FIRST CASE ON CALL MICROBIOLOGY
CASE
A 24 yr Caucasian female presents to CUMC ED @ 1AM with fever chill, body aches
WHAT DO YOU DO?
• Panic?
• Remember Micro orientation & refer to notes?
• Ask for travel history?
THINK MALARIA

CRITICAL VALUE
• Malaria is a potential medical emergency & should be treated accordingly
• Delay in diagnosis & treatment is a leading cause of death in malaria patients in the United States, particularly with *P. falciparum*

ON CALL PATH RESIDENT
*PLASMODIUM YES/NO
*P. FALCIPARUM YES/NO*
Confirmation in Microbiology following day

JUST THE FACTS!
GLOBAL
• 200-300 MILLION CASES GLOBALLY
• 1-2 MILLION DEATHS ANNUALLY
  ✓ 75% CHILDREN FROM SUBSAHARAN AFRICA
• MALARIA 4TH CAUSE OF DEATH IN CHILDREN IN DEVELOPING COUNTRIES

SOUTH OF THE BORDER
MALARIA-ENDEMIC COUNTRIES
Africa, Middle East, Asia & South Pacific

MALARIA ON THE HOMEFRONT
• Between 1857 and 2003, in the U.S., 63 outbreaks of locally transmitted mosquito-borne malaria occurred
  ✓ Transmission: Local mosquitoes infected by biting persons infected with malaria parasites (acquired in endemic areas)
• In 2003, 7 pts in West Palm Beach within 10 miles of Palm Beach International Airport
  ✓ Same strain *P. vivax* by multilocus genotyping
• 11 outbreaks involving 20 cases of probable locally acquired malaria reported to CDC since 1992
• Travelers/Immigrants from Dominican Republic

Malaria-Endemic Areas
Chloroquine-Resistant
Chloroquine-Sensitive
Note
**JUST THE FACTS!**

**U.S.A 2006**
- 1,564 CASES OF MALARIA
  - AFRICA 72%
  - Mainly *P. falciparum*
  - ASIA 16%
  - Mainly *P. vivax*
  - CENTRAL AMERICA & CARIBBEAN
  - *P. falciparum & P. vivax*
- **6 DEATHS**
  - 4 PTS NO CHEMOPROPHYLAXIS
  - 2 PTS LACK OF INFORMATION

**WHO CRITERIA FOR SEVERE MALARIA**

**PARASITEMIA**
- 2%, 100,000/µL
  - MAX PARASITEMIA FOR *P. VIVAX, P. OVALE* – RARELY GO ABOVE 2%
- 2-5%, UP TO 250,000/µL,
  - SEVERE MALARIA, MORTALITY
- 10%, 500,000/µL
  - EXCHANGE TRANSFUSION

**2006 U.S. DEATHS**

<table>
<thead>
<tr>
<th>CASES</th>
<th>Country</th>
<th>Symptoms</th>
<th>Initial Dx</th>
<th>Final Dx</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thailand</td>
<td>FEVER</td>
<td>Hepatic Enceph &amp; Peritonitis</td>
<td><em>P. falciparum</em> Renal failure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lethargy</td>
<td>In differen</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hx: alcoholic cirrhosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Kenya</td>
<td>FEVER Chills</td>
<td>1st ED URI</td>
<td><em>P. falciparum</em> Respiratory failure</td>
</tr>
<tr>
<td></td>
<td>Tanzania</td>
<td>Nausea</td>
<td>2nd ED Malaria</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Nigeria</td>
<td>FEVER Vomiting</td>
<td>Malaria</td>
<td><em>P. falciparum</em> 1.4% Parasitemia Next day 27%</td>
</tr>
<tr>
<td></td>
<td>2 yo No prophyl</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**2006 DEATHS**

<table>
<thead>
<tr>
<th>CASES</th>
<th>Country</th>
<th>Symptoms</th>
<th>Initial Dx</th>
<th>Final Dx</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Ghana &amp; India</td>
<td>FEVER</td>
<td>1st ED CAP Vomiting</td>
<td><em>P. falciparum</em> 10% parasitemia Exchange transfusion</td>
</tr>
<tr>
<td></td>
<td>59 yo No prophyl</td>
<td></td>
<td>2nd ED malaria</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>India</td>
<td>FEVER Neurogenic bladder</td>
<td>1st ED pyelonephritis</td>
<td><em>P. falciparum</em> Renal failure Respiratory distress</td>
</tr>
<tr>
<td></td>
<td>75 yo</td>
<td>Cerebral malaria</td>
<td>2nd ED malaria</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Uganda</td>
<td>FEVER</td>
<td>Dengue Malaria</td>
<td><em>P. falciparum</em></td>
</tr>
<tr>
<td></td>
<td>55 yo No prophyl</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**U.S.A. MALARIA CASES**

<table>
<thead>
<tr>
<th>Plasmodium</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>falciparum</em></td>
<td>656 (50%)</td>
<td>742 (49%)</td>
<td>615 (39%)</td>
</tr>
<tr>
<td><em>vivax</em></td>
<td>315 (24%)</td>
<td>337 (22%)</td>
<td>275 (18%)</td>
</tr>
<tr>
<td><em>malariae</em></td>
<td>47 (4%)</td>
<td>27 (3%)</td>
<td>46 (3%)</td>
</tr>
<tr>
<td><em>ovale</em></td>
<td>27 (2%)</td>
<td>23 (3%)</td>
<td>47 (3%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>17 (1.3%)</td>
<td>10 (1%)</td>
<td>10 (0.6%)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>262 (20%)</td>
<td>345 (23%)</td>
<td>573 (37%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1,324</td>
<td>1,528</td>
<td>1,564</td>
</tr>
</tbody>
</table>

**THE MOSQUITO**

- Approximately 430 known species of *Anopheles*
  - only 30-50 transmit malaria in nature
- Successful development of the *Plasmodium sp* in the mosquito (from the "gametocyte" stage to the "sporozoite" stage) depends on several factors ambient temperature and humidity
- Malaria is transmitted among humans by female mosquitoes of the genus *Anopheles*
- Female mosquitoes take blood meals to carry out egg production
THE PATIENT
MULTIPLE SYMPTOMS

- FEVER
- CHILLS
- Sweats
- Headaches
- Nausea and vomiting
- Body aches
- Hepatosplenomegaly

NON-SPECIFIC FLU-LIKE SYMPTOMS
Could be ANYTHING!!
Can mimic gastroenteritis, pneumonia, meningitis, encephalitis, hepatitis

THERAPY

<table>
<thead>
<tr>
<th>PLASMODIUM</th>
<th>REGION</th>
<th>TX</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em></td>
<td>Chloroquine S</td>
<td>Chloroquine</td>
</tr>
<tr>
<td></td>
<td>Chloroquine R</td>
<td>Quinine Sulfate + doxy/tet/clinda</td>
</tr>
<tr>
<td><em>P. malariae</em></td>
<td>All</td>
<td>Chloroquine</td>
</tr>
<tr>
<td><em>P. vivax/ovale</em></td>
<td>Chloroquine S</td>
<td>Chloroquine + Primaquine</td>
</tr>
<tr>
<td><em>P. vivax</em></td>
<td>Chloroquine R</td>
<td>Quinine + doxy/tet+ Primaquine</td>
</tr>
</tbody>
</table>

SEVERE MALARIA

All
Quinidine gluconate + doxy/tet/clinda
INVESTIGATIONAL
Artesunate followed by Atovaquone-proguanil or Doxy or Mefloquine

PROPHYLAXIS

- North American travelers lack immunity to malaria
- Personal protection measures against mosquitoes is as important as anti-malarial drugs
  ✓ DEET, mosquito nets, clothing to cover body
  ✓ Avoid outdoor activity in dusk & dawn
  ✓ Peak biting time

DRUGS
- Primaquine
  ✓ REGIONS WITH *P. vivax* OR *Ovale* PREDOMINATE
- Mefloquine or doxycycline followed by Primaquine 2 wks
  ✓ REGIONS WITH *P. vivax*

MEFLOQUIN RESISTANT MALARIA

LABORATORY DX
MICROBIOLOGY SPECIMENS

BLOOD SMEAR
- LAVENDER TOP TUBE
- THICK & THIN SMEARS
- WRIGHT-GIEMSA STAIN
  ✓ MANUAL IN MICROBIOLOGY
  ✓ AUTOMATED HEMATOLOGY
- 200-300 OIL IMMERSION FIELDS EXAMINED
- ONE SET OF NEGATIVE FILMS WILL NOT RULE OUT MALARIA
- 4-5 ADDITIONAL BLOOD FILMS MUST BE EXAMINED
  ✓ EVERY 6 HR OVER 36 HR

MICROSCOPY
✓ GOLD STANDARD

PCR
- SPECIES-SPECIFIC
- PERFORMED IN REFERENCE LAB/DOH
- NO COMMERCIAL ASSAY AVAILABLE

SEROLOGY - IFA
- DETECTS IgG
- NOT PRACTICAL FOR ROUTINE DX
- POSITIVE FOR > 1 YR
- CANNOT DISTINGUISH ACTIVE FROM PAST INFECTION

NEW FDA-APPROVED RAPID TEST
- Binax NOW Malaria
- FEW DROPS OF WHOLE BLOOD PLACED ON DIPSTICK
- RESULTS AVAILABLE IN 15 MINUTES
- DIFFERENTIATES *P. falciparum* FROM OTHER SPECIES
- MICROSCOPIC CONFIRMATION REQUIRED
THE MALARIA PARASITE: who’s who?

- 4 SPECIES CAN INFECT HUMANS
  - *Plasmodium falciparum*
  - *P. vivax*
  - *P. ovale*
  - *P. malariae*

- *Plasmodium vivax* & *P. ovale*
  - Dormant liver stage parasites ("hypnozoites")
  - Reactivate malaria mths or yrs after the infecting mosquito bite
  - *P. vivax* causes severe anemia, respiratory distress, neurological manifestations

- *Plasmodium malariae*
  - Long-lasting infections
  - Untreated cases persist asymptotically in the human host for yrs, even a lifetime

---

PLASMODIUM KNOWLESI

The Fifth Human Malaria (Monkeys)
Cox-Singh et al. 2008, Clin Infect Dis 46:165-171

- UNTIL RECENTLY, MISDIAGNOSED AS *P. MALARIAE*
- WIDELY DISTRIBUTED ACROSS MALAYSIA BORNEO
  - EXTENDS TO PENINSULAR MALAYSIA – MACAQUES
  - *P. KNOWLESI* REPLICATES EVERY 24 HR
- PATIENTS WHO RESIDE IN OR TRAVEL TO SOUTHEAST ASIA WITH *P. KNOWLESI*
  - HYPERPARASITEMIA DIAGNOSIS BY MICROSCOPY
  - RECEIVE INTENSIVE MANAGEMENT AS APPROPRIATE FOR SEVERE FALCIPARUM MALARIA

---

PLASMODIUM FALCIPARUM

- MOST AGGRESSIVE FORM OF MALARIA
  - UNTREATED CAUSES SEVERE ANEMIA, RENAL FAILURE, PULMONARY EDEMA AND DEATH
  - HIGHEST PARASITEMIA RATE
  - INVADES UP TO 30% ERYTHROCYTES
  - OFTEN NEED TRANSFUSIONS
  - ANEMIA & STICKY RBC
  - PLUGS POST-CAPILLARY VENULES IN KIDNEY, LUNG, BRAIN
  - HEPATOMEGALY & SPLENOMEGALY
  - PRESENCE OF SICKLE CELL ANEMIA TRAIT (HEMOGLOBIN AS) PROTECTIVE

---

LAB ASPECTS

BLOOD COLLECTION

- Draw blood immediately
  - (DO NOT WAIT for periodicity)
  - Finger stick or EDTA anticoagulant tube
  - Prepare thick and thin blood films immediately after blood collection
  - If first set of blood films negative, collect again in 4-6 h
  - MULTIPLE SETS MAY BE REQUIRED TO DEMONSTRATE PARASITES

---

EXAMINATION OF BLOOD FILMS

- What is significance of finding only ring forms on 2 sets of blood films drawn 6 h apart?
  - All life cycle stages (rings, developing trophozoites, early schizonts, late schizonts, mature schizonts, gametocytes) seen on the blood films with *P. vivax*, *P. ovale*, and *P. malariae*
  - Only rings & gametocytes with *P. falciparum*
  - IF 2 SETS OF BLOOD FILMS (COLLECTED 6 H APART) WITH RING FORMS ONLY, THEN PROBABLY *P. FALCIPARUM*

---

THIN BLOOD FILMS

- Advantages
  - RBC morphology easily seen
  - Size comparison of infected RBCs to uninfected RBCs easy
  - Much easier to identify to species level
  - Easier to calculate parasitemia
- Disadvantages
  - Lower sensitivity than thick blood film
  - Low parasitemia may be missed
THICK BLOOD FILMS

- **Advantages**
  - Can examine greater volume of blood
  - See malaria pigment within WBCs better

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

---

THIN FILMS vs THICK FILMS

<table>
<thead>
<tr>
<th>THIN FILMS</th>
<th>THICK FILMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Thin Film Image]</td>
<td>![Thick Film Image]</td>
</tr>
</tbody>
</table>

---

PLASMODIUM STAGES IN PERIPHERAL BLOOD

- **RINGS & TROPHozoITES**
- **SCHIZONTS**
- **GAMETOCYTES**

---

LAB IDENTIFICATION

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>HOST RBC</th>
<th>TROPHS</th>
<th>GAMETOCYTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. vivax</td>
<td>Enlarged &amp; pale</td>
<td>Large rings; Mature trophs; Ameboid form fill entire RBC</td>
<td>Crescent (banana) shaped</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>Normal</td>
<td>Small rings; Multiple rings/RBC</td>
<td>Round to oval; almost fill the RBC</td>
</tr>
<tr>
<td>P. malariae</td>
<td>Normal</td>
<td>Mature trophs: Ribbon/band shaped</td>
<td>Round to oval; almost fill the RBC</td>
</tr>
</tbody>
</table>

---

P. Falciparum Trophozoites

- Multiply infected RBC's
- Rings more common
- Accole forms, chromatin dots

---

Plasmodium Falciparum Gametocytes

- Thin smears
- Thick smear
- Crescent or sausage shaped
**Plasmodium falciparum**

- Note: Appliqué or Accolé forms

**P. vivax trophozoites**

- Enlarged RBC's
- Amoeboid rings
- Schüffner's dots (stippling) after 8-10 h
- Delicate ring, amoeboid trophozoites
- Mature schizont has 12-24 merozoites

**P. vivax gametocytes**

- Round to oval
- May fill the entire RBC (may be distorted)
- Some scattered brown pigment

**Plasmodium vivax**

- Benign tertian malaria
  - Not really “benign” causes fatality
- 48 hr cycle
- Infects young RBCs
- Enlarged RBCs as ring grows
- Schüffner’s dots (stippling) after 8-10 h
- Delicate ring, amoeboid trophozoites
- Mature schizont has 12-24 merozoites

**P. malariae trophozoites**

- Band forms
- Basket form
**Plasmodium Malariae**
- Quartan malaria
- 72 hr cycle (long incubation period)
- Infects old RBCs
- Normal size or slightly smaller RBCs
- No stippling
- Thick ring, large nucleus; band forms
- Mature schizont has 6-12 merozoites

**Plasmodium Ovale**
- 48 hr cycle
- Infect young RBCs
- Enlarged RBCs as ring grows; fimbriated edges; cells often oval
- Schüffner's dots (stippling) immediately
- Smaller ring than *P. vivax*; non ameboid
- Mature schizont has 8 merozoites; rosette form, merozoites around pigment
**MALARIA ON CALL**

HEMATOLOGY RESIDENT REVIEW

THICK SMEAR

THIN SMEAR

POSITIVE

(malaria yes/No)
P.falciparum:Yes/No
Determine % Parasitemia

NEGATIVE
Malaria unlikely
NEW SPECIMEN?

CONFIRM WITH MICROBIOLOGY
POSITIVITY & SPECIATION

---

**YOUR FIRST CASE**

The 24 yr Caucasian female presents to CUMC ED @1 AM with fever, chills, myalgia

PMH
- TRAVEL TO INDIA 2 MTH PRIOR
- 2 WKS AFTER RETURN DEVELOPED FEVER & CHILLS
- DX WITH "MALARIA" BY AN ID FRIEND & TREATED
- REMAINED WELL FOR 1 MTH

PHYSICAL EXAM
- VITAL SIGNS
  - Temperature 38.9° C, pulse 100 bpm, blood pressure 110/60 mm Hg
- SKIN
  - No rash or lesions
- CHEST, HEART & ABDOMEN
  - Normal

---

**PT LAB RESULTS**

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULTS</th>
<th>NORMAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>9.2</td>
<td>5 - 16</td>
</tr>
<tr>
<td>RBC</td>
<td>2.9</td>
<td>3.7 – 5.3</td>
</tr>
<tr>
<td>HGB</td>
<td>8.1</td>
<td>11 - 13</td>
</tr>
<tr>
<td>HCT</td>
<td>26.8</td>
<td>33 - 39</td>
</tr>
<tr>
<td>% Neutrophils</td>
<td>64</td>
<td>165 - 415</td>
</tr>
<tr>
<td>% Lymphs</td>
<td>25</td>
<td>20 - 45</td>
</tr>
<tr>
<td>% Monos</td>
<td>10</td>
<td>40 - 65</td>
</tr>
<tr>
<td>% Eosin</td>
<td>1</td>
<td>2 - 6</td>
</tr>
<tr>
<td>Parasitemia</td>
<td>2%</td>
<td></td>
</tr>
</tbody>
</table>

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**YOUR PATIENT DIAGNOSIS & TREATMENT**

- P.vivax
- TX WITH PRIMAQUINE & CHLOROQUINE

---

**PLASMODIUM COMPARISON**

<table>
<thead>
<tr>
<th>CLINICAL PRESENTATION</th>
<th>P.falciparum</th>
<th>P.vivax</th>
<th>P. malariae</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEVER, CHILLS</td>
<td>Usually continuous</td>
<td>48 hr intervals</td>
<td>72 hr intervals</td>
</tr>
<tr>
<td>HYPNOZOITE</td>
<td>NO</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>ANEMIA</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>PARASITEMIA</td>
<td>May exceed 50%</td>
<td>2 - 5%</td>
<td>2 – 5%</td>
</tr>
<tr>
<td>SEVERITY</td>
<td>YES</td>
<td>YES/NO</td>
<td>NO</td>
</tr>
</tbody>
</table>

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**THE IMITATORS**
DIAGNOSIS???