The Great Filter
Parasitic Disease of the Liver

Professor PL Chiodini
The Hospital for Tropical Diseases
London

- Imported parasitic and infectious diseases
- From any part of the globe
- Tourists, refugees, migrants, business people etc
- 8,000,000 people in London
  - so a window on the world
- Important in sentinel surveillance
Outline

• Liver anatomy, blood supply and drainage
• Routes of parasite entry
• Liver as the definitive, intermediate or dead-end host
• Some key problems
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Three-dimensional structure of a liver lobule

Adams et al. Nature Reviews Immunology 6, 244–251 (March 2006) | doi:10.1038/nri1784
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Routes of Parasite Entry
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Parasite Life Cycle Stages Present in the Liver
Liver hosting an intermediate life cycle stage

- *Plasmodium* pre-erythrocytic schizont
- *P. vivax* and *P. ovale* hypnozoites
- *Schistosoma* maturation and pairing
Liver as the definitive site

- *Fasciola*
- *Clonorchis / Opisthorchis*
- *Capillaria hepatica*
Liver as a dead end stage (1)

- *Echinococcus granulosus* (usually)
- *E. multilocularis*
- *Capillaria hepatica* (usually)
Liver as a dead end stage (2)

- *Entamoeba histolytica*
- *Toxocara* sp. eggs
- *Enterobius* adult females
Generalised parasitic infection involving the liver

- Visceral leishmaniasis
- Asexual stages of malaria
Hepatic complications of parasitic infection elsewhere

- **Systemic**
  - Malaria

- **Gastrointestinal**
  - Amoebic liver abscess
  - Cholangitis or pyogenic abscess secondary to ascariasis
Malaria Life Cycle
www.malaria-reference.co.uk

1. MOSQUITO
   - Sporozoites injected
   - Taken up by the liver
   - Causal prophylaxis acts here
   - All 5 species develop in the liver
   - Vivax and ovale malaria also have a dormant (hypnozoite) stage in the liver
   - Schizont in the liver
   - Parasites released to invade and develop in red blood cells
   - Hypnozoites "awake" and become schizonts (months after exposure)

2. Parasites develop to sporozoite stage in mosquito
   - Some do not multiply but become gametocytes to be taken up by mosquitoes
   - Parthenogenesis in red blood cells leading to clinical illness
   - "Suppressive" prophylaxis acts here

3. Bite prevention
Sporozoite entry to the liver

Plasmodium falciparum
pre-erythrocytic schizont
See Garnham (1966) Malaria Parasites and Other Haemosporidia, Blackwell
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Patterns of Malaria Relapses

Pre-erythrocytic stages and malaria prevention

- Causal versus suppressive prophylaxis
- Understanding hypnozoite reactivation
- Treatment of relapsing malarias
*Plasmodium cynomolgi* as a model for *P. vivax* hypnozoites


- Long term *in vitro* cultivation
- *P. cynomolgi*-infected *Macaca fascicularis* hepatocytes
- Hypnozoites persist and reactivate to form schizonts
Sporozoite entry to the liver

Plasmodium cynomolgi as a model for *P. vivax* hypnozoites


- Potential uses
  - Direct study of drug action on hypnozoites
  - Drugs to induce hypnozoite maturation then treat with a schizonticide ("wake and kill")
  - Evaluate genetic, epigenetic and environmental cues to hypnozoite reactivation
So what does cause hypnozoite activation?

- Stimulus not yet known
- Hypothesis
  

  - Malaria itself (eg. *P. falciparum*)
  - Typhoid fever (probably)
  - Possibly other systemic bacterial infections eg relapsing fever
  - Probably not viral infections
Preventing or Treating Liver Stages

• Primaquine
  – Synthesized in 1946
  – Precise mode of action unknown
    • Mitochondrial metabolism (respiratory chain)
    • Metabolites generate intracellular oxidative potentials
  – Very little resistance recorded
  – Adverse events
    • Methaemoglobinaemia; Haemolysis; Abdominal pain
Drugs for Hypnozoites


Primaquine

Tafenoquine
Tafenoquine

• Longer half life (approx 14 days)
• Under development for
  – Chemoprophylaxis
  – Terminal prophylaxis
  – Radical cure of relapsing malarias
• G6PD deficiency remains an issue
Fasciola hepatica – Life cycle

Image from Carlo Denegri Foundation

Contamination by ingesting metacercariae encysted in water plants (Watercress)

Cercariae in water

Excretion in duodenum and migration to the liver

Adult in biliary ducts

Eggs in faeces

Embryonation in water

Micronidium in water

LIFE CYCLE of Fasciola hepatica

Adapted and redrawn from NCDC

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Global Burden of Fascioliasis


• 91.1 million people at risk
• Infects 2.5 to 17 million people annually
• Sheep and cattle-rearing communities
  – Bolivia
  – Ecuador
  – Egypt
  – Iran
  – Peru
  – Vietnam
Fasciola issues in Europe

• Increase in livestock infection
• Triclabendazole treatment failure
Fasciola hepatica ovum
Fasciola hepatica in Dairy Herds in England and Wales
Fasciola hepatica in Dairy Herds in Germany
Kuerpick et al (2013) Parasitology 140, 1051-1060
Fasciola hepatica in Sheep

See Taylor MA (2012) Vet Parasitol 189, 2-7

- Can infect all grazing animals
- Mainly sheep and cattle
- Most pathogenic in sheep
- More prevalent with high summer rainfall
- If milder winter, acute fluke disease appears much earlier in the year
Fasciola and UK Climate Change


- Increased rainfall in Autumn and Winter
- Warmer average temperatures throughout the year
- 4 week extension of herbage growing season over past 40 years
Triclabendazole-resistant *Fasciola* in UK Sheep


- Two female sheep
- Live, undamaged flukes at necropsy 7 days after a 3\textsuperscript{rd} dose of triclabendazole
Triclabendazole-resistant *Fasciola* in UK Sheep

See Gordon et al (2014) *Veterinary Record* August 11, 159-160

• 6 sheep infected with *F. hepatica* eggs from two naturally infected ewes
• Treated with of triclabendazole at 12 weeks when infection patent; second dose repeated 3 weeks later
• Post-mortem 14 days after second dose showed between 19 and 70 live adult fluke in each animal
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Treatment Failure in Humans (1)

- 70 y old male patient
- Right upper quadrant abdominal pain; 10kg weight loss
- Alk phos 347 (75-140); Eosinophils 3.44
- CT scan suggested liver metastases
- Biopsy: eosinophilic infiltrates; no cancer
Treatment Failure in Humans (2)

- “Lover of wild watercress”
- *Fasciola* eggs in stool
- Fasciola IFAT 1 in 1024 (<1 in 28)
- Two doses of triclabendazole given
Treatment Failure in Humans (3)

• Follow-up one year later
  – Much better
  – Eosinophil count 0.34 (normal)
  – Alkaline phosphatase 103 (normal)
  – Fasciola IFAT 1 in 128 (much improved)
  – **BUT** Fasciola eggs in stool
Bithionol

- No longer obtainable in England for human use
- Dosage regimen
  - 30 to 50 mg/kg po alt die in 2 divided doses to a total of 10 to 15 doses
Alternative Drugs


• Triclabendazole (TCBZ) acts against mature and immature *F. hepatica*
• Albendazole (ABZ) is active against flukes older than 12 weeks
• Some isolates resistant to TCBZ but sensitive to ABZ
• Some isolates resistant to ABZ but sensitive to TCBZ (ABZ drug pressure in the field)
RCT of Artesunate vs Triclabendazole


- Triclabendazole 10 mg/kg
  2 doses, 12 hours apart
- Artesunate 4 mg/kg/d daily for 10 days
Artesunate vs Triclabendazole

<table>
<thead>
<tr>
<th>Endpoint at 3m, ITT</th>
<th>Triclabendazole</th>
<th>Artesunate</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No symptoms</td>
<td>46/50 (92%)</td>
<td>38/50 (76%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Eosinophils &lt;400/μL</td>
<td>21/50 (42%)</td>
<td>8/50 (16%)</td>
<td>0.008</td>
</tr>
<tr>
<td>Improved U/S scan</td>
<td>35/50 (70%)</td>
<td>33/50 (66%)</td>
<td>0.83</td>
</tr>
<tr>
<td>Complete response</td>
<td>18/50 (36%)</td>
<td>5/50 (10%)</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Nitazoxanide vs Placebo

- 50 Adults (40 active Rx)
  Nitazoxanide 500 mg bd for 7 days
- 50 Children (40 active Rx)
  Age 2-3 y 100 mg bd for 7 days
  Age 4-11 y 200 mg bd for 7 days
  Age >12 y 500 mg bd for 7 days
Nitazoxanide vs Placebo

Cure based on egg counts

<table>
<thead>
<tr>
<th>Age group</th>
<th>Nitazoxanide</th>
<th>Placebo</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>18/30 (60%)</td>
<td>1/8 (12.5)</td>
<td>0.042</td>
</tr>
<tr>
<td>Children</td>
<td>14/35 (40%)</td>
<td>0/8</td>
<td>0.038</td>
</tr>
</tbody>
</table>
Experimental Chemotherapy for Fascioliasis

• Triclabendazole plus artesunate

• Triclabendazole plus ivermectin

• Triclabendazole plus verapamil

• Triclabendazole plus ketoconazole
Clonorchis sinensis

© Korean Society of Parasitology

- Geographical distribution:
  - China, Korea, Russia, Taiwan, Hong Kong, and Vietnam
Global Burden of Clonorchiasis


601 million people at risk
Infests 35 million people annually
Liver fluke infection in Asia

© IARC Monographs – 100B
Issues with *Clonorchis*


- Eating raw freshwater fish is well established
- Most infected individuals are asymptomatic
- Humans susceptible to infection, re-infection and super-infection
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• Endemicity-related significant risk factor for cholangiocarcinoma
• Classed as a Group 1 biocarcinogen by International Agency of Cancer Research
• No vaccine in sight
• Tribendimidine reported to have similar cure rate to praziquantel
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Amoebic liver abscess

- Textbook presentation of fever, tender enlarged liver and tender intercostal space far from universal
- Fever may be the only presentation
- Polymorph leucocytosis, raised ESR
- Diagnosis and Rx are straightforward
- The real issue is prevention
Acquired immunity in children to intestinal amoebiasis

- Associated with mucosal IgA response to the carbohydrate recognition domain of the Gal/GalNAc lectin
- Children with this response had 86% fewer new infections over one year

- IgA anti-CRD (+)
- IgA anti-CRD (-)

Children infected (%) vs. Months of observation.

- Gal-lectin based intranasal synthetic peptide vaccine
- Cholera toxin as adjuvant
- 6 vaccinated and 6 control baboons
- 4 immunisations at 7 day intervals
- Challenged with amoebic trophozoites via colonoscopy
90 days follow up

- **6 controls**
  - 250/415 (60.24%) stool samples PCR +ve
  - 4/6 inflammatory colitis; 2 with amoebae seen on histology

- **6 vaccinated**
  - 36/423 (8.51%) stool samples PCR +ve
  - 0/6 with inflammatory colitis or parasite invasion

- Inverse correlation between presence of intestinal anti-peptide IgA antibodies and positive faecal PCR

An alternative adjuvant will be needed (potential neurotoxicity)

- Recombinant LecA protective in mouse model of intestinal amoebiasis
Stanley SL. Vaccines for amoebiasis: barriers and opportunities. *Parasitology*. 2006; 133: S81-S86

- Little or no market in developed countries
- Amoebiasis vaccine must be highly cost-effective in resource-poor settings
- Multivalent anti-diarrhoeal vaccine may be way forward
  - amoebiasis
  - Cryptosporidiosis
  - Shigellosis
  - pathogenic *E.coli et al.*
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## Issues with cystic echinococcosis

Limited choice of drugs

<table>
<thead>
<tr>
<th></th>
<th>1987</th>
<th>2014</th>
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</thead>
<tbody>
<tr>
<td><strong>Hydatid drugs</strong></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>(albendazole/mebendazole; praziquantel)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Antiretrovirals</strong>¹</td>
<td>1</td>
<td>25</td>
</tr>
</tbody>
</table>

1. [http://www.fda.gov/ForConsumers/byAudience/ForPatientAdvocates/HIVandAIDSActivities/ucm118915.htm](http://www.fda.gov/ForConsumers/byAudience/ForPatientAdvocates/HIVandAIDSActivities/ucm118915.htm)
Issues with cystic echinococcosis

How to use the drugs we have

• Single or combination?
• Duration
  – If used alone
  – Pre and post intervention
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Nabarro L, Amin Z, Chiodini PL (2014)
Submitted for publication

Current Management of Cystic Echinococcosis; a Survey of Specialist Practice
Adapted from the WHO IWGE Expert Consensus for the Diagnosis and Treatment of Cystic and Alveolar Echinococcosis in Humans

<table>
<thead>
<tr>
<th>Cyst type</th>
<th>Stage</th>
<th>Image</th>
<th>Imaging features</th>
<th>Expert consensus recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE1</td>
<td>Active</td>
<td><img src="image1.png" alt="Image" /></td>
<td>Unilocular simple cyst, Uniform anechoic content or shifting echoes (snow flake sign)</td>
<td>&lt;5cm albendazole (ABZ) &gt;5cm PAIR* and ABZ</td>
</tr>
<tr>
<td>CE2</td>
<td>Active</td>
<td><img src="image2.png" alt="Image" /></td>
<td>Multivesicular, multiseptate cyst, Daughter cysts partly or totally filling mother cyst, “wheel”, “rosette”, “honeycomb”</td>
<td>Surgery and ABZ Or Other percutaneous procedure ** and ABZ</td>
</tr>
<tr>
<td>CE3a</td>
<td>Transitional</td>
<td><img src="image3.png" alt="Image" /></td>
<td>Anechoic content, Detached laminated membrane floats in cyst (water lily sign)</td>
<td>&lt;5 cm ABZ &gt;5 cm PAIR* and ABZ</td>
</tr>
<tr>
<td>CE3b</td>
<td>Transitional</td>
<td><img src="image4.png" alt="Image" /></td>
<td>Complex mass, Anechoic daughter cysts and echogenic areas of disrupted membranes or degenerating daughter cysts</td>
<td>Surgery and ABZ Or Other percutaneous procedure ** and ABZ</td>
</tr>
<tr>
<td>CE4</td>
<td>Inactive</td>
<td><img src="image5.png" alt="Image" /></td>
<td>Heterogeneous hypoechoic cyst, No daughter cysts, Degenerating membranes may look like a ball of wool sign</td>
<td>Watch and wait</td>
</tr>
<tr>
<td>CE5</td>
<td>Inactive</td>
<td><img src="image6.png" alt="Image" /></td>
<td>Thick calcified wall</td>
<td>Watch and wait</td>
</tr>
</tbody>
</table>
On line Survey

• Five clinical cases from HTD
  – History and examination
  – Serology and other key blood test results
  – Imaging

• Clinicians asked how they would manage each

• 41 replies; 23 countries; 5 continents
  – 46% physicians; 44% surgeons; 10 % other
34 year old M
Abdominal pain
9x9x10cm hepatic cyst
ALT 197, ALP 257, EO 2
Hydatid IgG positive

<table>
<thead>
<tr>
<th></th>
<th>% used</th>
<th>Pre procedure BMZ therapy</th>
<th>Post procedure BMZ therapy</th>
<th>Expert consensus guidance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watch and wait</td>
<td>2.5</td>
<td></td>
<td></td>
<td>Contraindicated (progression)</td>
</tr>
<tr>
<td>Drug therapy alone</td>
<td>0</td>
<td></td>
<td></td>
<td>Contraindicated (failure)</td>
</tr>
<tr>
<td>PAIR</td>
<td>12.5</td>
<td>4d to 6 months</td>
<td>3 weeks to 6 months</td>
<td>Contraindicated (recurrence)</td>
</tr>
<tr>
<td>Other percutaneous intervention (PEVAC)</td>
<td>10</td>
<td>1 week to 1 month</td>
<td>1 month to 6 months</td>
<td>With drug cover (length not specified)</td>
</tr>
<tr>
<td>Surgery</td>
<td>62.5</td>
<td>0-6 months</td>
<td>1 month to 1 year</td>
<td>With drug cover (1 day pre to 1 month post)</td>
</tr>
<tr>
<td>Other</td>
<td>12.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

KEY CONCERN
• PAIR for type 2
76 year old M, multiple co-morbidities
2 previous surgeries for CE.
Previous hepatitis with Albendazole
Now abdominal pain and eosinophilia
MRCP: hepatic hydatid cyst
ERCP: communication between cyst and biliary duct

<table>
<thead>
<tr>
<th>Procedures</th>
<th>% Used</th>
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</thead>
<tbody>
<tr>
<td>Watch and wait</td>
<td>3.6</td>
</tr>
<tr>
<td>Drug therapy alone</td>
<td>10.7</td>
</tr>
<tr>
<td>PAIR</td>
<td>0</td>
</tr>
<tr>
<td>Other percutaneous intervention</td>
<td>14.3</td>
</tr>
<tr>
<td>Surgery</td>
<td>71.5</td>
</tr>
</tbody>
</table>

### Key Points
- Scolicidal agent used by 55%
- Praziquantel as sole agent
- Low dose Albendazole

### Drug Therapy

<table>
<thead>
<tr>
<th>Drug</th>
<th>% Used</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole</td>
<td>48</td>
<td>200mg OD – 400mg BD</td>
</tr>
<tr>
<td>Mebendazole</td>
<td>8</td>
<td>50mg/kg/d</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>12</td>
<td>20-50mg/kg/d</td>
</tr>
<tr>
<td>Albendazole/Praziquantel</td>
<td>8</td>
<td>200mg OD-400mg BD</td>
</tr>
<tr>
<td>Nil</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>
Conclusions

• Areas of concern
  – PAIR - type 2, lung cysts
  – Albendazole
    • Variable doses
    • Unclear length of treatment
    • Interrupted regimens
    • Under treatment in disseminated disease
  – Lack of clarity over praziquantel use
  – Scolicidal agents in biliary fistulae

• Where next?
  – Improved international co-operation
  – More prescriptive guidelines
  – Global hydatid registry collecting prospective data
  – Clinical trials in particular areas
    • Length of albendazole treatment
    • Role of praziquantel
    • Surgery vs PEVAC for type 2/3Bs
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Time to see things from the Liver’s point of view!

- Relatively neglected by malariologists
- Under increasing threat from *Fasciola*
- Treatment of cystic echinococcosis remains unsatisfactory
http://www.htd.org