Having exotic types for dinner – parasitology on your plate

Frits Franssen and Joke van der Giessen, Dutch NRL-P
Having exotic types for dinner – parasitology on your plate
Import of frog legs with exotic parasites
Frits Franssen and Joke van der Giessen*

Case history:
• Import into EU of deep frozen frog legs from Indonesia via Belgium and The Netherlands and further shipment to Croatia
• Croatia: parasitic infestations, container back to Rotterdam
• Request Dutch FSA to NRL-P: investigate presence of parasites and report possible public health threats
• Three batches of 50-150 frog legs

Rana macrodon

export volume 4 million kilos/year
± 80.000.000 frogs

* paper accepted for publication in Infectious Diseases Bulletin (RIVM and Health Services) and Dutch Veterinary Journal
Parasitological examination

- Method: macroscopic and microscopic examination, digestion and molecular typing

- Majority showed macroscopic lesions from which parasites could be isolated

- In total four types of parasites were found: cestode, nematode, trematode, pentastomid
Frog legs from Indonesia: parasite 1

Morphology:

- *Spirometra* sp. (cestode)

DNA isolation for PCR and sequencing
Molecular typing *Spirometra* sp. larvae

12 = Larva 1
13 = Larva 2
14 = Larva 3

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TGAAAATGAGCCACCACAAACCACGTGTCATGCAAAATTTTATCCTCAACACACAAAGCGAGAAGATT
ATACCAGTAACACCACCCCATAGTAAAAACACAAATAAAAGATAAAACCACATCAATAACACAGGCTCA
CGCAACGAAACACGACTATTTAAAATCATATAAAGCCAAGAAAACACCTTTTATACCCTGGGAAACC
CCAAATAATCATGGTCACAGAAGTTAAAGAAACAGCAGTCTCTCAGCTCAGCCACACACAGTAACATG
TGTTGCCCACAAACCACACTACTAAACACACTATAAGAAAACATAGCAAAACAGTAAACCCATAAAAA
CCAAAGGTATCATATGAAACAACCTAATTATCTACTACACATGACCTTACATACAAACACACGGGCAAAA
TCAATACATAAACCTCAGGAT

>**gb|AF096238.2** *Spirometra erinaceieuropaei*
cytochrome c oxidase subunit 1 (COI) gene, Identities = 428/428 (100%), Gaps = 0/428 (0%) Strand=Plus/Minus
Life cycle *Spirometra* sp.
Spirometra mansoni - pathology

 Spirometra mansoni
 larva migrans

รูปที่ 1 ระยะตัวอ่อน sparganum รูปที่ 2 เนื้อเยื่อตับ
(ที่มา: ภาควิชาประสิทธิยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่)

รูปที่ 3 sparganum ในสมองคน รูปที่ 4 sparganum ในตา
Frog legs from Indonesia: parasite 2

Molecular typing

• No PCR product with cestode CO1 primers nor with 5S rRNA nematode primers
• 18S rRNA nematode primers

GCCGCGGATTTGCTGTTTGCATCGTTATGTTTAGAACTAGGGCGTATCTGATCGCCTTACAACACACACACTCGATGAAAGTCATCGGCCAGCAACCGCC
TGTTTCTTGCATCGATCGAAACCAGTCAGCGATCGTCCGACCGACTTACCCCAATTTAGAACAACTGATTTAATGAGCCATTC

TACTTCCGAGTTAAGGCCTCATATGAGTCCTAGCGTATCGTTATTTTCTGCTACTACTCCGCTCGGAGATGAGTTTGCGGC

CTCGTCTCTTGGATGGTAGGAGGTCCCCGATTCTCTCCGATCGGAAATCTGAGTTCCCCTGAGTACCAGTAACCCATCTGTAACACAGCAACGTGAT
GGCGCTTAACACAGCATCGACAGTTAGATAGAGGACATCGTCCGACCGACTTACCCCAATTTAGAACAACTGATTTAATGAGCCATTC
Molecular typing parasite 2

**993 bp stretch**

[Sequence alignment and comparison details]

*Raillietella* sp. of gheeko (91% homology)

DNA sequence of the isolated pentastomid larva and homologies with sequences of *Porocephalus crotali* (EF583871, 99% homology) and *Armillifer agkristodontis* (FJ607339, 98.2% homology).

Porocephalus crotali life cycle
Fig. 28.7 (A) Pentastomiasis with innumerable tiny calcified nymphs of *Armillifer* (*Porocephalus*) *moniliformis* throughout the liver, spleen, mesentry and peritoneal cavity of a 55-year-old Filipino man. Most of these nymphs show the characteristic semilunar or C-shaped outline of this parasite. These were an incidental finding on the intravenous pyelogram study. (B,C) Hundreds more of these calcified nymphs are present within the lungs and pleural cavity of the same patient. (Courtesy of Dr. Howard Steinbach and Radiology).
Frog legs from Indonesia: parasite 3

- Artificial digestion of 2 gram lesion samples of 16 frog legs in Trichromatic 35
- Parasite 3 (fragment): *Gnathostoma* sp.
- Not enough for DNA isolation but unmistakable morphology

Larva *Gnathostoma* reference

Larva, frog legs, close-up

Complete *Gnathostoma* sp. larva
Life cycle Gnathostoma spinigerum

1. Egg hatches and releases L1 larva.
2. Embryonated egg in water.
3. Copepod first intermediate host L1 develops into L2.
4. Second intermediate host L2 develops into L3.
5. Infected second intermediate host ingested by definitive host.
6. Paratenic host
7. L3 develop into adult worms.

Sources: Emerg Infect Dis © 2003 Centers for Disease Control and Prevention (CDC)
Frog legs from Indonesia: parasite 4

• Artificial digestion of 2 gram lesions samples of 45 frog legs in Trichromatic 35

(trematode) metacercaria isolated from frog legs

Reference species

Paragonimus miyazakii

Metagonimus yokogawai

Clonorchis sinensis
Life cycle of two flukes

Life cycle *Opistorchis*

1. Embryonated eggs passed in feces
2. Minicida
3. Sporocysts
4. Rediae
5. Cercariae
6. Exeyst in duodenum
7. Cercariae invade the crustacean and encyst into metacercariae
8. Metacercariae in flesh or skin of fresh water fish are ingested by human host
9. Eggs are ingested by the snail
10. Eggs are swallowed and passed with stool
11. Adults in biliary duct
12. Adults in cystic cavities in lungs
13. Lay eggs which are excreted in sputum

Life cycle *Clonorchis*

1. Unembryonated eggs
2. Embryonated eggs
3. Minicida
4. Sporocysts
5. Rediae
6. Cercariae
7. Metacercariae in flesh or skin of fresh water fish are ingested by human host
8. Eggs are ingested by the snail
9. Eggs are swallowed and passed with stool
Public health implications

- If eaten without cold or heat inactivation: infestation with nematodes, cestodes, trematodes, pentastomids

Gnathostomiasis: An Emerging Imported Disease

David A.J. Moore, Janice McCroddan, Paron Dekumoy, Peter L. Chioldini

Emerg Infect Dis 9(6), 2003. © 2003 Centers for Disease Control and Prevention (CDC)

The Infectivity of Frozen Gnathostoma spinigerum Encysted Larvae in Mice

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Table 1

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Annelids</th>
<th>Nematodes</th>
</tr>
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<tbody>
<tr>
<td>Gnathostoma</td>
<td>Capillaria p</td>
<td>Capillaria p</td>
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<tr>
<td></td>
<td>Opisthorchis</td>
<td>Opisthorchis</td>
</tr>
<tr>
<td></td>
<td>Metagonimus</td>
<td>Metagonimus</td>
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<tr>
<td></td>
<td>Megwardi</td>
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<td></td>
<td>Angiostrongylus</td>
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<td></td>
<td>Schistorna</td>
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<td></td>
<td>Fasciola</td>
<td>Fasciola</td>
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</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Organ (time)</th>
<th>No. of mice</th>
<th>Larvae Infected</th>
<th>Larvae Recovered</th>
<th>Infectivity</th>
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</thead>
<tbody>
<tr>
<td>Eyes and CNS</td>
<td>10</td>
<td>35</td>
<td>0.0</td>
<td>Occasional</td>
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<tr>
<td>Intestine</td>
<td>10</td>
<td>23</td>
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</tr>
<tr>
<td>Nervous</td>
<td>10</td>
<td>24</td>
<td>0.0</td>
<td>CNS</td>
</tr>
<tr>
<td>Skin</td>
<td>10</td>
<td>10</td>
<td>0.0</td>
<td>Skin and CNS</td>
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</tbody>
</table>

* Encysted larvae of G. spinigerum at the age of 6 months, maintained in laboratory rats and mice. The larvae were injected into the flesh of mice, frozen at -2 to -4°C and then fed to clean mice.

The species endemic in Asia is classified as Fasciola gigantica.
Public health implications

• Ingestion of large numbers of dead parasites: possible allergenicity

Rapid Alert System for Food and Feed (RASFF) - Week 11, 2009

12/03/2009 Spain 2009.ANC parasitic infestation (Gymnorhynchus gigas) of chilled ray’s bream (Brama brama) from Morocco product (to be) redispached or destroyed

Oral inoculation with Gymnorhynchus gigas induces anti-parasite anaphylactic antibody production in both mice and rats and adverse reactions in challenge mice

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Several allergens from Anisakis simplex are highly resistant to heat and pepsin treatments

Anisakis simplex Allergy After Eating Chicken Meat

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Brama brama (Brasem)
International action

HACCP: Hazard Analysis Critical Control Points
Conclusions

• NRLs confronted with import of exotic food of animal origin from all over the world

• broad expertise on parasite-host relations and knowledge of food production proces (breeding under QAS or gathering from the wild)

• potential increase of infection risks with changing food habits (short time great heat or low temp long time cooking or no cooking at all)

• Fast and close cooperation between NRLs / CRL advantageous for parasitological identification and insight in import-export routes
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