**MALARIA – KEY FACTS**

- 300-500 million cases/year
- 700,000 – 2.7 million die/year
- 600 U.S. cases 1914, none since 1940's
- Counterfeit drugs, worldwide issue
- World’s population (41%), endemic
- *Africa, Asia, Middle East, Central, South America, Hispaniola, Oceania*
- Mosquito (new species), blood/blood products, congenital, shared needles

**MALARIA PATIENT: LABORATORY**

- ER – Evening & night shifts (STAT request)
- Hematology - microbiology? (late shift ?)
  - Finger stick to venipuncture (morphology changes) (parasites continue to grow in EDTA!!)
  - Automated hematology vs manual examination; low parasitemias missed
  - Thick/thin blood films (read, report); controls
- Lack of technical expertise (generalist/specialist)
- Risk management issues (recognition of STAT!)

**MALARIA PATIENT: LABORATORY**

- Low grade fever, malaise, diarrhea; poor history
- Traveler, poor prophylaxis, self-medication
- Malaria never considered; no STAT coverage
- Automated exam, no manual review
- Failure to prepare/read thick & thin films
- Low parasitemia (<0.1% to 0.0001%) – traveler immunologically naïve = symptomatic early
- Reported false negative, patient released; 2016 submicroscopic and mixed asymptomatic cases

**Parasitemia Light Microscopy**

- 0.0001-0.0004% (5-20/µl) = Positive thick film
- 0.002 % (100/µl) = Naive patients may be symptomatic below this level; remember ER patients - travelers
- 0.2% (10,000/µl) = Level above which immune patients exhibit symptoms; 0.1% (5,000) = BinaxNOW (maximum sensitivity)
- 2% (100,000/µl) = Maximum parasitemia of *P. vivax, P. ovale* (young RBCs only) – rarely exceeds 2%
- 2-5% (100,000-250,000/µl) = Hyperparasitemia, severe malaria, increased mortality
- 10% (500,000/µl) = Exchange transfusion, high mortality
QUALITY CONTROL SLIDES
EDTA (With or without *Plasmodium*

PATIENT BLOOD FILMS CAN SERVE AS CONTROLS (IF WBCS OK, THEN PARASITES WILL BE OK); positive blood films with *Plasmodium* spp. NOT REQUIRED

♦ EDTA: Prepare thick and thin films immediately; distortion occurs if >1 to 2 h (Remember when reviewing PT slides)
♦ EDTA: Parasites may be lost if >4 to 6 h delay in smear preparation
♦ EDTA: Adhesion to slide poor if ratio of EDTA/blood too high or blood held too long

BLOOD PARASITE STAINS
Stain Options

♦ GIEMSA: Historically the blood stain of choice
♦ WRIGHT: Commonly used for hematology
♦ WRIGHT-GIEMSA: Automated hematology
♦ RAPID STAINS: Results in very short time
  – Diff-Quik: (American Scientific Products, McGraw Park, IL)
  – Wright's Dip Stat Stain Set (Medical Chemical Corp., Torrance, CA)
♦ OTHER: Field's stain

BLOOD PARASITE STAINS
Color Variations

INTRAERYTHROCYTIC PARASITES

♦ Determine the size of the infected RBCs vs the uninfected RBCs
  – Normal size infected RBCs: *P. falciparum*, *P. malariae*, *P. knowlesi*
  – Enlarged infected RBCs: *P. vivax*, *P. ovale*
♦ Check to see if any extracellular forms
  – *Babesia* spp., *Plasmodium* (extremely rare)

NORMOCYTIC VS MACROCYTIC RBCS

Plasmodium falciparum
Normocytic

Plasmodium vivax
Macrocytic

EXTRACELLULAR FORMS
Parasites and Platelets

*Babesia* spp. Extracellular Forms - May be *Plasmodium* rings in very heavy infections.

Make sure you know what platelets look like – artifact situation. Actual parasites must have both nuclear and cytoplasmic colors.
**THIN BLOOD FILMS**
*Remember microfilariae - PT*

- **Advantages**, 300 oil immersion fields (100x)
  - RBC morphology can be seen; RBCs preserved
  - Compare size of infected RBCs to uninfected RBCs
  - Much easier to identify to species level
  - Easier to calculate parasitemia (%/100 RBCs)

- **Disadvantages**, screen using 60x oil
  - Much lower sensitivity than thick blood film
  - Failure to use new methanol for fixation
  - Infections with low parasitemia may be missed

**Examination Possibilities**

- **RBCs**
  - Intraerythrocytic protozoa
    - *Plasmodium* spp.
    - *Babesia* spp.
  - Extraerythrocytic protozoa
    - *Babesia* spp.
    - *Trypanosoma* spp.
  - Extraerythrocytic helminths
    - Microfilariae

**Examination Possibilities**

- **WBCs**
  - Within WBCs
    - *Leishmania donovani*
    - *Toxoplasma gondii*
  - Outside of WBCs
    - *Toxoplasma gondii*
    - *Trypanosoma* spp.

Top: *Leishmania*; Bottom: *Toxo*, *Tryp*

**THICK BLOOD FILMS**

- **Advantages**
  - Examining greater volume of blood
  - May be able to see malaria pigment within WBCs
  - May be able to see Schüffner's dots

- **Disadvantages**
  - Can’t compare sizes of infected and uninfected RBCs
  - Organism distortion is difficult to recognize
  - Identification to species level is more difficult

**POSITIVE THICK FILMS**

- *Plasmodium vivax*
  - 48 hr cycle (tertian malaria)
  - Tends to infect young RBCs (2 rings/cell)
  - Enlarged RBCs, Duffy *pos/neg* Africa 2017
  - Schüffner’s dots (stippling) after 8-10 hrs
  - Delicate ring (EDTA will continue to grow)
  - Ameboid rings; severe complications +/-
  - Mature schizont contains 12-24 merozoites

- *Loa loa*
- *Wuchereria bancrofti*
- *Trypanosoma cruzi*
**Plasmodium vivax**

- 48 hr cycle (tertian cycle like *P. vivax*)
- Tends to infect young RBCs
- Enlarged RBCs with fimbriated edges
- Schüffner’s dots (stippling) beginning of cycle; severe complications rare
- Smaller ring than *P. vivax* (EDTA changes)
- Trophozoites less ameboid than *P. vivax*
- Mature schizont contains ave. 8 merozoites

**Plasmodium ovale**

- 48 hr cycle (tertian cycle like *P. vivax*)
- Tends to infect young RBCs
- Enlarged RBCs with fimbriated edges
- Schüffner’s dots (stippling) beginning of cycle; severe complications rare
- Smaller ring than *P. vivax* (EDTA changes)
- Trophozoites less ameboid than *P. vivax*
- Mature schizont contains ave. 8 merozoites

**Plasmodium ovale**

- *Plasmodium ovale curtisi*
- *Plasmodium ovale wallikeri*

**Plasmodium malariae**

- 72 hr cycle (quartan malaria)
- Tends to infect old RBCs
- Normal to small size RBCs as ring grows
- No true stippling present
- Thick ring, large nucleus
- Trophozoite forms “bands” across RBC
- Mature schizont contains 6-12 merozoites

**Plasmodium malariae**

- 72 hr cycle (quartan malaria)
- Tends to infect old RBCs
- Normal to small size RBCs as ring grows
- No true stippling present
- Thick ring, large nucleus
- Trophozoite forms “bands” across RBC
- Mature schizont contains 6-12 merozoites

**Plasmodium falciparum**

- 36-48 hr (malignant tertian malaria)
- Tends to any RBC regardless of age; very heavy infection
- All sizes of RBCs; severe complications occur
- No Schüffner’s dots (stippling); Maurer’s dots (bluish, single dots)
- Delicate rings, may have two dots of chromatin/ring, appliqué or accolé forms; may have two rings/RBC
- Very ameboid trophozoite
- Crescent-shaped gametocytes (may take 10 days+)
Plasmodium falciparum

Clinical Disease (All RBCs)

- Primary attack 8-12 days: preceded by 3-4 days vague
- Symptoms: headache, photophobia, muscle aches, anorexia, nausea, vomiting; onset symptoms more severe
- Early infection: steady low-grade fever, no periodicity AND no crescent-shaped gametocytes (ER patients)
- True relapses: relapse NO, recrudescences up to a year
- Severe complications: can occur any time during infection; related to plugging of capillaries in various internal organs; symptoms may be quite severe even with LOW PARASITEMIA (cerebral malaria very dangerous)
- Late symptoms: Extreme fevers, different organs impacted
- Paroxysms: severe, anemia severe (all age RBCs)

Plasmodium knowlesi


- 24 hr (simian malaria); most rapid cycle of the 5
- Infects any RBC; heavy infection; Southeast Asia
- All sizes of RBCs; Duffy factor like P. vivax
- No Schüffner’s dots (stippling); clumpy dots later on
- Delicate rings, may have two dots of chromatin/ring, appliqué or accolé forms; may have two rings/RBC
- Band form trophozoites common; gametocytes round
- New isolates act a bit differently from lab strains
- Early stages = P. falciparum; later stages = P. malariae

MIXED MALARIAL INFECTIONS

- More common than thought
  - Thailand: 30% both P. falciparum, P. vivax
  - Africa: P. falciparum, P. malariae (Malaysia/P. knowlesi)
  - Gambian children: <1% to >60% mixed infections
  - New Guinea: all four species (confirmed by PCR)
  - Anopheles mosquitoes: can transmit two species at the same time
- Difficult to detect
  - Different parasite levels, low organism densities, confusion among morphologic criteria
  - PCR becoming test of choice for ID to species level
The BinaxNOW Malaria Test is a rapid immuno-diagnostic assay for differentiation and detection of circulating Plasmodium falciparum (P.f) antigen and the antigen common to all to Pan malarial species: Plasmodium vivax (P.v.), Plasmodium ovale (P.o.), and Plasmodium malariae (P.m.) in whole blood. It is now FDA approved (June, 2007).

Test line 1 Positive = P. falciparum
Test line 2 Positive = P. vivax, P. malariae, or P. ovale
Test lines 1,2 Positive = P. falciparum and possible mixed infection

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**PLASMODIUM SPP. Report Comments**

- *Plasmodium spp. seen:* Unable to “rule out” Plasmodium falciparum or Plasmodium knowlesi
- *Plasmodium spp. not seen:* One negative set of blood films will NOT “rule out” malaria; submit additional blood specimens every 4-6 hours.
- *Plasmodium spp. seen, possible mixed infection:* Unable to “rule out” Plasmodium falciparum or Plasmodium knowlesi

**NOTE:** It is mandatory that the parasitemia be calculated and reported for every initial and subsequent positive set of malarial films – the same method for determining parasitemia must be used for all blood film sets on that particular patient.

**Anticoagulant Artifacts Organism Changes**

- Helicosporium
- Unknown
- Nucleated RBC

**Blood Parasite Artifacts**

- Helicosporium
- Fish Tank
- Howell-Jolly bodies
- EDTA >48 hrs
**Blood Parasite Artifacts**

- Unknown
- Platelet
- Poor Staining
- Debris
- Bacteria artifact
- Artifact

**Blood Parasite Artifacts**

- *P. falciparum* gametocyte
- Processing delay
- Malarial pigment
- Platelets - EDTA
- Yeast in WBC
- Hypersegmented neutrophil
- Basophilic stippling

**BABESIA SPP.**

- **Babesia divergens**: Europe
  - Rare, but 42% mortality
- **Babesia microti**: US, parts of Europe, Japan
  - Northeast, south to New Jersey; 5% mortality
  - Most cases mild; more severe in immunosuppressed
- **WA1, CA1, MO1** (*Babesia divergens*-like), states
  - Very serious in splenectomized patients
  - Related to canine pathogen, *B. gibsoni*
- **WA1, CA5**: Now *Babesia duncanii*
  - More pathogenic than *B. microti*

**Other blood parasites: Babesia spp.**

- Artifacts
- Amastigotes
- Drying Artifacts
- Platelets
- Toxoplasma

**LEISHMANIASIS – KEY FACTS**

- ~350 Million in 88 countries at risk

- **Cutaneous**: macrophages of skin
- **Old World**: *L. tropica, L. major, L. aethiopica*
- **New World**: *L. mexicana, L. braziliensis*
- **Mucocutaneous**: macrophages of skin and buccal cavity or nasopharynx
  - *L. braziliensis*
- **Visceral**: macrophages of spleen, liver, bone marrow (reticuloendothelial system)
  - *L. donovani, L. infantum*
- **American integumentary**: cutaneous/mucocutaneous presentations on American continent
  - South U.S. to North Argentina
**CUTANEOUS LEISHMANIASIS**

: CLINICAL PRESENTATION

1. **Common** throughout Mexico, Belize, Guatemala and southern United States (Arizona, Texas)
2. **Forest rodents** (wood rats) are important hosts
3. **Prolonged exposure** = “chicleros” collect chewing gum latex (30% first year), timber cutters, road builders, farm workers
4. Two culture-positive and four PCR-pos rodents Leishmania-positive. Isolates extend geographic and ecologic range of enzootic leishmaniasis in the U. S. and is new host record. Tucson, Az

**MUCOCUTANEOUS LEISHMANIASIS**

- Leishmania: *L. mexicana* Complex

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**LEISHMANIASIS: DIAGNOSIS**

- **Organism isolation** by smear or culture; histology
- **Cutaneous, mucocutaneous only** (lesion site)
- **Lesion sampling**: dental broach, slit scrape method, aspiration of lesion edge (multiple samples required), biopsy
- **Tissue sampling** better than other smearing techniques; **culture positive** in 80% of cases
- **LD bodies** seen in about 30% of patients; many lymphocytes/plasma cells in wet ulcerative lesions; dry lesions = granulomas / fewer cells

**LEISHMANIASIS**

Cure – Yes or No?

- **Currently**, no reliable way to assess cure
- **Most researchers** think sterile cure is **unlikely** and/or **uncommon**
- **Evidence**:
  1. Reactivation years later (immunosuppressed or “traumatized” persons & animals)
  2. Demonstration of organisms years after exposure, self-healing, or treatment
  3. Persistence of immunologic markers
  4. Extrapolation of information about other intracellular pathogens

Vaccine: couple Leishmania + sand fly salivary proteins

**TRYPANOSOMIASIS, AFRICAN**

- **Caused by** *Trypanosoma brucei gambiense* (West) and *T. b. rhodesiense* (East)
- **Transmitted by** bite of tsetse fly (*Glossina* spp.)
- *T. b. gambiense* in West, Central Africa by rivers and streams; animal reservoirs insignificant
- *T. b. rhodesiense* in East, Central Africa in the savanna; wild game major reservoir hosts
- After initial development in skin, trypomastigotes found in blood, lymph notes, and CSF
AMERICAN TRYPANOSOMIASIS
(No longer South American)
- Chagas' Disease – Trypanosoma cruzi, MD suspicion
- Transmission – feces of blood-sucking triatomid bugs (kissing bugs, reduviid bugs); U.S. bugs infected; POTENTIAL; possible anaphylaxis; 2/3 bugs POS in Texas - 2016
- Bug's feces contact site of bug bite or mucus membranes; transfusions, congenital infections, ingestion of acai juice
- Mexico, Central, South, and North America (Texas, LA, Georgia, California); rural areas: dogs, cats, opossums, rodents, armadillos important reservoir hosts; >300,000 cases in U.S.
- Latin America: #1 impact on health, social systems; dogs sentinel animals for organism reintroduction; Benznidazole first choice, Nifurtimox second

CONFIRMED BLOOD DONATIONS: CHAGAS' DISEASE
Spread of Triatomid Bugs

>300,000 infected with T. cruzi in US; 300 new congenital infections in US/yr

AMERICAN TRYPANOSOMIASIS – CLINICAL DISEASE
- Initial infection often asymptomatic – years later chronic form of disease; acute disease, children
- Unilateral periorbital edema (Romana’s sign)
- Lesion, fever, lymphadenopathy, myocarditis, hepatosplenomegaly, meningoencephalitis
- Chronic disease = cardiomyopathy, congestive heart failure or arrhythmias; dilation of esophagus or colon; “MEGASYNDROME”

TRYPANOSOMIASIS, AMERICAN – LABORATORY DIAGNOSIS
- Trypomastigotes in blood in acute cases; very few in chronic cases
- Lab workers use blood-borne pathogen precautions; tryps are infective
- Blood concentrations can be performed.
- Immunoassays for antigen detection sensitive and specific (interpretation problems with leishmaniasis).
- African tryps divide in blood; T. cruzi do not

BLOOD PARASITES REVIEW
- Malaria: problems with diagnosis, risk management
- Babesia: differences in pathogenesis, blood problems; East Coast – immunocompromised; West – more severe (like Europe)
- Trypanosomes: American spread into areas of US; T. cruzi (Chagas' disease); organ transplantation; >300,000 U.S.
- Leishmaniasis: American spread into areas of US (cutaneous and mucocutaneous)
- Toxoplasmosis: Neglected in many areas of world
- Filarial Infections: Other than Dirofilaria spp., not common in U.S.; primarily eye infections
THANKS – QUESTIONS?