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Falciparum malaria in a child from Ghana with neutrophils showing phagocytosis of trophozoites, merozoites and possibly schizont

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ABSTRACT

Malaria remains a significant health threat in tropical and subtropical regions. The immune response to *Plasmodium falciparum* involves both humoral and cellular components, including phagocytosis by neutrophils. However, observing phagocytosis through light microscopy is uncommon. This report presents a rare case of falciparum malaria in a Ghanaian twin, highlighting intracellular *P. falciparum* forms in neutrophils. Two 7-year-old male twins from Ghana were admitted with fever, vomiting, and abdominal pain. They tested positive for *P. falciparum* infection via rapid diagnostic tests and blood smears. Child 1 displayed significant neutrophilia and intracellular parasites, including trophozoites, merozoites, and a potential schizont, within neutrophils. Child 2 showed no phagocytosis but exhibited other signs of systemic inflammation and hemolysis. Both children were treated successfully with piperaquine tetraphosphate/artenimol (Eurartesim®). This case provides rare microscopic evidence of *P. falciparum* phagocytosis by neutrophils, suggesting prior antibody development in the patient. While the findings highlight the role of phagocytosis in parasite clearance, the absence of similar observations in the second twin raises questions about individual immune responses and genetic factors. Further research is needed to explore the underlying biological mechanisms.

1. Background

Malaria affects numerous tropical and subtropical countries worldwide. In 2023, the World Health Organization (WHO) reported 247 million cases, resulting in 619,000 deaths [1]. Malaria infection triggers both humoral and cellular immune responses. The humoral response involves the maturation of immunoglobulins, driven by continuous stimulation with parasite antigens, leading to partial immunity [2]. The cellular response is primarily mediated by cytokines such as CD56+ gamma T cells and tumor necrosis factor. Additionally, phagocytosis by monocytes or macrophages plays a crucial role in parasite clearance, although observing this process through light microscopy is rare.

We present a case involving a young male from Ghana diagnosed with malaria. Their blood smear exhibited trophozoites, merozoites, and potentially a phagocytosed schizont by neutrophils.

2. Case presentation

We present a case involving fraternal twin males, aged 7, from Ghana who were admitted to our hospital presenting with fever, vomiting, and abdominal pain persisting for the last 4 days. They arrived in Spain one week before their admission. Their father reported prior episodes of malaria in their home country.

Laboratory test results revealed that Child 1 exhibited neutrophilia,

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Table 1
Comprehensive Laboratory Test Results for Two Pediatric Patients.

Variable	Child #1	Child #2
Red Blood Cells (million/mm^3)	4.68	–
Hemoglobin (g/dL)	12.1	11.9
Hematocrit (%)	37.2	–
Mean Corpuscular Volume (fl)	79.4	–
White Blood Cells (/mm^3)	7200	–
Neutrophils (%)	81.3	–
Lymphocytes (%)	9.8	–
Monocytes (%)	8.2	–
Platelets (/mm^3)	84,000	92,000
Alkaline Phosphatase (IU/L)	195	–
Lactate Dehydrogenase (IU/L)	–	351
C-reactive Protein (mg/L)	–	199.1
Prothrombin Time (seconds)	–	17
Prothrombin Activity (%)	–	59
INR	–	1.39
Partial Thromboplastin Time (seconds)	–	31.9
Fibrinogen (mg/dL)	–	703

lymphopenia, and thrombocytopenia, while Child 2 presented with thrombocytopenia, elevated lactate dehydrogenase (LDH) levels, increased C-reactive protein (CRP) and fibrinogen, prolonged prothrombin time (PT), elevated INR, and reduced prothrombin activity (PA). The specific values for both children are detailed in Table 1.

Rapid diagnostic tests using BinaxNOW® Malaria (Abbott, USA) showed positive results for aldolase and HRP-II antigens in both children. Giemsa-stained thin blood smears revealed typical *Plasmodium falciparum* rings and few schizonts, indicating parasitemia of 3.8 % in Child #1 and 1.56 % in Child #2. Notably, in Child #1, 5.5 % of neutrophils contained malaria pigment. Some neutrophils were observed harboring intracellular *P. falciparum* trophozoites (Fig. 1A), merozoites (Fig. 1B and 1D), and occasional neutrophils concurrently containing both trophozoites and merozoites (Fig. 1C). The number of intracellular merozoites ranged from 1 to a maximum of 15. In some neutrophils, malaria pigment coexisted with merozoites (Fig. 1D). A parasitophorous vacuole was observed within one neutrophil (Fig. 1E), along with another neutrophil containing an intracellular schizont, though its intracellular or extracellular location was unclear (Fig. 1F). Conversely, for Child #2, neither malaria pigment nor intracellular parasites were detected within neutrophils. Both patients received a three-day treatment regimen of piperaquine tetraphosphate/artenimol (Eurartesim®). Post-treatment evaluation of thin blood smears revealed the absence of parasitic forms.

3. Discussion

This study presents a case of falciparum malaria exhibiting very inusual microscopic findings of intracellular merozoites, trophozoites, and potentially, schizonts. Previous research has shown that neutrophils and neutrophil metamyelocytes can phagocytize merozoites, and to a lesser extent, trophozoites in bone marrow aspirates [3]. Notably, in 1985, Sun et al. reported a case involving two Nigerian siblings (ages 4 and 10) infected with *P. falciparum* [4]. The 4-year-old male exhibited a 7 % parasitemia, numerous schizonts, and free merozoites. The authors documented intracellular phagocytized merozoites in both monocytes and neutrophils, alongside an intact schizont within a neutrophil's cytoplasm. A parasitophorous vacuole was observed within a monocyte. In this case, the child experienced severe complications, including a seizure and subsequent cardiac arrest following the administration of chloroquine, which led to his death. This was likely due to the lack of immunity, and the severity of the infection as demonstrated by the large number of schizonts in the blood. Lübbert et al. (2013) detailed a case of a 37-year-old Mozambican man diagnosed with 50 % parasitemia after a brief visit to his home country, showcasing a single schizont phagocytosed by a monocyte [5]. In this case, the patient was treated with quinine and doxycycline, showing a good prognosis with fever resolution and no need for intensive care despite the hyperparasitemia. No parasitic forms were detected by the end of the follow-up. In addition, Diallo et al. (2017) reported a case of a six-year-old boy from Guinea-Bissau with 3.6 % parasitemia, noting malaria pigment in monocytes and neutrophils, multiple merozoites within a neutrophil's cytoplasm, and an intracellular schizont in a monocyte [6]. This patient was treated with a combination of artemisinin and lumefantrine. The headaches, fever, chills, and abdominal pain resolved by the third day, and all parasitic forms were cleared.

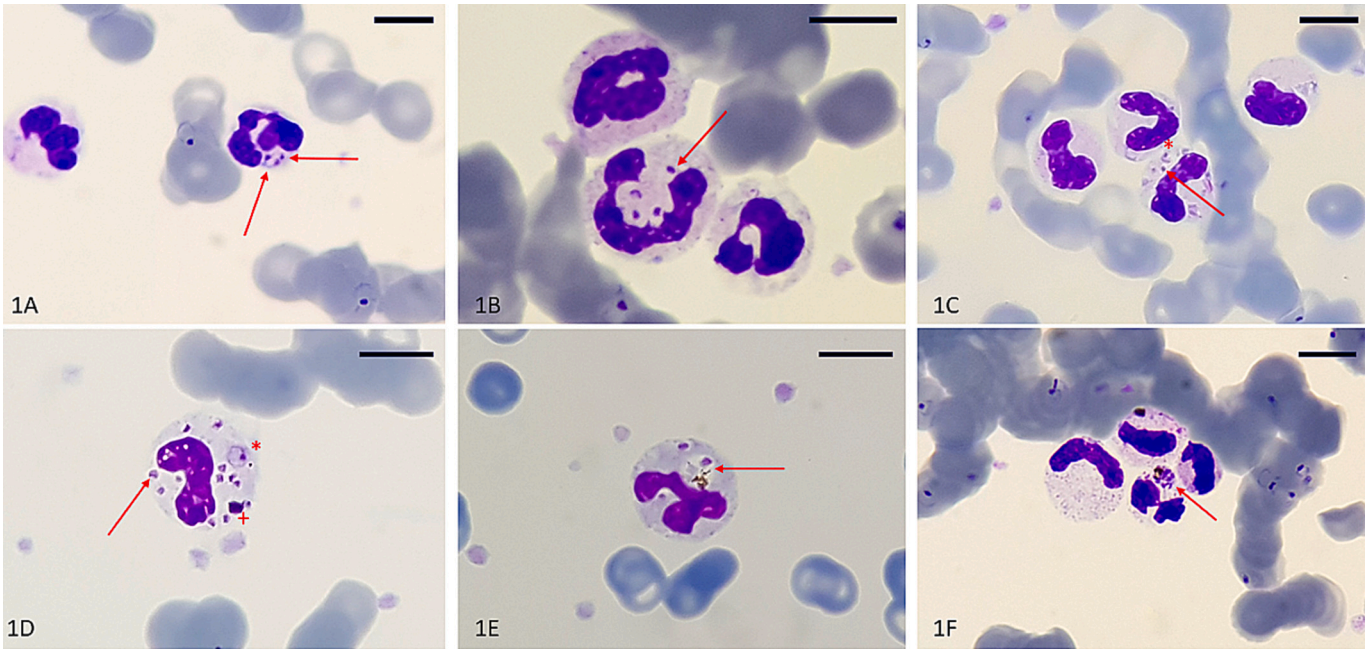

Fig. 1. 1A: Two ring stage trophozoites inside a neutrophil are visible one with doble-chromatin dots (⟵); 1B: Neutrophil with four visible merozoites (⟵); 1C: Neutrophil with one trophozoite (*) and few merozoites (⟵); 1D: Neutrophil with 12 merozoites (⟵); a trophozoite (*) and malaria pigment (+); 1E: A parasitophorous vacuole is clearly visible inside a neutrophil (⟵); 1F: Schizont is visible probably inside a neutrophil (⟵). Scale bars: 10 μm.

Phagocytosis plays a pivotal role in parasite clearance, with research suggesting that merozoite phagocytosis involves both immunoglobulins (Igs) and complement, while trophozoite phagocytosis is predominantly Ig-mediated [7]. Our patient, displaying trophozoites within neutrophils, indicates prior antibody development, as indicated by the patient's father due to previous infections. However, this doesn't explain the absence of phagocytized parasitic forms in Child #2's neutrophils. Studies propose that the primary cell involved in merozoite phagocytosis depends on specific antibody titers, with monocytes dominating at lower titers and neutrophils activated at higher titers, consistent with our case [7]. As a limitation, detection of antibodies against *P. falciparum* was not available in our laboratory, which could have helped confirm this hypothesis.

The association of Ig-mediated phagocytosis by neutrophils with disease protection remains uncertain [8]. Literature suggests that intracellular parasites can indicate both potential outcomes [4–6]. The rarity of these microscopic findings may be attributed to predisposing biological factors, potentially specific HLA alleles or certain *P. falciparum* genotypes, or a combination thereof. However, further research is warranted for conclusive insights.

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CRediT authorship contribution statement

Antonio Beltrán-Rosel: Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Jorge Ligero-López:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Beatriz López-Alonso:** Writing – review & editing. **Amparo Boquera-Albert:** Visualization, Methodology, Investigation. **Ingrid Ferreira-Ferreira:** Visualization, Methodology, Investigation. **Pilar Collado-Hernández:** Writing – review & editing. **José Miguel Rubio-Muñoz:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

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