



The Pathophysiology of Trypanosomiasis Associated Anaemia: A Multifactorial Phenomenon

Sudan V^{1*}, Sumbria D¹, Kaur J², Kour R¹ and Gupta KK²

¹Department of Veterinary Parasitology, College of Veterinary Science, India

²Department of Veterinary Medicine, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), India

***Corresponding author:** Vikrant Sudan, Department of Veterinary Parasitology, College of Veterinary Science, Rampura Phul, Punjab 151103, India, Email: viks.sudan@gmail.com

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Abstract

The pathobiology of anaemia in trypanosomiasis remains one of the aspects that still lacks thorough understanding. Extra vascular haemolysis is often identified as a major contributor to anaemia, primarily due to the mononuclear phagocytic activity of the host. This sets off a series of reactions, ultimately resulting in significant alterations in haemato-biochemical indices. The magnitude of these alterations becomes more pronounced during successive waves of fluctuating parasitemia. However, it's important to note that the basic cause of anaemia is intricate and typically multifaceted, mainly affecting the cellular integrity of red blood cells, terminating into hemolytic anemia and/or increased erythrophagocytosis. Injuries inflicted by the flagella and microtubules of the parasite onto the membranes of erythrocytes further facilitate erythrophagocytosis of damaged RBCs. Additionally, erythrocytes, reticulocytes, and platelets often bind to trypanosomes via sialic acid receptors, causing injuries to the cells promoting their rupture. A number of factors attributing to haemolytic anaemia include trypanosome are discussed in the present review. This review elaborates on the details and interactions of all these contributing factors.

Keywords: Anaemia; Multifactorial Etiology; Trypanosomiasis

Introduction

Many aspects of trypanosomiasis pathogenicity require further elucidation. While immunosuppression and hypoglycemia are well-documented in the literature, anaemia remains a less understood aspect of trypanosomiasis pathogenesis. Despite being a pivotal immunopathological feature of animal trypanosomiasis, anaemia remains a topic of ongoing debate. The complexity of anaemia in trypanosomiasis is exacerbated by its multifactorial origin [1], adding to its intricacy. This review aims to consolidate information concerning anaemia in trypanosomiasis,

including its stages, a glimpse into its multifactorial etiology, and a multidimensional exploration to enhance our comprehension of the disease.

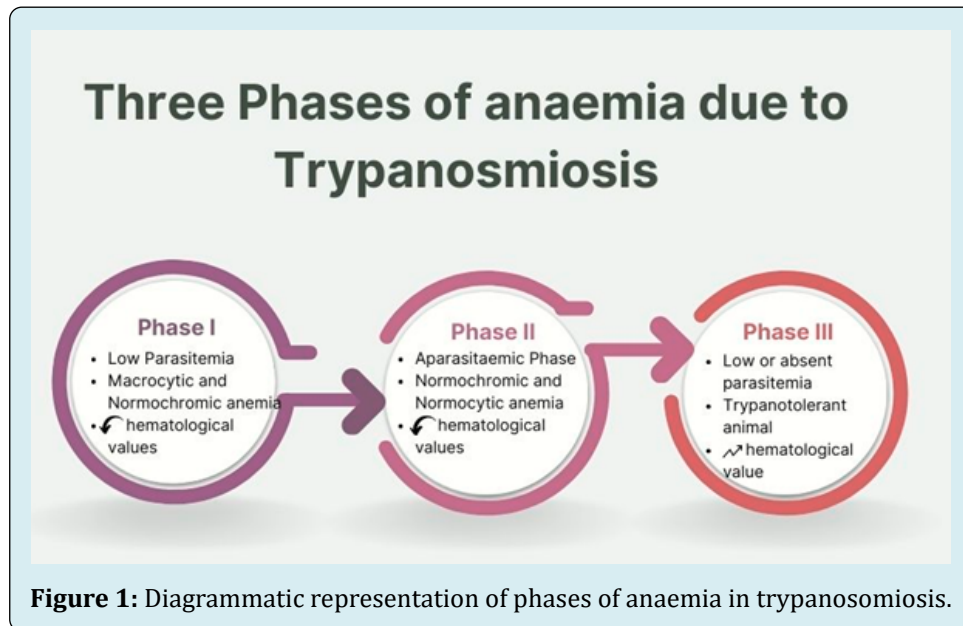
The Complexity of Trypanosomiasis Inflicted Anaemia

The pathophysiology of anaemia associated with trypanosomiasis is exceedingly intricate and surpasses with those caused by other infectious agents due to its multifaceted origins [1]. In several other parasitic diseases, anaemia directly results from blood loss caused by the parasite, as

seen in hookworms [2], or the blood-sucking habits of the parasite, as in haemonchosis [2]. Alternatively, it may arise from haematophagia and subsequent hemorrhages during the migratory phase, or injurious nature of parasite involved, as observed in immature liver flukes [3]. Additionally, anaemia can be induced by the discharge of antihematinic metabolites into the host circulation by the parasite, as seen in fasciolosis [4]. However, in trypanosomiasis, the

etiology of anaemia is notably intricate. It is assumed that in trypanosomiasis cascade of events culminate into cardiovascular collapse owing to haemolytic anaemia [5].

To facilitate comprehension, the anaemia in trypanosomiasis has been categorized into three distinct phases (Figure 1).



Phase I: The Acute Crisis Initiation Phase: This phase marks the initial onset of anaemia, and coincide with initial symptoms and establishment of trypanosomes inside the host's blood stream. Following the establishment of the infection, parasitemia periodically surges with characteristic fluctuations in its levels [6,7]. During this phase, the anaemia is macrocytic, normochromic [8]. Additionally, a decline in hematological and erythrocytic values becomes evident. Sub-acute phases are seen in experimental infection of rodents with *Trypanosoma congolense* [9] and *T. brucei* [7].

Phase II: The Progression or the Chronic Phase: Following the acute crisis phase, this stage depicts lower levels of fluctuating parasitemia, spanning up to months. In the case of tissue-dwelling trypanosomes like the *T. brucei* group, this phase is also referred to as the aparasitaemic phase, wherein the parasites tends to establish themselves outside the bloodstream and are either found in reduced numbers [10] entirely absent in blood smear [11]. Throughout this phase, the anaemia transitions to a normochromic and normocytic type [12]. Hematological and erythrocytic values are generally depressed during this period.

Phase III: The Stabilizing or the Recovery Phase: This phase is distinguished by either the low, infrequent, or complete absence of parasitemia. During this stage, the previously depressed erythrocytic values start to normalize, accompanied by the resolution of other pathological alterations [5]. This phase leads to self-recovery and is commonly referred to as the trypanotolerant stage [11]. Wild animals often demonstrate a moderate level of trypanotolerance, reflecting their relative ability to control the excessive proliferation of trypanosomes within their bodies and limit their pathogenic effects [11].

Multifactorial Etiology of Trypanosomiasis Associated Anaemia

The primary subtype of anaemia prevalent in trypanosomiasis is hemolytic anaemia. It is end result of complex interactions between various host and parasite-associated factors, which can act independently or synergistically, ultimately leading to a fatal form of hemolytic anaemia. Common attributes (Figure 2).

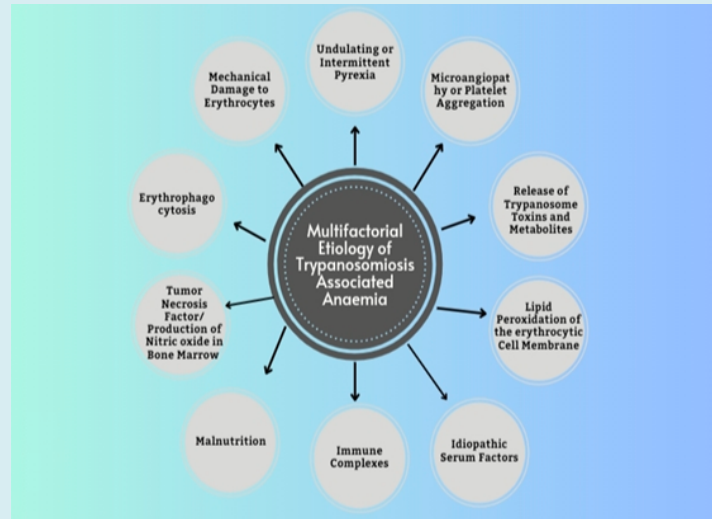


Figure 2: Diagrammatic representation of multiple etiology of anaemia in trypanosomiasis leading to haemolytical anaemia include.

Anaemia Arising Due to Mechanical Damage to Erythrocytes:

In acute infection of trypanosomiasis, the forceful movement of flagella and microtubule-reinforced bodies in millions of parasites causes significant mechanical damage to erythrocytic cell membrane [13]. This erythrocytic cell membrane damage is further exacerbated by adhesion of various blood cells like erythrocyte, platelets, and reticulocytes to the trypanosome surfaces through sialic acid mediated receptors, resulting in additional cell membrane damage [14]. As a result, multiple points of disruption emerge on the surface of erythrocyte membranes at the points of attachment with the body of trypanosomes. Furthermore, there have been documented instances of mechanical harm to the vascular endothelium by the invasion of trypanosomes penetrating the tissues [15]. Together, these elements contribute to the mechanical damage of erythrocytes, ultimately resulting in extensive rupture of erythrocytes and subsequent anemia.

Anaemia Caused due to Undulating or Intermittent Pyrexia:

The Variant Surface Glycoproteins (VSGs) of trypanosomes are recognized to exert a notable influence on the degrees of recurring fevers and changing levels of parasitic presence [11]. Remarkably, there have been substantial elevations in measures of osmotic fragility and the permeability of erythrocytes, particularly at temperatures exceeding the host's typical body temperature [16]. Furthermore, elevated body temperature reduces the flexibility and lifespan of erythrocytes in vivo [17]. As a result, heightened temperatures have been linked to an augmented occurrence of immunochemical reactions, culminating in the lipid peroxidation of erythrocytes [18]. Hence, in phases of recurring fevers when temperatures reach their peak, the stability of erythrocytes is diminished, making them more

prone to rupture [16].

Anaemia Due to Microangiopathy or Aggregation of Platelets:

Microangiopathy or platelet aggregation is commonly linked with both intact as well as fragmented trypanosomes [19]. The harm inflicted on trypanosomes is more frequent during trypanocidal drug treatment. This harm causes the burst of auto-platelet antibodies, subsequently initiating procoagulant release leading to fibrin deposition. This ultimately terminates into formation of microthrombi further attributing to dispersed intravascular coagulopathies [18]. Weakening of erythrocytes, owing to reasons discussed above, tends to their breakdown into fragments as they move through the fibrin deposits of microthrombi [15], eventually leading to dispersed sites of intravascular coagulation [11].

Anaemia Caused by Release of Toxins and/or Metabolites of Trypanosomes:

It has been well understood for a significant duration that both viable and deceased trypanosomes secrete a range of bioactive chemical compounds like neuraminidase, pyruvates, free fatty acids, proteases, phospholipase, etc. capable of harming erythrocytes [1]. Neuraminidases are produced in laboratory settings by various trypanosome species. They are recognized for elevating the susceptibility of erythrocytes [20] to get phagocytosed by the mononuclear phagocytic system (MPS). Neuraminidases enhance the vulnerability of erythrocytes by splitting the sialic acid component found on the cell surface [21]. They are also known to hinder erythropoiesis by causing harm to the erythropoietin hormone [22]. Trypanosomes have the ability to release proteases from their reservoirs and also from the bodies of compromised or deceased parasite [18]. This feature is very common post therapy when a large number of trypanosomes are available inside the host's

body. Proteases cause harm to erythrocytes and vascular endothelium by cleaving sialic acid segments from the cell membrane in the glycopeptide form [23]. Reports indicate that toxic by-products, generated through the metabolism of aromatic amino acids by trypanosomes, directly affect the erythrocyte cell membrane, leading to imbalances associated with osmotic fragility and eventual lysis of erythrocytes [18]. Phenylalanine undergoes catabolism to phenylpyruvate that hampers mitochondrial gluconeogenesis [18]. Moreover, in the context of trypanosomiasis, tryptophan is metabolized into indole-ethanol that harms erythrocyte cell membranes [18], rendering them more vulnerable to rupture.

Anaemia due to Lipo-Peroxidation of Erythrocytic Cell Membrane: Irrespective of the factors involved, the process of anemia in trypanosomiasis is closely connected to the production of free radicals and superoxides stemming from lipid peroxidation. These oxidative substances are known to impact the erythrocytes [18,24] by targeting polyunsaturated fatty acids and proteins found in the membrane of erythrocytes [25,26]. On occasion, red blood cells experience direct influence, resulting in oxidative hemolysis [22,24]. During trypanosomiasis, the formation of antigen-antibody complexes containing sialic acids plays a role in erythrophagocytosis [27] and the ensuing anemia [26]. Animals infected with trypanosomiasis have been noted to exhibit a significant deficit in their systemic antioxidant capacity [28]. The vulnerability of erythrocytes to harm from free radicals after exposure to hydrogen peroxide is extensively documented in several trypanosome species [22]. The peroxidation of erythrocytes leads to the generation of substantial quantities of lipid peroxidation byproducts, which diminish antioxidant capacity and worsen the erythrocytes' diminished ability to resist oxidative stress [18]. Trypanosomes generate neuraminidase, which removes sialic acid from the surface of erythrocytes, rendering red blood cells more susceptible to phagocytosis, elevating serum sialic acid levels and frequently coinciding with the development of anemia [29]. Erythropoietin levels are known to undergo regression owing to sialic acid fragmentation, thereby aggravating anaemia [22]. As parasitemia increases in trypanosomiasis, glycolysis leads to the buildup of pyruvate in the host's body [30]. This buildup leads to acidosis and a decreased affinity of hemoglobin for oxygen [31], thereby increasing the strain on erythrocyte membranes. Acidosis is once more linked to the suppression of the tricarboxylic acid cycle (TCA) within the mitochondria [32], further exacerbating anemia. After the autolysis of dead trypanosomes, a substantial quantity of phospholipase A1 and lysophospholipase A1 [33] is discharged into the bloodstream, leading to erythrocyte deterioration, impairment of vascular endothelial cells, and hemolysis [34]. These phospholipases are predominantly characterized

by their palmitic, stearic, and linoleic acid composition [35], and they subsequently liberate free fatty acids (FFA) from phosphatidylcholine. Inoleic acid exhibits detergent-like properties and is responsible for causing pronounced hemolysis and cytotoxic effects [33]. The peroxidation of erythrocyte membranes by lipids is frequently connected with reduced membrane flexibility and a decrease in the activities of membrane-bound enzymes [18], thereby worsening anemia. Lipid peroxidation and oxidative harm to erythrocyte membranes are indicators of oxidative stress, which is associated with *T. evansi* infection in murine models [36].

Anaemia Caused due to Idiopathic Serum Factors: In trypanosomiasis, there have been observations of two unknown (idiopathic) serum factors, which are essentially heat-stable proteins not originating from trypanosomes. These proteins inhibit erythropoiesis activity [37], indirectly affecting erythroid colony functions [22], thereby aggravating anemia.

Anaemia Caused by Immune Complexes: Within trypanosomiasis, immunological processes result in the surface removal of autologous IgM and IgG antibodies and factors related with C3 complement from erythrocytes [1]. The emergence of autoantibodies usually occurs after the initial surge in parasitemia, concurrently with lower Packed Cell Volume (PCV). Consequently, erythrocyte surfaces become capable of binding auto- or polyreactive antibodies, and they may also become sensitized through the absorption of immune complexes [1]. Additionally, erythrocytes absorb trypanosome molecules passively, anti-trypanosome antibodies attachment occurs leading to their removal from host [1]. The remarkable increase in tissue macrophages during trypanosomiasis has been well-documented [18] and is facilitated through the interplay of various lymphokines, antigen-antibody complexes, and C3b complement fragments [38], thereby playing a role in cytokine-mediated erythrocyte depletion in the context of trypanosomiasis [1]. Cytokines such as TNF- α , IFN- γ , etc. [39] are often implicated as contributing factors to anemia. Additionally, hypoferrremia is sometimes associated with IL-6 and hepcidin response [40].

Anaemia Arising Due to Malnutrition: Trypanosomiasis triggers inappetence and anorexia, leading to lower energy and depleted body score and tissue reserves. This depletion is attributed to the glycogenesis, deficiencies in essential amino acids, vitamin C, and B vitamins [18]. Poor ATP supply to erythrocytes causes alterations to the erythrocytic membrane surface, weakening it, increasing its osmotic fragility, and promoting hemolysis [41], consequently accelerating erythrocytic destruction.

Anaemia Caused by Tumor Necrosis Factor/ Production of Nitric Oxide in Bone Marrow:

The production of Tumor Necrosis Factor (TNF- α) by monocytes is a significant contributor of anemia in trypanosomiasis [42]. In this condition, there's an observed increase in proliferation of bone marrow tissue, leading to elevated levels of nitric oxide (NO) within the bone marrow. This coincides with the inhibition of T-cell proliferation when stimulated with the mitogen concanavalin, both in experimental conditions and within the living organism. NO also inhibits the proliferation of hematopoietic precursors, exacerbating anemia in subsequent phase trypanosomiasis [43]. *T. evansi* causes the generation of NO in camels as well [44]. When NO is created, it reacts with superoxide to make peroxynitrate anion or hydroxyl radicals. Both of these produced compounds have a necrotic impact [45]. NO enters the RBC, where it causes the synthesis of methemoglobin and changes the permeability of the membrane, increasing cellular fragility [46].

Anaemia Caused by Erythrophagocytosis:

Erythrophagocytosis by monocytes and macrophages, significantly contributes to anemia in trypanosomiasis through an immunological mechanism. This process is initiated by antibody-antigen (Ab-Ag) complexes generation on RBC membrane proceeded by trypanosome destruction. These complexes transform the erythrocytes into foreign proteins, making them susceptible to engulfment by macrophages [47]. During trypanosomiasis, there's a modification in the antigenicity of the host animal's erythrocytes, triggering immunoglobulin-mediated erythrophagocytosis facilitated by macrophage fragment crystallizable receptors for IgG [48]. The host animal produces IgG and IgM, which coat the modified erythrocytes, often involving IgM and IgG complexes and complement C-3 [49], thereby promoting their erythrophagocytosis by macrophages.

Besides the details of anaemia in trypanosomiasis are very much explained in detail in classical work of Mbaya, et al. [50].

Conclusion

In conclusion, trypanosomiasis-associated anemia is undoubtedly a multifactorial condition, with multiple factors working synergistically to trigger a series of reactions leading to significant anemia in affected hosts. The pathobiology of anemia in trypanosomiasis is complex, but deeper understanding of the condition aids in assessing the state of infected hosts. While extensive research has postulated many theories, addressing the challenges associated with anemia in animals remains a complex issue. Effective management of anemia in trypanosomiasis-affected hosts is a detrimental factor for their survival and reducing the overall impact of the disease.

Conflict of Interest

None of the authors is having any conflict of interest.

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