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# Prevalence of circulating microfilariae detected by thin smear and associated haematological and biochemical alterations in hunting dogs in Jos, Nigeria

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## Abstract

Canine filarial infections are emerging vector-borne diseases of both veterinary and zoonotic importance. This study determined the prevalence of circulating microfilariae and evaluated their haematological and serum biochemical effects in hunting dogs in Jos, Nigeria. Forty (40) adult hunting dogs were sampled, and microfilariae were detected using thin blood smear examination. Haematological and biochemical parameters were analysed using standard methods, and group comparisons were performed using an independent samples t-test. Circulating microfilariae were detected in 17.5% of dogs. Infected dogs showed a significant increase in packed cell volume, haemoglobin, total white blood cells, neutrophil, lymphocyte, monocyte, and eosinophil counts. Total protein and globulin levels were also significantly elevated, while albumin and liver enzymes showed no significant differences. Haematological ratios, including eosinophil-neutrophil ratio (ENR), and eosinophil-lymphocyte ratio (ELR) were significantly elevated in infected dogs, while eosinophil-monocyte ratio (EMR) did not show any significant change between the two groups. This therefore suggests the need for clinicians and researchers to consider microfilariae when monitoring the health of dogs.

**Keywords:** Filarial worm; Hunting dogs; Jos Plateau; Zoonosis; Haematology

## 1. Introduction

Pathogenic filarial nematodes are known to produce a wide range of infections in animals as well as humans, resulting in lung and skin diseases in humans and heartworm disease in wild and domestic canids [1]. Dog filarioids are nematodes of the superfamily Filarioidea whose transmission is mediated by various arthropod vectors, including mosquitoes, fleas, flies, lice, and ticks, depending on the

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parasite's genus [2]. While numerous mosquito species have been suspected as possible vectors, only a limited number can support the complete growth of filarial larvae through to the infective third stage (L3) and successfully transmit them to new hosts. The developmental process involves ingestion of microfilariae with a blood meal, migration of the mosquito midgut to the thoracic muscles, and successive moults to the L3 stage. These processes that are significantly regulated by both parasite as well as mosquito species [3, 4]. Laboratory and field investigations have also established that *Aedes aegypti*, *Aedes albopictus*, and *Culex pipiens* can act as vectors for the transformation of *Dirofilaria immitis* (*D. immitis)* (the dog heartworm) from microfilariae to infective L3 larvae [5].

Several filarioid species infect dogs, among which *D. immitis* is the most pathogenic, causing canine heartworm disease. Other species, such as *Acanthocheilonema spp.* and *Dirofilaria repens (D. repens)*, mature in the subcutaneous tissues, forming skin nodules, while *Brugia spp.* adults colonise the lymphatics of the mandible, retropharynx, and the axilla. Although infections by *D. repens*, *Acanthocheilonema*, and *Brugia* are often of limited clinical relevance to veterinarians, these species are zoonotically significant because of their capacity to infect humans [6]. In Nigeria, *Acanthocheilonema/Dipetalonema* spp. and *D. immitis* epidemiology remains largely undefined despite their worldwide distribution, which has been known with zoonotic implications unfolding. The resurgence of canine filariasis as a public and veterinary health concern worldwide has been attributed to increased dog mobility, the spatial expansion of vectors, and advancement in the availability of more effective molecular diagnostic tests that indicate previously hidden infections [7]. Field surveys and reviews have revealed *D. immitis, D. repens,* and *A. reconditum/Dipetalonema* spp. in Nigeria, with their reported prevalence rates ranging extensively from below 1% to double-digit figures due to variations in the used diagnostics, including wet mount, modified Knott's test, morphological examination, antigen test, and limited molecular tests [8].

Dogs for hunting are a particularly vulnerable group for vector-borne disease because of their lifestyle and husbandry practices. The animals are frequently taken into rural and bush environments by their owners, where there is higher exposure to vectors such as mosquitoes, ticks, and fleas. Their housing conditions are typically extensive, increasing vector exposure, while preventive measures, such as frequent application of antiparasitic products are irregularly used [9, 10]. Besides the intense physical exertion involved in hunting, these dogs undergo physiological stress, which will tend to intensify signs of disease. Hunters have reported clinical signs such as weakness or exercise intolerance, coughing, and weight loss in their dogs, and these can be attributed to other diseases, including cardiopulmonary disease, metabolic, or other parasitic diseases [11, 12]. Notably previous studies suggest an association between filarial infection and decreased exercise tolerance in dogs, emphasizing the importance of considering filariasis as a differential diagnosis in hunting dogs with such clinical signs [11]. There are a few studies that explored haematological and biochemical alterations associated with canine filarial infections, especially in hunting dogs in Nigeria. Similar studies from other climes revealed peripheral eosinophilia in the acute or invasive stage, and lymphocytosis in chronic infection [13]. Naturally infected animals also had regenerative hypochromic anemia, leukocytosis with neutrophilia, eosinophilia, and monocytosis. In addition, rises in total bilirubin, serum liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), as well as renal parameters such as blood urea nitrogen (BUN), creatinine, and inorganic phosphate [14, 15]. In Human and Veterinary medicine, there is a rising use of haematological ratios as valuable, easily calculated from complete blood count (CBC) and less expensive biomarkers for assessing inflammation and disease prognosis. Some ratios that have been used as potential diagnostic and prognostic tools to evaluate various inflammatory and allergic conditions include ELR and ENR. Examples of conditions for which these ratios were helpful include dermatological and immune-mediated diseases, e.g., atopic dermatitis, psoriasis vulgaris, lupus erythematosus, and urticaria [16-18]. Despite increasing reports of canine filariasis in Nigeria, there is limited information on associated haematological and biochemical alterations, particularly in high-risk populations such as hunting dogs. Hence, this study aimed to determine the prevalence of circulating microfilariae and their haematological and serum biochemical changes observed in hunting dogs.

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# 2. Materials and methods

## 2.1. Study area

The study was conducted in Jos, Plateau State, within Jos North, South, East and Bassa Local Government Areas (LGAs). Plateau State is a high plateau with well-documented, lovely geological structures. The crest of this height is 1,829 meters with an average elevation of approximately 1,280 meters. The Plateau is characterized by diverse geological formations, including granitic, volcanic, and sedimentary rock structures. Rainfall occurs from April to October, and June to September is the rainiest season. Annual rainfall is 1,233 mm (48.54 inches) and reaches a maximum of 284 mm (11.18 inches) in July. It has regular and excessive rainfall that improves soil moisture and creates many water bodies [19].

## 2.2. Study population

40 adult hunting dogs were enrolled in this study from homes in Jos South, Jos North, and Bassa Local Government Areas (LGAs) of Plateau State, Nigeria. Dogs were enrolled using a convenience sampling approach based on accessibility and owner consent. Inclusion criteria included hunting dogs (≥ 1 year old) with no reported antiparasitic treatment within the previous three months and whose owners provided consent. Dogs that had received recent antihelmintic therapy or appeared clinically unstable were excluded.

## 2.3. Sample collection

Blood samples were drawn between 19:00 and 21:00 hr, the time of maximum microfilarial circulation. Approximately 7 mL of blood was collected by cephalic venipuncture; 2 mL into ethylenediaminetetraacetic acid (EDTA) tubes to be used for haematological analysis, and 5 mL were dispensed into a plain bottle for serum separation. The samples were forwarded to the National Veterinary Research Institute laboratory within 2 h of sample collection in cold packs. The clotted blood samples in plan were centrifuged at 1500 × g for 10 min, and the transparent supernatant serum was extracted into sterile Eppendorf tubes and preserved at −20 °C until biochemical analysis [19].

## 2.4. Filarial parasite screening

Circulating microfilariae were detected using direct thin blood smear stained with Leishman, which was microscopically examined at ×10 and ×40 magnifications. It is acknowledged that direct thin blood smear has limited sensitivity, particularly in the case of low parasitaemia, and it may underestimate the true prevalence compared to concentrated techniques such as the modified Knott's test or molecular diagnostics.

## 2.5. Species identification

Species -level identification was not performed in this study. Morphological differentiation of canine filaria species requires detailed examination of sheathing, cephalic space, and tail morphology, and may require concentration or molecular techniques for confirmation. Therefore, the specific filarial species involved could not be determined.

## 2.6. Haematological analysis

Complete blood counts (CBC) were done using standard manual techniques. Packed cell volume (PCV) was measured by microhematocrit, and total leukocytes were counted as described by [20] using hemocytometer method while haemoglobin concentration was determined using cyanmethemoglobin method. Differential leukocyte counts were made using thin blood smears with a light microscope under oil immersion.

## 2.7. Serum biochemistry

Total protein, albumin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured through colorimetric assays as described by [21], while globulin was calculated by subtracting albumin from total protein.

## 2.8. Statistical analysis

Data were analysed using IBM SPSS statistics version 27. Continuous variables were expressed as mean ± standard deviation. An independent samples t-test was used to compare haematological and biochemical parameters between microfilarial-positive and microfilaria-negative dogs. Levene's test was applied to assess the homogeneity of variances. Statistical significance was set at $p < 0.05$.

## 2.9. Animal ethics and consent declarations

All procedures associated with the animals were carried out in line with institutional and national guidelines for the care and use of animals in research as approved by the University of Ibadan Animal Care and Use Research Ethics Committee care (UI-ACUREC) with approval number UI_ACUREC/152-0924/30. Samples were collected from the

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hunting dogs with the agreement of the owners. Animals were restrained humanely to minimize injury and stress during sampling. A qualified veterinarian collected blood by aseptic venipuncture by using standard techniques. Information obtained from this study was kept confidential

# 3. Results

## 3.1. Prevalence of filarial infection in hunting dogs

Prevalence of circulating microfilariae was detected in 7/40 (17.5%) of the examined hunting dogs, while 33/40 (82.5%) were negative (Table 1). Microfilariae were detected on Leishman-stained thin blood smears and appeared as slender, elongated organisms consistent with filarial worms (Figure 1).

**Table 1. Prevalence of filarial worms in hunting dogs in Jos, Plateau State.**

| Status | Frequency | Percent (%) |
|---|---|---|
| Positive for filarial worm | 7 | 17.5 |
| Negative | 33 | 82.5 |
| Total | 40 | 100 |

## 3.2. Haematological changes associated with filarial infections

Marked differences were observed in several haematological parameters between filaria-positive and filaria-negative dogs (Figure 2). Dogs positive for filarial worms showed a significant increase in PCV (48.7 ± 3.59%) compared with uninfected dogs (43.4 ± 4.34%; $p = 0.005$). Similarly, haemoglobin (Hgb) was significantly increased in infected dogs (16.2 ± 1.2 g/dL) compared to non-infected dogs (14.5 ± 1.45 g/dL; $p = 0.005$), while RBC counts did not show a significant difference.

There was a significantly higher WBCs count of dogs positive for filarial worms than those negative (18.52 ± 1.25×$10^9$/L vs 8.65 ± 1.45×$10^9$/L; $p < 0.0001$). Figure 2 shows the differential leukocyte analysis revealing a significant increase in neutrophils, lymphocytes, monocytes and eosinophils in infected dogs ($p < 0.0001$ for all) with eosinophils showing the most marked increase, from 0.19 ± 0.14 ×$10^9$/L in negative dogs to 3.04 ± 0.58 ×$10^9$/L in dogs positive for filarial worms.

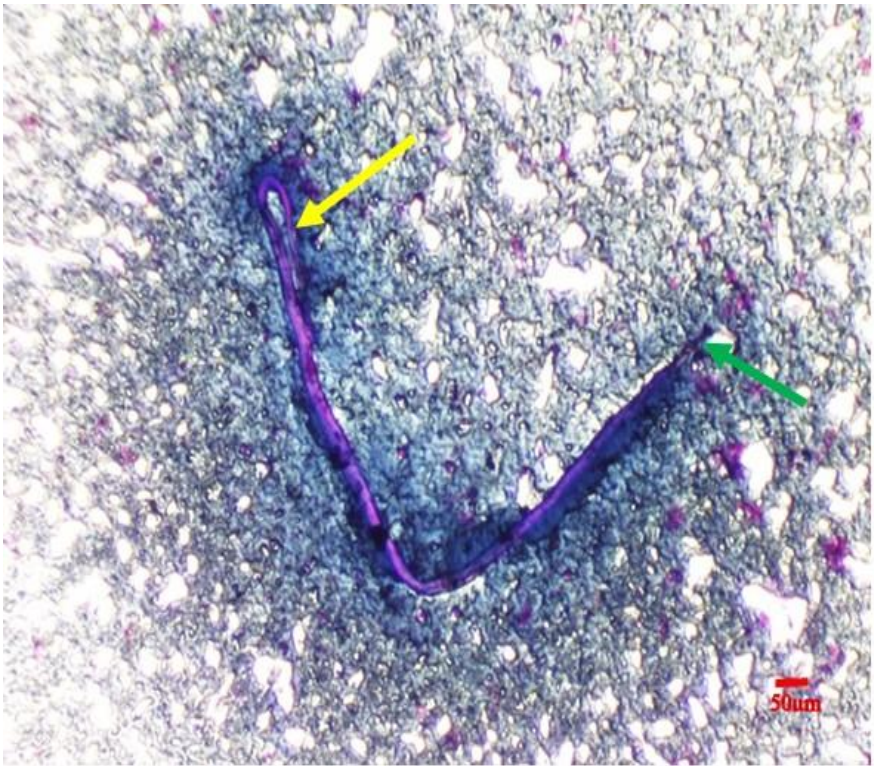


**Figure 1**. Presence of slender and elongated, tapered anterior ends (green arrow) and a gradually narrowing posterior extremity (yellow arrow) consistent with filaria worm on thin blood smear stained with Leishman stain.

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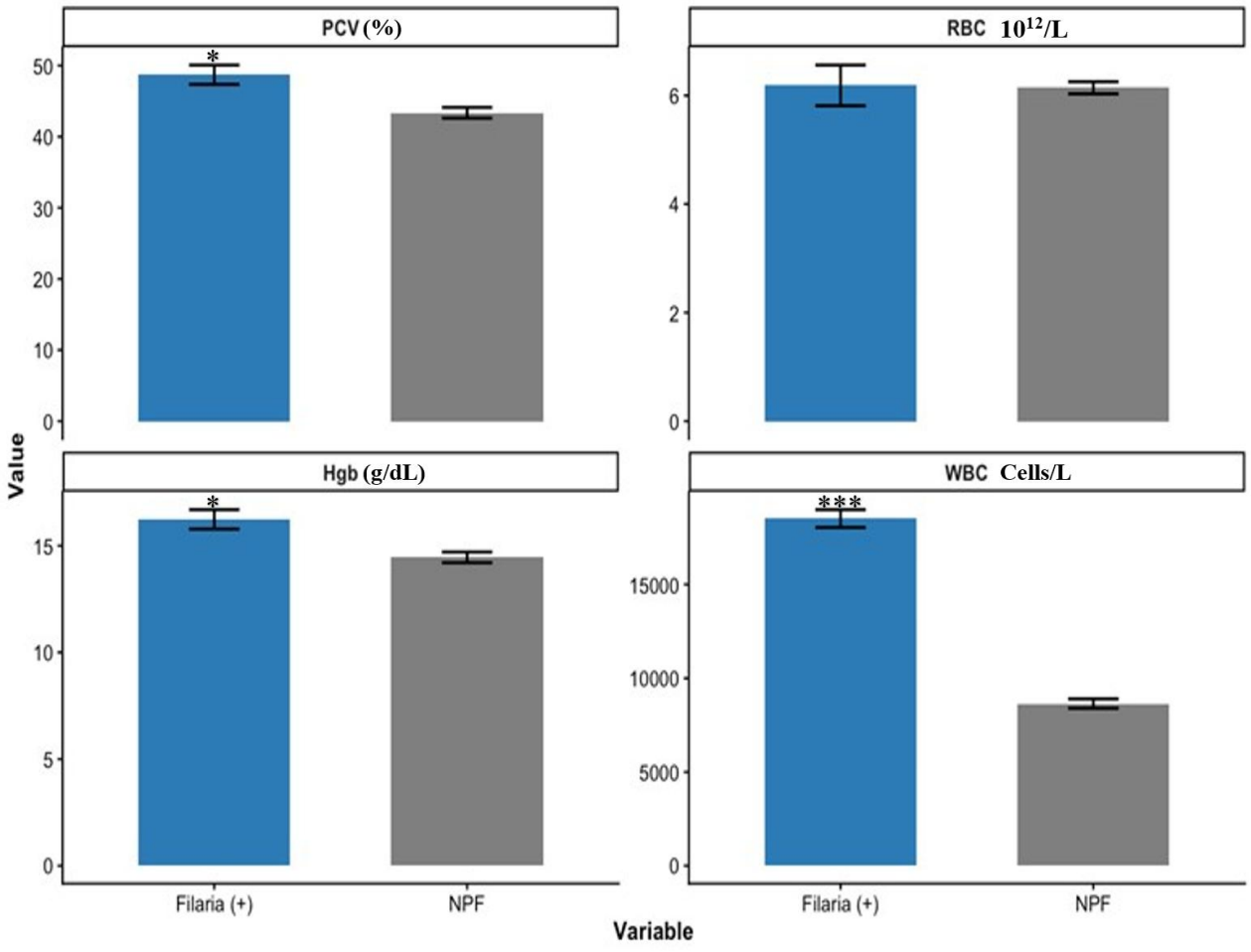


*= p< 0.005, ***= p< 0.0001, PCV= Pack Cell Volume, RBC= Red Blood Cell count, Hgb=Haemoglobin, WBC = White Blood Cell count, NPF = Filaria negative

**Figure 2.** Haematological parameters associated with filarial worm in hunting dogs in Jos, Plateau State.

## 3.3. Haematological ratios

Filarial infection was also associated with significant alterations in calculated haematological ratios (Figure 3). There was a significant increase in the ENR in infected dogs (0.33 ± 0.10) compared to uninfected dogs (0.04±0.03; $p < 0.0001$). Similarly, the ELR was markedly elevated in filaria-positive dogs (0.78 ± 0.18) compared to filaria-negative dogs (0.11 ± 0.08; $p < 0.0001$). Although the EMR was numerically higher in infected dogs, the difference was not statistically significant ($p = 0.145$).

## 3.4. Serum biochemical changes associated with filarial infection

Significant biochemical differences were observed between filaria-positive and negative dogs (Figure 4). Hunting dogs with filarial worms had a markedly higher total serum protein (87.1±5.43g/L) compared to uninfected dogs (48.3 ± 8.92 g/L; $p < 0.0001$). This increase is a consequence of the significant elevation in globulin levels in infected dogs (60.4± 4.51 g/L) than in non-infected dogs (19.7 ± 8.77 g/L; $p < 0.0001$). Serum albumin did not differ significantly between filaria positive and negative dogs ($p = 0.26$). The liver enzymes showed no significant differences between the two groups.

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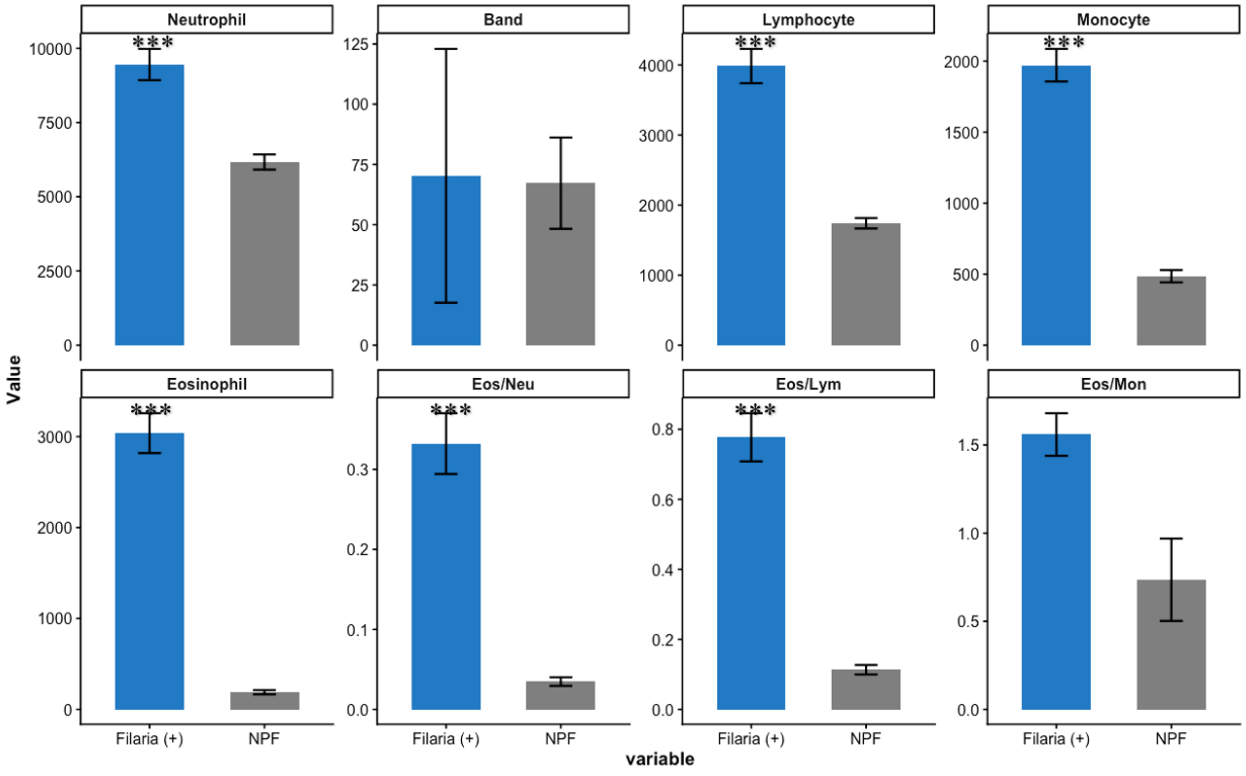


***= p< 0.0001, Eos/Neu= Eosinophil:Neutrophil ratio. Eos/Lym= Eosinophil:Lymphocyte ratio. Eos/Mon= Eosinophil:Monocyte ratio, NPF= Filaria negative

**Figure 3.** Leukogram and inflammatory ratios associated with filarial worm in hunting dogs in Jos, Plateau State.

## 4. Discussion

This study reports a 17.5% prevalence of filarial infection in hunting dogs sampled from Jos, Nigeria. This prevalence aligns with earlier reports from tropical regions where vector-borne parasitic infections are endemic, indicating the high exposure risk of hunting dogs to hematophagous arthropods such as mosquitoes and biting flies [2, 22, 23]. The outdoor and predatory behavior of hunting dogs inherently predisposes them to filarial transmission, especially in rural and peri-urban settings where vector control is minimal [8].

The haematological indices, as shown in this study, have been linked to the severity of microfilaremia [24]. They reported that an increase in the intensity of microfilariae infestation results in decreased erythrocytes. The observed increase in haemoglobin concentration may be attributed to haemoconcentration, possibly resulting from dehydration or physiological adaptation to chronic parasitic stress.

The changes in leukogram as seen in these studies can be attributed to the parasite and its metabolic products' effects on the animals.

The above prevalence indicates how important it is to evaluate the haematological indicators as well as serum chemistry to aid in the consideration of the disease. The remarkable finding from this study showed that dogs with filarial worm infections had significantly higher amounts of total blood protein and globulin than those who were free of the disease. This pattern agrees with the chronic antigenic stimulation produced by persistent parasite infections, which results in increased immunoglobulin production and prolonged B-cell activation [25, 26]. By continually releasing excretory-secretory antigens that trigger polyclonal gammopathy, filarial nematodes are known to provoke strong humoral immune responses [26-28]. The presence of high levels of globulins may be related to the existence of immune complex formation and a prolonged

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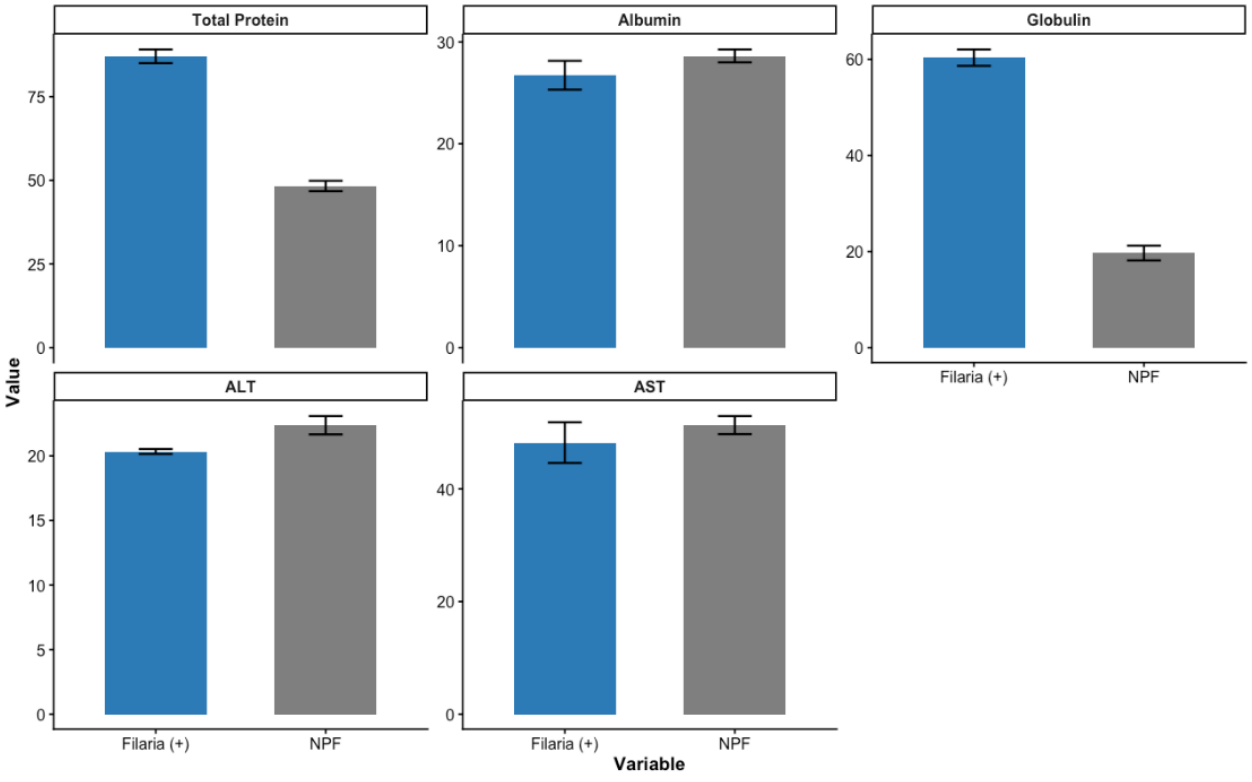
inflammatory response, which are hallmarks of filarial infections in both humans and dogs [13, 29].

Similar research showed that dogs infected with *D. immitis* had large increases in serum total protein, gamma globulins, and acute-phase proteins, which were indicative of hepatic stimulation of protein synthesis and immunological activation. Chronic inflammation has been linked to elevated beta globulin levels, which may be related to beta-gamma bridging, leading to increased beta-motility proteins, such as complement fractions or immunoglobulin subclasses, of which IgM is the most prevalent [30].

The significant eosinophilia observed in dogs with filarial infection confirms the established haematological characteristic associated with helminthic diseases. By releasing eosinophil cationic proteins and causing antibody-dependent cytotoxicity, eosinophils are vital to the host's defense against tissue-migrating parasites [26, 31]. Increased neutrophil and eosinophil counts, which are indicative of type I hypersensitivity and Th2-mediated immune responses, have been consistently documented in canine filariasis, including infections with *Dipetalonema reconditum* and *D. repens* [13, 29]. Therefore, the high level of eosinophil count in this study indicates persistent tissue inflammation and an active infestation of parasites. The significant elevation in the ENR and ELR of dogs infected with filarial worms demonstrates the likely immuno-inflammatory response which is novel. Although this response has been reported by some scholars associated with other diseases [17], it has not been reported in filarial infection. Therefore, ENR and ELR may warrant further investigation as potential haematological biomarkers of filarial infection, especially in high-risk areas with limited access to molecular diagnostics.

From the perspective of clinical pathology, a combination of eosinophilia and hyperglobulinemia, and possibly ENR and ELR with clinical presentation, will serve as a good marker for the diagnosis of filarial infection in dogs. The observations in this study have significant diagnostic and epidemiological implications to help clinicians consider this disease as one of their tentative diagnoses.




***= p< 0.0001, ALT=Alanine aminotransferase, AST=Aspartate aminotransferase, NPF= Filaria negative.

**Figure 4.** Serum Chemistry changes associated with filarial worms in hunting dogs in Jos, Plateau State.

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## 5. Conclusion

Hunting dogs in Nigeria demonstrated a 17.5% prevalence of circulating microfilariae. A combination of eosinophilia and hyperglobulinemia, and possibly ENR and ELR with clinical presentation, will serve as supportive haematological indicators pending further validation in dogs. This work indicates the need to carry out deliberate screening of hunting in peri-urban areas using serology or molecular means to ascertain the status of these dogs and the zoonotic implications. Given that hunting dogs may act as reservoirs for zoonotic filarial species, these findings emphasize the importance of incorporating vector control with routine screening.

## Authors' contributions

G. Y. Gurumyen: Conceptualization, writing of original draft.
S. N. Salisu: Sample collections and laboratory analysis.
I. Musa: data curation.
S. J. Titus: Arranged for resources.
P. Tanko: Review and editing.
E. V. Tizhe: Review and editing.
D.M Buba: Laboratory analysis.
I.J Gosomji: Project administration.
A.A Usman: Validation and arranged resources.
A.N Zoclanclounon: Data analysis.
B.O Emikpe: Supervision.

## Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

Data will be available upon request. The data generated in this study were only used to write the manuscript.

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