Case report:

A Ten Year Old Boy with Unexplained Hemolytic Anemia

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ABSTRACT

Babesiosis is a tick - borne zoonotic disease transmitted by the intracellular protozoan from genus Babesia. Infection with is uncommon and symptomatic disease is mostly confined to splenectomized patients. It may be transmitted by blood transfusion.

In splenectomized patients, the disease has an acute onset and is often fatal. Unsplenectomized patients may experience a milder self - limiting disease, although intravascular hemolysis does occur. The diagnosis is made from the peripheral blood film, where the parasites, looking very similar to malaria, are seen inside the red cells.

In this case report, we describe a child with unexplained hemolytic anemia with presumptive diagnosis of autoimmune hemolytic anemia treated with Steroids and immunosuppressive treatment with no response. After review of his investigation, surprisingly, his peripheral blood film revealed Babesiosis.

CASE PRESENTATION

A Ten years old boy presented to the general pediatrician at October 2013 with 2-day history of pallor, jaundice, abdominal distension and dark-colored urine. He received 1 unit of packed red cells once. His condition remained stable for 1 month and deteriorated again with

pallor and change in urine color and later on was admitted to a hospital for investigations and follow up for 1 week, and discharged home without any complications. He kept on follow up only for 2 months. Since 24th of January 2014, treatment started with small dose prednisolone and increase gradually. A consultation to Child Welfare Teaching Hospital Hemato-oncology outpatient clinic was done on 24th March 2014 in which full investigation done and revealed a picture of autoimmune hemolytic anemia . For that reason prednisolone started at a dose of 2mg/kg /day for 2 weeks at the beginning then dose was adjusted according to the clinical criteria and the result of investigations. After 6 months of follow up, immunosuppressive treatment with mycophenolate mofetil (*Celcept*)® started with gradual tapering of steroids. The anemia worsened with declining in hemoglobin level , increasing in reticulocyte count with deepening of jaundice ; so an online consultation to Swinfen Charitable Trust website *http://www.humanitariantelemed.org* was established to Dr. Peter Wood , who say : " *it certainly sounds like a hemolytic anemia although hemoglobin nadir is not too bad (92g/L) . I note the initial positive Coomb's test, but then several negative tests subsequently. If the initial positive was only weak, it may not be significant. Certainly the predominantly negative Coomb's suggest it is NOT an immune hemolysis and hence unlikely to respond to immunosuppression. Non-immune cause particularly hereditary spherocytosis should be considered (there may be a family history; other causes would be G6PD or pyruvate kinase deficiency. If you are convinced, the hemolysis is immune then Rituximab is a reasonable option, as is cyclosporine A. The response to Rituximab usually only lasts for a couple of years if it responds".*

Our interpretation, we have more than one patient who has negative coomb's test turned to be positive when checked abroad so we didn't rely on the lab. In determining the accuracy of Coomb's test. It wasn't a case of hereditary spherocytosis so we continue treatment as autoimmune hemolytic anemia ignoring the comment of the expert which was a valid one.

On August 2014, courses of Rituximab (375 mg/m^2 weekly for 4

doses) had been started with steroid and *Celcept*®; the latter was discontinued with gradual taper . The patient was kept on Prednisolone only after finishing the forth course of Rituximab. Deterioration of the clinical condition with progressive anemia, worsening of jaundice and change in the urine color happened after starting the slow tapering of steroid (5mg per month). In April 2015, Cyclosporine A (*Sandimmune*)® 5 mg/Kg/day in 2 divided doses had been started with small dose of steroid but the condition remained stationary with element of mild hemolysis (high reticulocyte count and hemoglobin around 9.0 g/dl with jaundice) .

At 1st of October 2015, a suggestion was contemplated to transfer the patient outside Iraq for further investigation unavailable in our country to diagnose the type of hemolytic anemia to prescribe the correct treatment according to his investigation.

Consultation to Dr. Azadeh Kiumarsi in Iran had been done and her notes:

*" Surprisingly, in his PBS we saw some ringed shaped organisms within the RBCs which we sent it to a parasitologist and he approved that there was evidence for "**Babesiosis"**. We recommend that you treat the organism; it may help the hemolytic anemia to stop!!! but if this surprising diagnosis and its treatment would not stop the lysis we recommend splenectomy as the next step".*

At 12.10.2015 Swinfen review for Dr. Peter Wood had been done , who answered:" Babesiosis is a red cell parasite like malaria and can certainly cause intravascular hemolysis as in your case It would have a negative coomb's test and would not respond to steroid therapy .I have looked at the images and there do appear to be intracellular inclusions that look like Babesia .The treatment of choice is quinine 25 mg/kg/day and clindamycin 20-40 mg/kg/day both for 7 days , you would expect to see a reduction in hemolysis with 1-2 weeks (falling bilirubin , LDH, and RTC)but the Hb. May take a little longer to recover .Splenectomy is a reasonable option and if effective will be simpler in the long term "

On Examination: He had cushingoid face, mild pallor, and jaundice. There is neither lymphadenopathy nor abnormal

pigmentation but there is palpable splenomegaly.

Results of laboratory investigations and Treatments are shown in tables 1 and 2.

Other Investigations

GUE= normal.

Autoimmune study = Negative

Blood Urea= 21 mg/dl, S. creatinine = 0.8 mg/dl

TSB= 2.8 mg/dl , Direct= 0.8 mg/dl, Indirect= 2 mg/dl, SGPT= 12 U/L, SGOT= 13 U/L

Serum Haptoglobin =377 mg/dl (N=32-205)

Serum LDH= 808 IU/ L (N=207-450)

Treatment

Briefly , the patient had been managed by the following treatment for presumed autoimmune hemolytic anemia during the course of his disease : Steroids in the form of prednisolone courses , Mycophenolate Mofetil (*Celcept*)®, Rituximab(4 courses) , Cyclosporine A *(Sandimmune)* ® as well as one unit of packed red blood cell transfusion .

After the diagnosis of Babisiosis has been established, a decision made to start antiparasitic treatment of Babesiosis based on the suggestion of Dr. Azadeh Kiumarsi with quinine 20-40 mg/kg /d & clindamycin 25mg/kg/d for 7-10 days.

SUMMERY AND DISCUSSION

Babesiosis is a worldwide tick borne hemolytic disease that is caused by intra erythrocytic protozoan parasites of the genus *Babesia*. Human infection is accidental; Babesiosis may also be acquired by blood transfusion, particularly in areas endemic for *B. microti* and *B. duncani* [1] It is a disease with a world-wide distribution affecting many species of mammals with a major impact on cattle and man .[2, 3]

History:

Babesiosis was first reported in 1888 by Viktor Babes in Romania who detected the presence of round, intra-erythrocytic bodies in the blood of infected cattle. [4]

Epidemiology

Babesiosis has rarely been reported outside the United States. Sporadic cases have been reported from a number of countries including France, the former Yugoslavia, United Kingdom, Ireland, the former Soviet Union and Mexico. In the United

States, infections have been reported from many states but the most endemic areas are the islands off the coast of Massachusetts and New York and in Connecticut.[5,6] Transfusion-associated Babesiosis has also been described.[7]

Patients receiving erythrocyte transfusions are at highest risk, while infection after transfusion of plasma has not been reported.[8]

Overall, the risk of acquiring Babesiosis from a blood transfusion is very low.

In Connecticut, the risk of acquiring babesiosis from a transfused unit of packed red blood cells was estimated at about 0.17 percent and was even lower from a transfused unit of platelets [9] .

Transplacental /perinatal transmission has been reported.[5,6]

Clinical Manifestations

Mild Illness [10,11,12,13] :

- Nonspecific symptoms (Fatigue, malaise, weakness)(common)
- Fever >38 C, Chills & sweats.(common)
- Headache (less common)
- Myalgia (less common)
- Arthralgia (less common)
- neck stiffness, sore throat, dry cough, weight loss, vomiting , diarrhoea & dark urine.(less common)
- Mild splenomegaly and hepatomegaly
- Lymphadenopathy is absent.
- Jaundice .(rare)
- Slight pharyngeal erythema .(rare)
- Retinopathy with splinter hemorrhage and retinal infarct (rare)
- Parasitemia less than 4-5%
- Hemolytic anemia (low hematocrit, Elevated LDH, low Hemoglobin, elevated total bilirubin, low Haptoglobin &/or reticulocytosis)
- Thrombocytopenia (common)
- White blood cell counts are normal , increased or mildly decreased
- Liver enzyme are elevated

Severe Illness

Severe Babesiosis was defined as a hospitalization ending in death, lasting longer than two weeks, or requiring a stay in the intensive care unit (ICU) of two days or longer[13].

- Parasitemia >4 percent
- alkaline phosphatase >125 U/L
- and white blood cell counts >5 x 10^9/L
- Malaise[12]
- Arthralgia or myalgia [12]

- Shortness of breath [12]
- Thrombocytopenia [12]
- Elevated liver enzymes are also common [12]

Differential Diagnosis of Babisiosis [14]

The diagnosis of Babesiosis should be considered in patients with flu-like symptoms in the setting of appropriate exposure (e.g. residents of endemic areas, or travelers returning from endemic area) .

These include malaria, meningitis, pneumonia, infective endocarditis, viral hepatitis, and noninfectious causes of hemolytic anemia.

Coinfection with other tick-borne illnesses should also be considered. Acute Anemia, Colorado Tick Fever, Ehrlichiosis , insect Bites , Lyme disease , malaria ,Q Fever , relapsing fever in emergency medicine , Rocky Mountain Spotted Fever ,tick-borne diseases ,typhoid fever .

Diagnosis of Babesiosis:

Microscopy [10,11,15]:

- Definitive diagnosis of babesiosis should be made by microscopic examination of thin blood smears (Wright or Giemsa staining under oil immersion).
- *B. microti* appear round, oval, or pear-shaped. The most common form is the ring, with a pale blue cytoplasm and one or two red chromatic dots. Multiple infections per cell may be observed. Ring forms may be mistaken for *Plasmodium falciparum* trophozoites.

Distinguishing features of *Babesia* include:

- Occasional merozoites arranged in tetrads, referred to as "Maltese Cross"
- Occasional exoerythrocytic parasites (when parasitemia is high)
- Absence of brownish pigment deposits (hemozoin) in ring forms
- Absence of schizonts and gametocytes

Polymerase Chain Reaction (PCR)

PCR-based amplification of the babesial gene is more sensitive than blood smear examination and results can be available within 24 hours.[16,17]

PCR is especially useful in the setting of low level parasitemia, (eg, at the *onset of symptoms* and *during convalescence*) [11]

PCR can be used to detect persistent Babesial DNA in the blood even when parasites are no longer visible on blood

smear. In one asymptomatic case, Babesial DNA persisted for as long as 17 months [16]

Serologic diagnosis with indirect immunofluorescent antibody testing (IFAT)

- Is most useful when parasites are not visualized by microscopy and DNA is not detected by PCR.[18,19]
- In cases of acute infection, serology is best used to confirm the diagnosis made by blood smear or PCR. In patients treated with rituximab (anti-CD20 antibody), B cells are depleted and serology is of no value.[15,20]

Treatment [21]

Asymptomatic individuals need not be treated, unless a Babesia species is detected on blood smear or by PCR assay for more than 3 months. Suspected Babisiosis should not be treated if reliable blood smear and PCR reaction results are negative. When Babesia is detected, symptomatic patients should be treated.

Severe *Babesia microti* Infection

First-Line Therapy

Based on the greatest cumulative clinical experience, it is recommended that patients with severe illness caused by *B. microti* be treated with intravenous clindamycin plus oral quinine for 7 to 10 days.

Persistent Relapsing Infection

A single course of standard antimicrobial therapy may fail to cure patients who are immunocompromised by one or several of the following conditions: splenectomy, HIV infection/AIDS, malignancy, and immunosuppressive therapy (in particular rituximab therapy)

In immunocompromised patients, antimicrobial therapy should be administered for a minimum of 6 weeks, including 2 weeks during which the parasite is no longer seen on blood smear. Drug regimens other than clindamycin (7-10 mg/kg q6-8h IV or 7-10 mg/kg q6-8h PO (maximum 600 mg/dose)) plus quinine (8 mg/kg q8h PO (maximum 650 mg/dose)) have been used, but no particular regimen is superior to another. In addition to the

standard regimen, atovaquone (20 mg/kg q12h PO (maximum 750 mg/dose) plus azithromycin 10 mg/kg PO on day 1 (maximum 500 mg/dose) and 5 mg/kg/day PO from day 2 on (maximum 250 mg/dose)

Exchange Transfusion

Partial or complete RBC exchange transfusion should be considered for patients with high-grade parasitemia (≥10%) RBC exchange transfusion removes *Babesia*-infected RBCs, toxic by-products of RBC lysis (e.g., hemoglobin), and circulating inflammatory mediators. Exchange transfusion also corrects the anemia caused by hemolysis of infected red blood cells as well as clearance of infected and uninfected red blood cells. Prompt use of RBC exchange transfusion is associated with favorable outcome. RBC exchange transfusion is also recommended for patients with severe anemia (hemoglobin ≤10 g/dL) or renal or hepatic compromise.

Mild Babesia microti Infection

Patients with non–life-threatening *B. microti* infection should be treated with atovaquone plus azithromycin for 7 to 10 days. In a randomized clinical trial, this regimen was found to be as effective as clindamycin plus quinine in resolving symptoms and clearing parasitemia. In patients with mild Babesiosis, symptoms typically improve within the first 48 hours of therapy and should resolve within 3 months.

Table 1 Laboratory Investigations and treatment/2014

Date	Hb.(g/dl)	WBC /cmm	PLT/cmm	RTC %	Coomb's test	TSB mg/dl	Direct	Indirect	SGPT U/L	SGOT U/L	Treatment
24.1.14	PCV=0.34			8	positive						PRD 2mg/kg/d
24.3.14	9.2	5.400	229.000	14	negative	3.4	0.9	2.5	8	10	=
9.4.14	11.8	28.600	308.000	9	negative						
22.4.14	11.1	16.200	196.000	21	negative	3.6					3 mg/kg/d
18.5.14	10.4	13.700	185.000	20							↓PRD2mg/kg+ Mycofenolate mofetile
1.6.14	10.2	11.300	193.000	21		3.0			27	19	↓↓PRD 1.5 mg/kg/d+ MMF
15.6.14	10.7	12.500	283.000	12		5.6					↓↓PRD 1mg/kg/d
29.6.14	9.8	13.700	222.000	18	negative	3.2					
13.7.14	10.3	13.200	240.000	23		3.4			21	7	
3.8.14	10.6	13.800	232.000	19	negative						PRD1mg/kg/d+ MMF+ Rituximab (started)
10.8.14	10.9	14.900	268.000	18		3.1					=
24.8.14	11.1	14.900	233.000								↓PRD +↓MMF+4th course Rituximab
9.9.14	9.9	14.600	241.000	14	negative	2.8			12	13	
25.12.14	9.4	6.700	260.000	23	negative	2.4			8	7	PRD only

Hb.=Hemoglobin, WBC=White blood cell, PLT=platelet, RTC=Reticulocyte count, TSB= Total serum bilirubin, SGPT= Serum glutamic pyruvic transaminase, SGOT=Serum glutamic oxaloacetic transaminase. PRD= prednisolone

Table 2 Laboratory Investigations and treatment/2015

Date	Hb.(g/dl)	WBC /cmm	PLT/cmm	RTC %	Coomb's test	TSB mg/dl	Direct	Indirect	SGPT U/L	SGOT U/L	Treatment
22.1.15	10.8	12.300	276.000	13	negative						
19.2.15	10.3	9.100	266.000	16.5	negative						
19.3.15	10.1	11.100	194.000			2.8	0.2	2.6			
16.4.15	8.9	9.300	277.000	18.5							PRD +cyclosporine
13.5.15	9.5	11.000	338.000	9.5					22		=
10.6.15	10.0	11.800	336.000	14							=
22.7.15	7.7	10.300	291000						13	29	=
28.7.15	9.3	13.000	515.000	20							

Hb.=Hemoglobin, WBC=White blood cell, PLT=platelet, RTC=Reticulocyte count, TSB= Total serum bilirubin, SGPT= Serum glutamic pyruvic transaminase, SGOT=Serum glutamic oxaloacetic transaminase. PRD= prednisolone

Table 3 Differential Diagnosis of Babisiosis

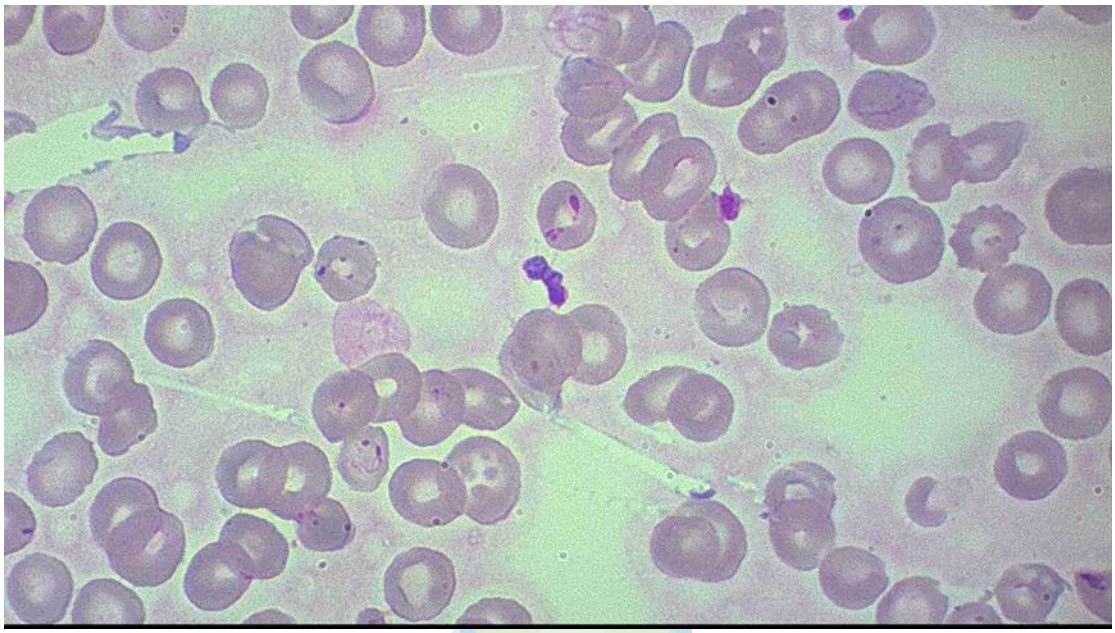
• **Acute Anemia**
• **Colorado Tick Fever**
• **Ehrlichiosis**
• **Insect Bites**
• **Lyme Disease**
• **Malaria**
• **Q Fever**
• **Relapsing Fever in Emergency Medicine**
• **Rocky Mountain Spotted Fever**
• **Tick-Borne Diseases**
• **Typhoid Fever**
• **Meningitis**
• **Pneumonia**
• **Infective endocarditis**
• **Viral hepatitis**

Table 4: Risk factors for developing severe illness due to Babesiosis [1,2,3-5]

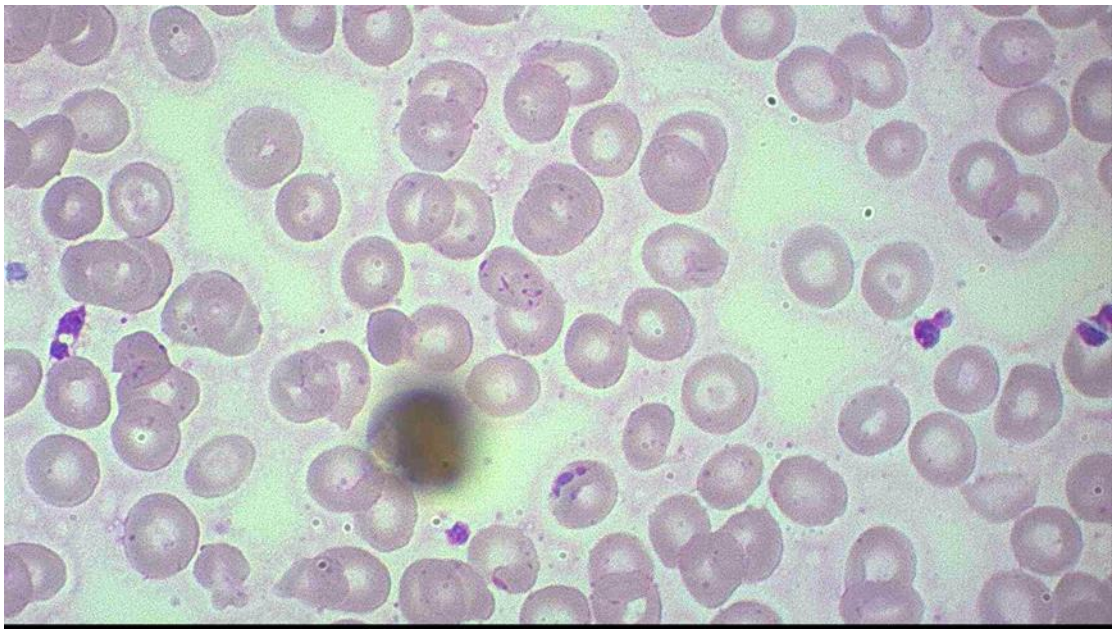
Age >50 years
Splenectomy
Coinfection with HIV or Borrelia burgdorferi
Immunosuppression caused by cancer chemotherapy or transplantation
Blockade of tumor necrosis factor alpha (TNF-alpha) activity (etanercept infliximab

Figure 1 – Peripheral blood film obtained at October 1 ,2015 showed: (A) ring forms red cell inclusions of Babisiosis with tetrad (Maltese cross) seen in one red cell (low power , 4x), (B) multiple ring forms red cell inclusions of Babisiosis are seen with Maltese cross seen in one red cell, (moderate power ,8x), (C) Maltese cross seen in one red cell (high power , 100x)

(A)



(B)



(C)

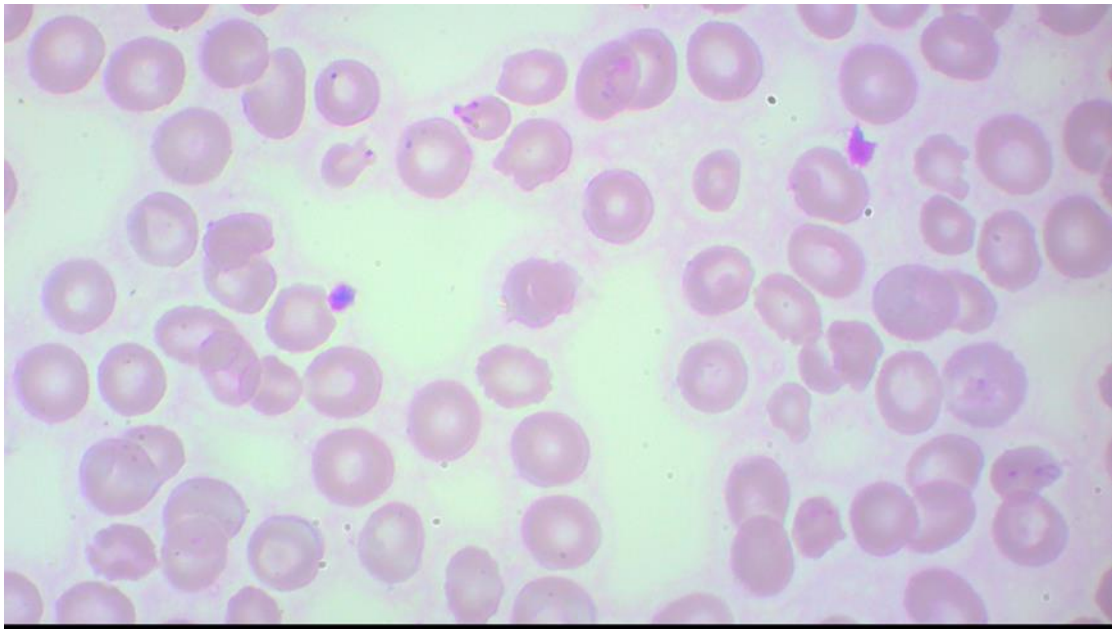
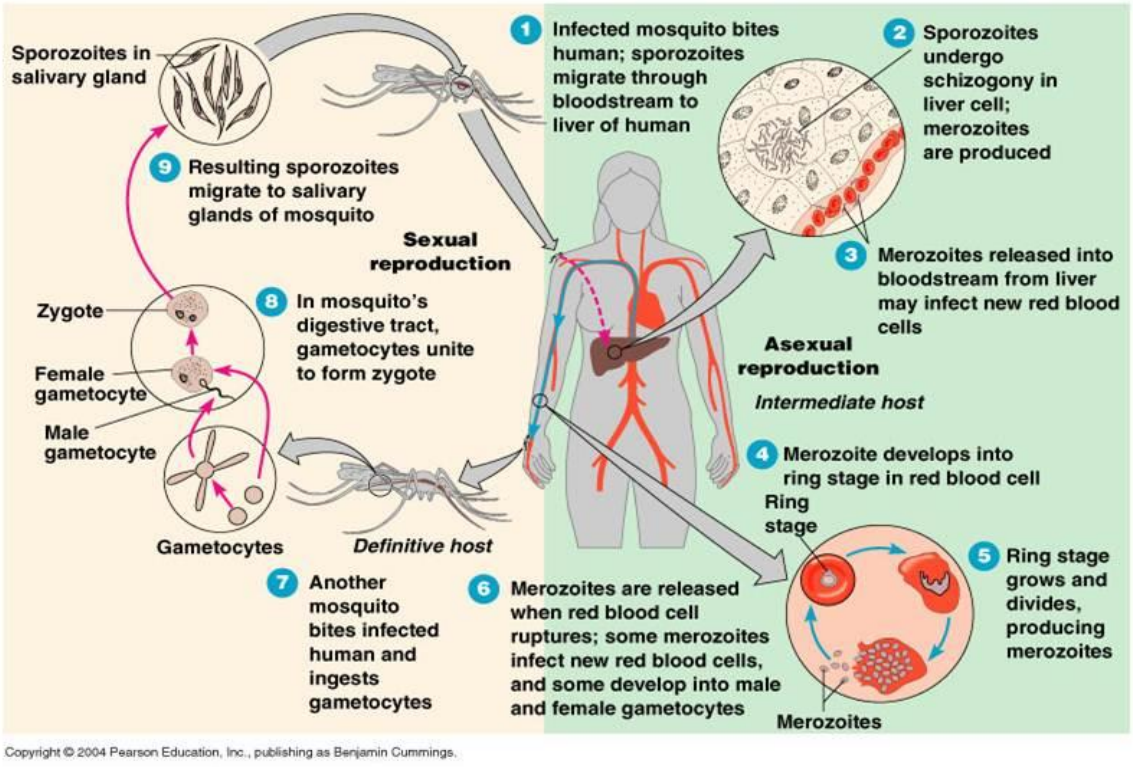


Figure 2 – The life cycle of Babisiosis



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