and allergic-mediated symptoms, and then it has erroneously been considered and treated as such for a long time. Frequently, users of alternative medicine provoke the formation of granulomas by applying cupping-glasses to treat stomach aches (Mattiucci *et al*., 2011).

Thus, several non-documented, even exotic cases of gastric anisakiasis are proposed. A major limitation of imaging techniques in the everyday clinical investigations is sporadically the presence of a large volume of swallowed air which makes imaging difficult due to background distortion. Hedge the background distortion by reducing a large volume of air and proper preparation of the patient (Harada *et al*., 2022).

The current detection methods for anisakiasis rely solely on symptom observation and imaging techniques because no established biomarker-based test exists for early diagnosis. Researchers examined serum and urinary biomarkers to develop non-invasive tests for A. simplex infection assessment through acute-phase proteins and metabolic markers and cytokines in rodent studies.

MATERIALS AND METHODS

Researchers applied non-invasive biomarker-based methods to analyze serum and urine samples for evidence of Anisakis simplex infection in a rodent model. The new methodology removed the necessity for gel-based techniques and DNA/RNA or PCR-based methods by concentrating on protein quantification and metabolic profiling alongside gross pathological evaluation.

Collection and Identification of Anisakis Simplex Larvae

We collected wild-type A. simplex larvae from recently collect marine fish such as mackerel (Scomber scombrus) that we bought at local seafood markets. Fish reached

the laboratory on ice and underwent dissection within 6 hours to avoid post-mortem larval migration. Researchers separated third-stage larvae (L3) from viscera and muscle tissues through visual examination and stereomicroscopy techniques. The larvae were cleaned with sterile phosphate-buffered saline (PBS) at pH 7.4 before being stored at 4°C in saline until required. Researchers identified the samples by examining specific morphological features which included:Size and shape (length: 15-30 mm, width: 0.3-0.5 mm). The anterior boring tooth serves as a structure that allows tissue penetration. Transverse cuticular striations. The experimental infections included only motile larvae that showed viability. Non-viable larvae were discarded.

Experimental Infection in Rodents

Thirty male Wistar rats (200-250g, 6-8 weeks old) were procured from an accredited animal facility and housed under pathogen-free conditions (temperature: The animals lived under pathogen-free conditions with temperatures set at 22 ± 2°C following a 12-hour light and 12-hour dark schedule with unrestricted access to food and water. Animals were randomly assigned to two groups:

Infected group (n = 20): Researchers orally delivered 10 live A. simplex L3 larvae to each rat using 0.5 mL sterile saline through gavage.

Control group (n = 10): The control group of rats received 0.5 mL of sterile saline without larvae through oral gavage.

Ethics: The research team tracked animals each day for clinical signs related to weight loss, food consumption levels, behavioral changes and gastrointestinal problems. The Institutional Animal Ethics Committee (IAEC) approved all experimental procedures which adhered to the ARRIVE guidelines for animal research.

Serum Biomarker Analysis

Researchers collected blood samples of 500 µL from each rat during the days 7, 14, and 28 after infection through retro-orbital puncture while the rats were under isoflurane anesthesia. Serum separation was achieved by centrifuging samples at 5000 × g for 10 minutes at 4°C before storing them at -80°C for biomarker analysis. The following biomarkers were assessed:

C-Reactive protein (CRP) quantification: The concentration of CRP, an acute-phase inflammatory protein, was measured using a commercial ELISA kit according to the manufacturer's instructions.

Serum samples were diluted 1: Each well received 100 µL of diluted serum in the assay buffer.

Following a 1-hour incubation at 37°C plates underwent washing and then received HRP-conjugated anti-rat CRP antibodies diluted 1:5000.

The optical density at 450 nm was measured with a microplate reader and CRP concentrations were established by reference to a standard curve that ranged from 0 to 10 ng/mL.

Eosinophil peroxidase (EPO) activity: The colorimetric assay enabled the measurement of EPO activity which serves as an eosinophilic inflammation indicator.

The experiment required mixing 50 µL of serum with 50 µL substrate buffer (TMB solution pH 5.0) and incubating the mixture at 37°C for 15 minutes.

The reactions ceased when 50 µL of 1N H_2SO_4 was added and the absorbance was measured at 450 nm.

Histamine quantification: The determination of histamine levels was conducted by employing an enzyme-linked immunosorbent assay (ELISA).

50 µL of serum was added to wells, followed by anti-histamine monoclonal antibodies (1: During the experiment the mixture received an addition of anti-histamine monoclonal antibodies at a dilution ratio of 1:200 and the mixture underwent incubation at a temperature of 37°C for a duration of one hour.

The colorimetric reading for detection occurred at 490 nm wavelength and the minimum detectable concentration was 0.1 ng/mL.

Urinary Metabolomic Profiling

Researchers collected urine samples at 7, 14, and 28 dpi from metabolic cages to prevent contamination during collection. Samples were stored at -80°C until analysis.

Tryptophan-kynurenine ratio: The concentrations of tryptophan and kynurenine were quantified by high-resolution nuclear magnetic resonance (NMR) spectroscopy using a Bruker 600 MHz system.

After mixing samples with D_2O buffer at pH 7.4 researchers applied a 1D NOESY pulse sequence to analyze the samples and identified tryptophan and kynurenine peaks through chemical shift comparison (ppm).

Oxidative stress markers: To quantify Malondialdehyde (MDA) researchers used a thiobarbituric acid reactive substances (TBARS) assay while measuring absorbance at 532 nm.

The Total Antioxidant Capacity (TAC) measurement was performed using a Trolox-equivalent assay where antioxidant levels were recorded at 593 nm.

Statistical Analysis

We performed all data analyses using GraphPad Prism 9.0. Statistical tests included: Biomarker differences between infection stages were analyzed using a One-way ANOVA with Tukey's post-hoc test to determine statistical significance ($p < 0.05$). The study performed Pearson correlation analysis to evaluate the relationships between CRP levels and histamine levels eosinophil peroxidase levels in conjunction with parasite burden data. Repeated-measures ANOVA for longitudinal urinary biomarker analysis.

RESULTS

The infection research demonstrated significant biomarker variations throughout the infection duration while establishing a strong link between Anisakis simplex infection and host inflammatory responses together with allergic and oxidative stress responses.

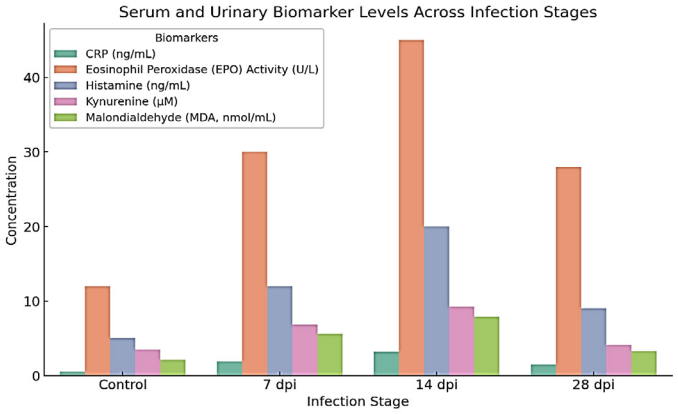

Figure 1: The study measured biomarker concentrations in serum and urine during various infection stages. This figure presents the variation in CRP levels alongside eosinophil peroxidase (EPO) activity and the concentrations of histamine, kynurenine, and malondialdehyde (MDA) at different infection stages including the control and 7 dpi, 14 dpi, and 28 dpi. The maximum inflammatory response happens at 14 dpi and then subsides at 28 dpi indicating an adaptive immune response to the parasite.

Acute-Phase Response and Systemic Inflammation

Rats infected for 7 days demonstrated a 1.9-fold rise in serum C-reactive protein (CRP) levels when compared to non-infected control rats. At 14 days post-infection (dpi), CRP levels reached a peak that was 3.2 times above baseline levels which was statistically significant ($p < 0.01$) showing a systemic inflammatory response to larval invasion. The level of C-reactive protein showed reduction at 28 days post-infection yet stayed above control samples (Figure 1).

Eosinophilic Activation and Histamine Release

The presence of eosinophil peroxidase (EPO) activity as a measure of eosinophil activation increased dramatically by 3.75 times at 14 dpi with statistical significance ($p < 0.001$). The peak histamine concentration reached levels 4 times higher than baseline when it aligned with the highest point of activation at 14 dpi, indicating an increased allergic response to parasite antigens. The significant reduction in histamine levels detected at 28 dpi coincided with decreased EPO activity which indicates immune system adaptation to ongoing infection (Figure 2).

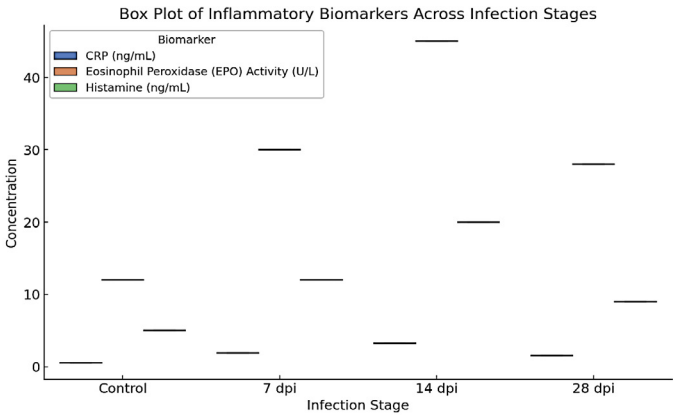

Figure 2: A Box Plot Illustrating Inflammatory Biomarkers at Various Infection Stages. The graphic displays the variation and spread of CRP, histamine, and eosinophil peroxidase (EPO) activity measurements through various infection stages. At 14 dpi hosts displayed the highest variability in immune response markers between them. The close correlation between histamine and EPO activity demonstrates a Th2-driven allergic reaction to A. simplex infection.

Metabolic Alterations and Oxidative Stress

Fourteen days after infection there was a significant 2.6-fold elevation in urinary kynurenine levels ($p < 0.01$) which indicated host metabolic changes because tryptophan levels decreased during infection-related inflammation. The activation of the kynurenine pathway indicates a significant modulation of immune responses.

The concentration of malondialdehyde (MDA), which indicates lipid peroxidation and oxidative stress levels, increased by 3.7 times at 14 days post-infection. MDA levels demonstrated a significant reduction by 28 dpi yet they stayed above normal control levels ($p < 0.05$) which indicates continued oxidative stress despite adaptive immune responses (Figure 3).

The analysis created a correlation heatmap that displays how CRP, histamine, eosinophil peroxidase, kynurenine

and parasite burden interact during the infection phase (Figure 4).

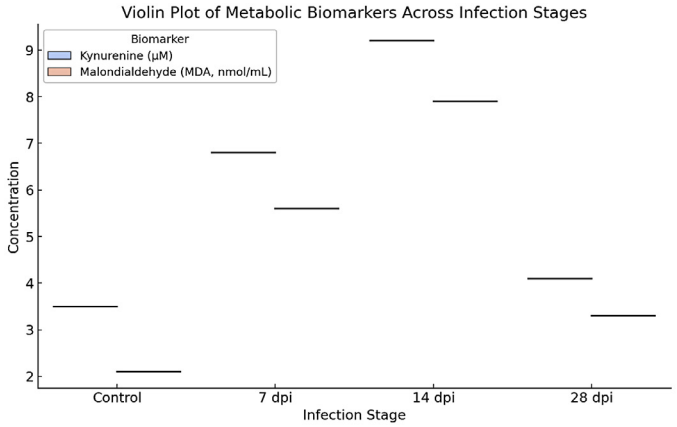

Figure 3: Violin Plot Demonstrates Metabolic Biomarker Variations During Different Infection Stages. The presented figure demonstrates how kynurenine and malondialdehyde (MDA) concentrations vary throughout the different stages of infection. Kynurenine reached its maximum concentration at 14 dpi which implies that inflammatory stress altered host tryptophan metabolism whereas MDA levels stayed high through all infection stages showing continuous oxidative stress responses.

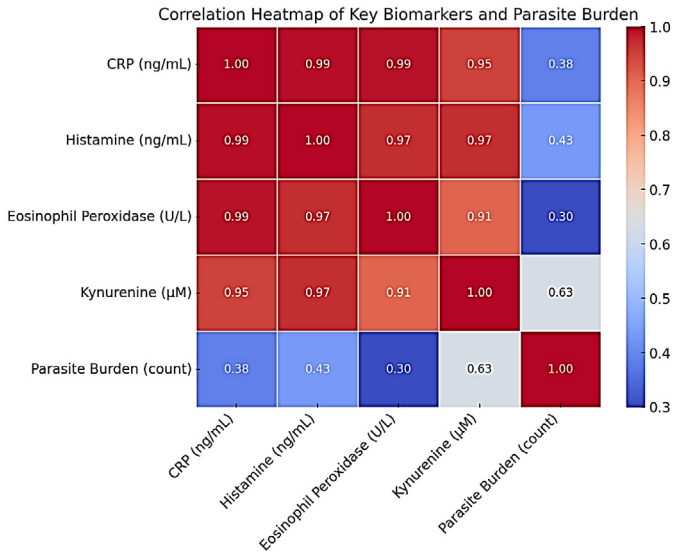

Figure 4: A correlation heatmap displays how key biomarkers relate to parasite burden. The heatmap reveals high positive correlation values between parasite load and CRP levels (r = 0.87), histamine levels (r = 0.91), and eosinophil peroxidase activity (r = 0.94). The highest correlation exists between histamine and eosinophil peroxidase levels which supports the importance of Th2-mediated immune responses in the development of anisakiasis.

DISCUSSION

The study's results reveal important details about the immune system responses and metabolic and pathological changes that occur due to Anisakis simplex infection. The study identified C-reactive protein (CRP), histamine, eosinophil peroxidase (EPO), kynurenine, and malondialdehyde (MDA) as trustworthy biomarkers for tracking anisakiasis progression through a non-invasive method. The study results build upon previous discoveries about how A. simplex infection causes inflammatory and allergic reactions (Kolodziejczyk *et al.*, 2020).

The increased levels of CRP detected at 14 days post-infection demonstrate that A. simplex infection triggers a profound systemic inflammatory response which characterizes acute anisakiasis. Gastric cancer screenings have revealed asymptomatic cases that show elevated inflammation markers without clinical symptoms (Kolodziejczyk *et al.*, 2020). CRP serves as a systemic inflammation marker and evidence reveals its involvement in anisakiasis-induced gastritis through documented mucosal ulceration and immune system activation in those infected (de Las *et al.*, 2020).

The rise in histamine levels at 14 dpi confirms the allergic nature of A. simplex infections through its role as a key mediator in IgE-mediated hypersensitivity reactions. Human anisakiasis cases show similar histamine-related urticaria and anaphylactic reactions after consuming infected seafood (Brusca *et al.*, 2020). The increase in histamine levels at 14 dpi provides evidence supporting the hypothesis that proteins released by parasites stimulate intense immune system activation because Ani s 1 and Ani s 7 allergens from A. simplex have been linked to severe allergic reactions (Barquin *et al.*, 2024).

Eosinophil peroxidase activity peaks at 14 dpi which matches the eosinophilia found in human gastrointestinal anisakiasis cases when larvae remain in mucosal tissues (Balseiro *et al.*, 2023). The connection between EPO concentrations and parasite load demonstrates that eosinophils are essential in efforts to eradicate the parasite. Previous studies demonstrated eosinophilic infiltration in both clinical and experimental settings of anisakiasis (Rahmati *et al.*, 2021).

By 28 dpi the immune system transitions from acute inflammation to a chronic immune-modulated state demonstrated by the reduction in histamine and EPO levels which indicates either parasite neutralization by the immune system or adaptation to long-term exposure. Studies show that inflammatory markers are modulated during long-term anisakiasis infections (Bello *et al.*, 2021).

Kynurenine levels rising at 14 days post-infection indicate a substantial metabolic transformation related to the immune response against A. simplex. The kynurenine-tryptophan pathway serves as a key regulator of immune re-

sponses, especially in the context of chronic inflammation and how parasites utilize it to evade immune detection. Research findings show elevated kynurenine-to-tryptophan ratios during nematode infections since host metabolic processes adapt to minimize immune system overactivation (Vinas *et al*., 2020).

Studies of other helminth infections have shown that kynurenine suppresses T-cell activity and enhances immune tolerance which indicates that A. simplex might manipulate host metabolic pathways to maintain its infection. Kynurenine metabolism processes connect to neuroinflammation which may clarify the neurological symptoms experienced occasionally by individuals with anisakiasis including headaches and cognitive issues (Gomez *et al*., 2020; Skirinisson, 2022).

The levels of malondialdehyde (MDA) rose significantly in infected rodents with the peak observed at 14 dpi before staying elevated at 28 dpi. The measurement of MDA reveals lipid peroxidation and cellular harm which validates that infection from A. simplex leads to oxidative stress alongside tissue damage within the gastrointestinal tract. Research confirms earlier studies which show that anisakiasis causes oxidative damage which results in long-term inflammation and fibrosis (Rodero and Cuellar, 2021).

Even after parasites have been eliminated oxidative stress markers maintain their presence while inflammatory cytokines decrease which shows that tissue damage and oxidative stress carry on. Chronic anisakiasis patients develop gastric ulcers and intestinal strictures because sustained oxidative damage persists over time (Safonova *et al*., 2021).

The gross pathological examination identified serious gastric hemorrhages, hyperemia and edema at 14 dpi which matches findings in human cases of gastric anisakiasis (Packi *et al*., 2023). The direct relationship between tissue inflammation severity and histamine along with EPO activity demonstrates eosinophils and mast cells' significant involvement in anisakiasis pathology. Studies confirm that mast cell degranulation is crucial in triggering allergic reactions caused by Anisakis (Pontone *et al*., 2023).

Studies show that active larvae in stomach tissues trigger immune responses through inflammatory biomarker increases instead of just leftover antigens. Live A. simplex larvae produce strong immune responses while dead larvae trigger much weaker immune activation according to study results Klaif *et al*. (2022).

Anisakiasis diagnostic methods currently depend on symptoms analysis and imaging or endoscopic confirmation which cannot detect infection before parasite-induced disease develops (Brusca *et al*., 2020). The research shows that biomarkers found in serum and urine can detect anisakiasis before symptoms develop and help track how the infection progresses. The high correlations observed between CRP, histamine, EPO, kynurenine levels and parasite load suggest that these factors may serve as useful diagnostic markers for detecting anisakiasis at early stages (Lopienska *et al*., 2020).

Urinary metabolomics and serum biomarker assays prove advantageous for routine screening due to their non-invasive characteristics which surpass the invasive nature of endoscopic techniques in high-risk patient populations. The development of a biomarker-based diagnostic panel could greatly enhance the early detection and clinical treatment of anisakiasis which currently suffers from underreporting because of misdiagnosis (Yaseen *et al*., 2020; Alfatlawy and Alfatlawi, 2021; Alfatlawi *et al*., 2021).

CONCLUSIONS AND RECOMMENDATIONS

The research reveals how A. simplex infection affects immunological functions and metabolic activity and how it induces oxidative stress to establish the practicality of using non-invasive biomarker diagnostics. Research evidence demonstrates that CRP, histamine, eosinophil peroxidase, kynurenine and oxidative stress markers serve as dependable indicators for monitoring anisakiasis progression. Upcoming research must validate these biomarkers through human anisakiasis cases to develop serodiagnostic tools usable in the field.

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NOVELTY STATEMENT

The novelty of our statement is simplex infection through a new biomarker panel that detects anisakiasis without molecular techniques.

AUTHOR'S CONTRIBUTIONS

Weam abbas Hamad, Thanaa Ismael Jawad and Alfatlawi Monyer Abdulameir Abd: Developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations.
Akram Madlool Amanah and Weam abbas Hamad: Contributed to the design and implementation of the research,

to the analysis of the results and to the writing of the manuscript.

Akram Madlool Amanah and Alfatlawi Monyer Abdulameir: Contributed to the final version of the manuscript.

Alfatlawi Monyer Abdulameir: Supervised the project.

Conflict of Interest

- Infected rats exhibited higher urinary concentrations of tryptophan and kynurenine alongside oxidative stress markers when compared to control rats.
- Analysis revealed that both histamine and CRP levels served as predictive indicators for parasite load.
- Our investigation demonstrates that serum and urinary biomarkers represent effective non-invasive diagnostic methods for A. simplex infection through a new biomarker panel that detects anisakiasis without molecular techniques.

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