Why do infectious diseases kill people?

<table>
<thead>
<tr>
<th>Viral disease</th>
<th>Case fatality ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ebola</td>
<td>~50-90%</td>
</tr>
<tr>
<td>H5N1 avian influenza</td>
<td>~60%</td>
</tr>
<tr>
<td>SARS</td>
<td>~10%</td>
</tr>
<tr>
<td>1918 influenza</td>
<td>~4-5%</td>
</tr>
<tr>
<td>seasonal influenza</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>pandemic H1N1 A flu</td>
<td>~0.05%</td>
</tr>
</tbody>
</table>

SARS-CoV-2
The Evolution of virulence

1.-4. Hypotheses assuming only parasite adaptation.
1. Expression of virulence in novel hosts
2. Evolution of virulence in novel hosts
3. Virulence is adaptive: *The trade-off model*
4. The role of multiple infections.

Can we use our knowledge about virulence to tame parasites?

5. Hypotheses assuming within host population variation

**Further aspects of the evolution of virulence**
6. Short-sighted evolution of virulence
7. Virulence and vaccination
8. Virulence and the mode of transmission
9. Condition dependent virulence
Part 1. Expression of virulence in novel hosts

- Virulence in accidental/coincidental infections (zoonosis)
- No parasite evolution yet!
- Evolutionary biology cannot explain/predict this.
- Definition of virulence centers around host fitness
- Can not be managed (but prevented).
1. Expression of virulence in novel hosts

Example: *Echinococcus multilocularis*

Alveolar echinococcosis, 1-3% mortality (80% if untreated).
1. Expression of virulence in novel hosts

Example: *Glugoides intestinalis* in *Daphnia*

Local parasites are locally adapted. They produce most transmission stages.

Novel associations produce less transmission stages.

$P < 0.001$
Local parasites are locally adapted. Virulence in novel associations varies greatly, but is on average lower.
Part 1. Expression of virulence in novel hosts

- Virulence can be accidental/coincidental (zoonosis).
- Not directly linked to adaptive parasite evolution! Thus, evolutionary biology cannot explain/predict this.
- Definition of virulence centers around host fitness
- Cannot be managed (but prevented).
- Establishment of disease is most likely rare, but can become important (SARS-CoV-2, HIV in early days, Ebola, ...)

Rule of thumb: Contact to novel parasites is mostly without harm, but may result in some cases in the expression of high virulence.
Part 2. Evolution of virulence in novel hosts

- Evolutionary biology can help to explain the course of the evolution of disease after disease emergence.
- Evolution may be influenced by treatments/management.
- Important to understand parasite evolution.
- Definition of virulence centers around parasite fitness.
Definitions of virulence
The host and the parasite view

To understand the evolution of virulence (which is typically done from the parasite's perspective) one has to consider those aspects of virulence that are fitness components of host and parasite (e.g. parasite induced host mortality).

Traits indicated with a Greek letter are commonly used in epidemiological models.
Part 2. Evolution of virulence in novel hosts

Example 1: Myxoma virus in rabbits.
Example 2: Tracing parasite evolution from sediment cores
Example 3: Serial passage experiments.
Example 1: Myxoma virus

Australia

Britain
Example 2: Tracing parasite evolution from sediment cores

Layers increase in age from top to bottom.

Photo by Jon Sweetman
Example 2: Tracing parasite evolution from sediment cores

Estimated year:
- 2001
- 1999
- 1997
- 1994
- 1992
- 1991
- 1989
- 1987
- 1984
- 1979
- 1973
- 1964

Pasteuria ramosa

Microsporidium 2
Example 2: Tracing parasite evolution from sediment cores

Parasite evolves higher virulence

| Photo by Jon Sweetman |

| young | old |

<table>
<thead>
<tr>
<th>Spore production (x1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
</tr>
<tr>
<td>Time (from old to young)</td>
</tr>
<tr>
<td>200</td>
</tr>
<tr>
<td>800</td>
</tr>
<tr>
<td>1600</td>
</tr>
<tr>
<td>2200</td>
</tr>
<tr>
<td>2800</td>
</tr>
<tr>
<td>3200</td>
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<tr>
<td>3600</td>
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<tr>
<td>4000</td>
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<td>4400</td>
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<td>4800</td>
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<td>5200</td>
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<tr>
<td>5600</td>
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<tr>
<td>6000</td>
</tr>
<tr>
<td>6400</td>
</tr>
<tr>
<td>6800</td>
</tr>
<tr>
<td>7200</td>
</tr>
<tr>
<td>Time to host castration (days)</td>
</tr>
<tr>
<td>(b)</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>14</td>
</tr>
<tr>
<td>16</td>
</tr>
<tr>
<td>18</td>
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<tr>
<td>20</td>
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<td>22</td>
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<td>24</td>
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<td>26</td>
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<td>28</td>
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<td>30</td>
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<td>32</td>
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<td>34</td>
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<td>36</td>
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<td>38</td>
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<td>40</td>
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<td>42</td>
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<td>44</td>
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<tr>
<td>46</td>
</tr>
<tr>
<td>48</td>
</tr>
<tr>
<td>50</td>
</tr>
<tr>
<td>Fecundity reduction (eggs)</td>
</tr>
<tr>
<td>(c)</td>
</tr>
<tr>
<td>old</td>
</tr>
<tr>
<td>young</td>
</tr>
<tr>
<td>Time (total 25 years)</td>
</tr>
</tbody>
</table>

Note: All parasites were tested in the same host genotype.

Pasteuria ramosa in Daphnia magna
Example 3: Serial passage experiments

- Various parasites (viruses, protozoa, fungi, bacteria, small herbivores) and all groups of host organisms (vertebrates, invertebrates, plants, bacteria) have been used.

- They are a common tool in medical, veterinary, and agricultural sciences. Only recently have they been used in evolutionary biology.

- Typically, hosts are genetically well defined (inbreed lines, clones, cell cultures).

- Ser. passage exp. mimic endless within-host growth. Experimentor warrants transmission.

- Evolved parasites are compared with ancestral parasite lines. Hosts do not evolve.
Example 3: Serial passage experiments

Salmonella typhimurium
(Bacteria)

Trypanosoma brucei
(Protozoa)

Parasite adaptation

VIRULENCE
Example 3: Serial passage experiments

**Salmonella typhimurium** (Bacteria)

- Virulence
- Feeding rates (~corn leaf damage)

**European corn borer** (moth)

- Attenuation
- Percentage dead mice

**Trypanosoma brucei** (Protozoa)

- Dose (log) necessary to kill 50% of mice (LD$_{50}$)

**Theileria annulata** (Protozoa)

- Days to detection of piroplasms in blood of cattle

(Sources: Zelle 1942; Diffley et al. 1987; Guthrie et al. 1974)
Example 3: Serial passage experiments

Poliomyelitis virus

![Graph showing percentage of prostrate or dead Cynomolgus monkeys against passages in cell culture.]

*Macaca fascicularis

(Sabin et al 1954)
Example 3: Serial passage experiments

<table>
<thead>
<tr>
<th>Parasite clade</th>
<th>Increase in fitness</th>
<th>Increase in virulence</th>
<th>Increase in within host growth</th>
<th>Attenuation in the former host (decline of viru.)</th>
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<tr>
<td>RNA Viruses (17)</td>
<td>4 yes</td>
<td>12 yes</td>
<td>7 yes</td>
<td>13 yes/1 no</td>
</tr>
<tr>
<td>DNA viruses (4)</td>
<td>-</td>
<td>3 yes</td>
<td>-</td>
<td>3 yes</td>
</tr>
<tr>
<td>Bacteria (5)</td>
<td>2 yes</td>
<td>1 yes</td>
<td>1 yes</td>
<td>4 yes</td>
</tr>
<tr>
<td>Fungi (4)</td>
<td>1 yes</td>
<td>3 yes</td>
<td>2 yes</td>
<td>4 yes</td>
</tr>
<tr>
<td>Protozoa (9)</td>
<td>1 yes</td>
<td>3 yes/1 no</td>
<td>3 yes</td>
<td>5 yes</td>
</tr>
<tr>
<td>Helminth (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 yes</td>
</tr>
<tr>
<td>Arthropods (3)</td>
<td>1 yes</td>
<td>1 yes</td>
<td>2 yes</td>
<td>2 yes</td>
</tr>
</tbody>
</table>

- Only studies with large transfere numbers (>100) included.
- Not all studies reported on all traits.
<table>
<thead>
<tr>
<th>Trait</th>
<th>Direction of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Trait measured in passaged host:</td>
<td></td>
</tr>
<tr>
<td>Virulence</td>
<td>increase</td>
</tr>
<tr>
<td>Within-host growth rate</td>
<td>increase</td>
</tr>
<tr>
<td>Competitive ability relative to ancestral host</td>
<td>increase</td>
</tr>
<tr>
<td>Transmissibility</td>
<td>increase / decrease</td>
</tr>
<tr>
<td>B) Trait measured in former host:</td>
<td></td>
</tr>
<tr>
<td>Virulence</td>
<td>decrease</td>
</tr>
<tr>
<td>Within-host growth rate</td>
<td>decrease</td>
</tr>
<tr>
<td>Infectivity</td>
<td>decrease</td>
</tr>
<tr>
<td>Clearance rate in vertebrate hosts</td>
<td>increase</td>
</tr>
</tbody>
</table>
Using serial passage experiments to produce vaccines

Extract from “Timeline: The development of vaccines: how the past led to the future” by SA. Plotkin & S.L. Plotkin (Nature Reviews Microbiology):

“In the early years of the twentieth century, it became clear that the passage of organisms in unnatural hosts results in genetic selection for avirulent strains. Thus, the Mycobacterium bovis bacille Calmette–Guérin vaccine was obtained by 230 serial passages of M. bovis over a period of 14 years, on artificial medium containing bile. Albert Calmette and Camille Guérin demonstrated that the resulting mutant protected animals and infants against Mycobacterium tuberculosis, although the basis for protection was unknown.

Filterable agents, which were subsequently called viruses, were also described in the last years of the nineteenth century. At this time, yellow fever was an important problem in Africa, and many scientists sought to attenuate the virus. The yellow fever virus strain 17D was selected from a virulent strain by Max Theiler by serial passage in minced chicken embryo and then in embryonated chicken eggs. The goal was to eliminate neurovirulence, and for animals this was lost between the 89 and 176 passages, but the attenuated virus still elicited neutralizing antibodies that protected monkeys from challenge with a virulent virus. The vaccine made with yellow fever virus strain 17D became a major public health success.”

Polio, measles, mumps and rubella virus vaccine were produce through passage in cell culture or embryonated eggs.
What did we learn about parasite adaptation?

- Novel parasites may show unnecessary virulence.
- Parasites adapt rapidly (Myxoma, Daphnia from sediment cores).
- During parasite adaptation, virulence may go up or down, depending on where it started and where the optimal virulence may be (Myxoma, Daphnia).
- Parasite virulence is linked to within host growth. Within-host evolution drives virulence up (Serial passage).
- Parasites evolve host specific adaptation (attenuation in former host). Antagonistic pleiotrophy constraints parasites to specialize: ‘The Jack-of-all-tops is a Master-of-none’ (Serial passage).
Part 3. Virulence is adaptive: The trade-off model

- Evolutionary biology can help to predict direction of change in virulence.
- Direction and magnitude of change may depend on the environment, which may be influenced by treatment.
- Definition of virulence centers around parasite fitness.

- Do parasite express an optimal level of virulence?
- Which factors influence the optimal level of virulence?
The trade-off model:

Virulence is a necessary consequence of evolution for parasite fitness. Virulence is genetically correlated with other fitness components of the parasite.
Example: Myxoma virus

Does the trade-off model explain the new apparent optimum?
Virus isolates with low virulence are more quickly cleared by the host immune defence.

<table>
<thead>
<tr>
<th>Strain I</th>
<th>Strain III</th>
<th>Strain V</th>
</tr>
</thead>
<tbody>
<tr>
<td>high virulence</td>
<td>intermediate virulence</td>
<td>low virulence</td>
</tr>
</tbody>
</table>

Host recovery rate /day vs. Parasite induced host mortality /day (virulence)
3. Virulence is adaptive: The trade-off model

\[ R_0 = \frac{\beta N}{\alpha + \mu + \rho} \]

- Parasite fitness
- Transmission rate
- Host density
- Parasite induced host mortality (= virulence)
- Host background mortality
- Host recovery rate

- \( \alpha = \alpha \) = alpha
- \( \mu = \mu \) = mu
- \( \rho = \rho \) = rho
Example: Myxoma virus

\[ R_0 \]

virulence, \( \alpha \)
3. Virulence is adaptive: The trade-off model is versatile

Established association

\[ R_0 = \frac{\beta N}{\alpha + \mu + \rho} \]

Possible trade-offs:
- between \( \alpha \) and \( \beta \) (+ corr.)
- between \( \alpha \) and \( \rho \) (- corr.)
- (between \( \rho \) and \( \beta \))

Parasite fitness
Transmission rate
Host density
Host background mortality
Host recovery rate
Parasite induced host mortality (= virulence)

beta, \( \beta \)
alpha, \( \alpha \)
Virulence is adaptive: The trade-off model

Parasite spore production (x 1000) (~beta)

Trade-off between transmission (beta) and virulence (alpha)

Time until host death (days) (~1/alpha)
Established association

3. Virulence is adaptive:
The trade-off model makes predictions

\[ R_0 = \frac{\beta N}{\alpha + \mu + \rho} \]

- Transmission rate
- Host density
- Parasite fitness
- Parasite induced host mortality (= virulence)
- Host background mortality
- Host recovery rate

Graph showing the relationship between \( R_0 \) and virulence, \( \alpha \), for \( \mu = 0.1 \) and \( \mu = 0.2 \).
Virulence is adaptive: The trade-off model

Monarch butterflies and the parasite *Ophryocystis elektroscirrha*

Parasite-infected monarch stuck to its pupal case

de Roode, et al. PNAS 2008
Relationships between parasite replication, virulence, and transmission

a, b: high spore load results in high virulence

c-e: high spore load leads to high rates of vertical transmission

f, g: high spore load leads to high rates of horizontal transmission

de Roode J C et al. PNAS 2008
Relationship between parasite replication and lifetime fitness.
Evidence for an optimal level of virulence

![Graphs showing the relationship between parasite replication and lifetime fitness.](image)
Experiments with malaria and mice

Transmission success (Gametocytes)

Transmission success is highest at an intermediate level of virulence.

Virulence (Anemia)

Mackinnon & Read (2002)
Part 4. The role of multiple infections

Competition of parasites within the same host is generally believed to favor mutants with higher competitive ability. This is often mediated by enhanced growth rates, which may correlate with a higher virulence.
Part 4. The role of multiple infections

The tragedy of the commons:

The tragedy of the commons is a dilemma arising from the situation in which multiple individuals, acting independently and rationally consulting their own self-interest, will ultimately deplete a shared limited resource, even when it is clear that it is not in anyone's long-term interest for this to happen. This dilemma was first described in an influential article titled "The Tragedy of the Commons", written by ecologist Garrett Hardin (Science, 1968).

4. The role of multiple infections

Multiple infections lead to competition among parasite genotypes within a host. The fastest replicating genotypes cause the most negative effect for the host and potentially outcompete the other genotypes. Thus, faster replicating and more virulent parasites have an advantage under conditions of frequent multiple infections. This results in the evolution of levels of virulence which are higher than predicted by the trade-off model for the evolution of virulence.

Multiple infections have been suggested to be the single most important factor for the evolution of high levels of virulence.
Multiple infections of the same parasite

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Pathogen/parasite</th>
<th>% multiple infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoa [8]</td>
<td>Blastocystis sp.</td>
<td>2.6%</td>
</tr>
<tr>
<td></td>
<td>Cryptosporidium parvum</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Plasmodium falciparum</td>
<td>19 - 83%</td>
</tr>
<tr>
<td></td>
<td>Plasmodium malariae</td>
<td>18 - 71%</td>
</tr>
<tr>
<td></td>
<td>Plasmodium vivax</td>
<td>6 - 65%</td>
</tr>
<tr>
<td></td>
<td>Trypanosoma brucei</td>
<td>9 - 43%</td>
</tr>
<tr>
<td></td>
<td>Trypanosoma cruzi</td>
<td>5 - 41%</td>
</tr>
<tr>
<td></td>
<td>Toxoplasma gondii</td>
<td>1 - 22%</td>
</tr>
<tr>
<td>Helminths [3]</td>
<td>Ascaris sp.</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Schistosoma haematobium</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Schistosoma mansoni</td>
<td>12 - 54%</td>
</tr>
<tr>
<td>Bacteria [10]</td>
<td>Borrelia burgdorferi</td>
<td>6 - 50%</td>
</tr>
<tr>
<td></td>
<td>Chlamydia trachomatis</td>
<td>41%</td>
</tr>
<tr>
<td></td>
<td>Escherichia coli</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Haemophilus influenzae</td>
<td>53%</td>
</tr>
<tr>
<td></td>
<td>Helicobacter pylori</td>
<td>13 - 24%</td>
</tr>
<tr>
<td></td>
<td>Mycobacterium tuberculosis</td>
<td>8 - 19%</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>9.5%</td>
</tr>
<tr>
<td></td>
<td>Streptococcus pneumoniae</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>Yersinia enterocolitica</td>
<td>-</td>
</tr>
<tr>
<td>Fungi [1]</td>
<td>Cryptococcus neoformans</td>
<td>-</td>
</tr>
<tr>
<td>Viruses [6]</td>
<td>Dengue virus</td>
<td>0 - 11%</td>
</tr>
<tr>
<td></td>
<td>Epstein-Barr virus</td>
<td>11%</td>
</tr>
<tr>
<td></td>
<td>Hepatitis C virus</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>HIV-1</td>
<td>9 - 15%</td>
</tr>
<tr>
<td></td>
<td>Human TT virus</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Influenza A virus</td>
<td>2%</td>
</tr>
</tbody>
</table>

**Human diseases**

- under-estimates
- multiple infection are frequently found

(Dalmer & Tanner 2011)
## Multiple infections of the same parasite

### Animal diseases

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Pathogen/parasite</th>
<th>% multiple infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoa [8]</td>
<td><em>Crithidia bombi</em></td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td><em>Plasmodium chabaudi</em></td>
<td>23%</td>
</tr>
<tr>
<td></td>
<td><em>Plasmodium mexicanum</em></td>
<td>79%</td>
</tr>
<tr>
<td></td>
<td><em>Plasmodium yoelii</em></td>
<td>9%</td>
</tr>
<tr>
<td></td>
<td><em>Plasmodium vinckei petteri</em></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Theileria annulata</em></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Theileria parva</em></td>
<td>45 - 81%</td>
</tr>
<tr>
<td></td>
<td><em>Trypanosoma congolense</em></td>
<td>30</td>
</tr>
<tr>
<td>Helminths [3]</td>
<td><em>Diplostomum pseudospathtaceum</em></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Fascioloides magna</em></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Maritrema novaezealandensis</em></td>
<td>48 - 100%</td>
</tr>
<tr>
<td>Bacteria [2]</td>
<td><em>Wolbachia spp.</em></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Pasteuria ramosa</em></td>
<td>28%</td>
</tr>
<tr>
<td>Viruses [1]</td>
<td><em>African horsesickness virus</em></td>
<td>-</td>
</tr>
</tbody>
</table>

*(Balmer & Tanner 2011)*
Part 3 + 4: Virulence is adaptive

The trade-off model and the role of multiple infections

Established association

Between-host evolution

Transmission rate

Host density

Parasite fitness

$R_0 = \frac{\beta N}{\alpha + \mu + \rho}$

Parasite induced host mortality (= virulence)

Host background mortality

Host recovery rate

maximizes life-time transmission

Within-host evolution

Salmonella typhimurium (Bacteria)

Percentage dead mice

maximizes within-host competition

Most changes in the environment will also affect the rate of multiple infections.
Part 3 + 4: Virulence is adaptive
The trade-off model and the role of multiple infections

- Evolutionary biology can help to predict changes in virulence.
- Direction and magnitude of change may depend on the environment, which may be influenced by treatment.
- Definition of virulence centers around parasite fitness.
- Multiple infections tend to drive virulence up.
- Multiple infections are very common.
- It is unclear if infectious diseases can be managed to become less harmful.
Can we use trade-off models to predict the evolution of virulence?
- There are no good examples of predicted changes in virulence associated with trade-offs.
- The few successful experiments published employed extreme conditions or found a weak response.
- Comparative evidence explains only small portion of the variance in virulence.
- Host mortality may not be important for the parasite (even in cases where a trade-off exists).
- Trade-off maybe multi-dimensional.
- The mechanism might work, but is too slow for virulence management.
- Genetic variation among hosts is ignored.
Part 5. Hypotheses allowing for variation in the host population

Hosts evolve to reduce virulence
Parasite adaptation is specific to host genotypes
=> Virulence is driven by host-parasite co-evolution
Parasite specialization: Serial passage experiments

**Salmonella typhimurium** (Bacteria)

- Percentage dead mice vs. Passages in mice

**European corn borer** (moth)

- Feeding rates vs. Passages on meridic diet

**Trypanosoma brucei** (Protozoa)

- Dose (log) necessary to kill 50% of mice (LD$_{50}$) vs. Passages in mice

**Poliomyelitis virus**

- Percentage prostrate or dead Cynomolgus monkeys vs. Passages in cell culture

- (Sources: Zelle 1942; Diffley et al. 1987; Guthrie et al. 1974; Sabin et al. 1954)
### Parasite specialization: Serial passage experiments

<table>
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<tr>
<th>Parasite clade</th>
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<td>3 yes</td>
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<tr>
<td>Bacteria (5)</td>
<td>2 yes</td>
<td>1 yes</td>
<td>1 yes</td>
<td>4 yes</td>
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<tr>
<td>Fungi (4)</td>
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<td>2 yes</td>
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<td>1 yes</td>
<td>3 yes/1 no</td>
<td>3 yes</td>
<td>5 yes</td>
</tr>
<tr>
<td>Helminth (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 yes</td>
</tr>
<tr>
<td>Arthropods (3)</td>
<td>1 yes</td>
<td>1 yes</td>
<td>2 yes</td>
<td>2 yes</td>
</tr>
</tbody>
</table>

- Only studies with large transfer numbers (>100) included.
- Not all studies reported on all traits.
Parasite specialization: costs of specialization

Schistosoma

Figure 5: Evolution in the parasite: parasite fitness in the intermediate and definitive hosts is negatively correlated. Negative correlation of parasite success in the snail intermediate (measured as the mean lifetime number of cercariae produced) and mouse definitive host (measured as the mean number of miracidia produced) across (a) five inbred lines of Schistosoma mansoni, $P < .001$

Webster et al. 2004. AMERICAN NATURALIST
Part 5. Hypotheses allowing for variation in the host population

- Hosts evolve to reduce virulence
- Parasite adaptation is specific host genotypes
  “The Jack-of-all-traits is a master of none” (the multiple fronts model)
- Virulence maybe driven by host-parasite co-evolution
- It may not be possible to understand the evolution of virulence without considering its interactions with the host.
Further aspects of the evolution of virulence

Part 6. Short-sighted evolution of virulence
Part 7. Virulence and vaccination
Part 8. Virulence and the mode of transmission
Part 9. Condition dependent virulence
Part 6. Short-sighted evolution of virulence

Characters that confer an advantage on an individual organism that express them at a given time in a given habitat will be favored. Whether the expression of those temporally or locally favored characters will increase or reduce the fitness of that organism at other times or in other habitats is irrelevant. Also irrelevant is whether this adaptation puts the entire population at risk. Parasites may evolve within host traits which are beneficial against local competitors, but detrimental for transmission. These mutants have a short term advantage, but a long term disadvantage.

Levin 1996 Emerging Infectious Diseases
6. Short-sighted evolution

**Bacterial meningitis**

- many human hosts are infected
- primarily *Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae*
- but very few manifest the symptoms of these infections
- normally the bacteria reside in the nasopharyngeal passages and are transmitted by droplet infection
- neurologically debilitating and sometimes fatal symptoms of the infection are a consequence of an inflammatory response against the bacteria entering and proliferating in the cerebral spinal fluid.
- bacteria capable of invading and proliferating in the brain could have a local advantage as there are no other bacteria and only modest host defenses
- the brain is a dead end for the parasite; there is no transmission.
6. Short-sighted evolution

Polio

- The poliovirus causes poliomyelitis
- Many hosts become infected, only few develop the disease
- Symptomatic infections are caused by virus invasion of and proliferation in the neurologic tissue of the central nervous system.
- Poliovirus normally replicates in the mucosal cells of the mouth, throat, and intestines and is transmitted by the oral-fecal route.
- Poliovirus virions proliferating in the central nervous system are not transmitted.
6. Short-sighted evolution

HIV

- AIDS is a deadly disease caused by HIV
- virtually every human infected with this retrovirus eventually manifests and succumbs to AIDS.
- Outbreak of AIDS takes many years (often 10 or more), with very strong variation among patients
- Transmission takes place early during infection, hardly after the onset of AIDS.
- In the course of infection, the HIV population undergoes continuous genetic changes. It is hypothesized that AIDS is a consequence of mutation and selection in the HIV population that occurs during the course of the infection in individual hosts.
Part 6. Short-sighted evolution of virulence

1. Adaptive evolution results from selection for immediate benefits
2. Sometimes benefits are detrimental for the entire population, but beneficial for the mutant
3. Short-sighted evolution of virulence describes the phenomenon that some parasites evolve high virulence in hosts, but this benefit is not transmitted to other hosts.
4. Short-sighted evolution is a de novo evolution of virulence
5. S. s. evo. can be explained with evolutionary theory, but it is hard to predict.
Part 7. Evolution in the face of imperfect vaccines

**The problem:**
Evolution is bound to replication and growth. Perfect treatments eradicate the parasite population within the patient, and thus reduce the evolution of resistance to the short phase during which the parasite is exposed and not yet eradicated. Perfect vaccines lead to rapid response of the immune system and fast elimination of the parasite population within the patient.

**Imperfect drugs and vaccines**, do not kill the parasite, but allow it to replicate and offer the possibility for further evolution of the parasite. Parasites may become more virulent or evolve to escape drugs or vaccines.
7. Evolution in the face of imperfect vaccines

**Diptheria toxoid vaccine and the evolution of avirulent *Corynebacterium diphtheriae***

The introduction of diphtheria toxoid vaccine (induces antitoxin immunity) at the beginning of the twentieth century led to a huge reduction in the number of people carrying the virulent form of this pathogen and to the persistence of non-virulent forms of the bacterium. The vaccine acts against strains carrying the toxin.

Diphtheria is caused by a toxin from *Corynebacterium diphtheriae*, which allows this bacterium to obtain nutrients. To produce the toxin the bacteria must carry a viral tox gene (tox+ strain). Toxin production is advantageous, but its production is costly. As the toxin is neutralized in people immunized with diphtheria toxoid (“imperfect vaccine”), its production is disadvantageous for the bacterium. Accordingly, diphtheria (“the disease”) has vanished from areas with long-standing and thorough diphtheria- toxoid vaccination programs, whereas the tox- *C. diphtheriae* strain has persisted, a change that is attributable to selection by the vaccine.

(Soubeyrand & Plotkin, NATURE)
7. Evolution in the face of imperfect vaccines

Virulence of *Plasmodium c. adami* that had undergone 30 passages in naïve mice (derived) with their progenitors (ancestral).

The panels shows the minimum red cell (RBC) densities in sham-(Naive) or AMA-1-vaccinated mice infected with ancestral or derived parasites. Vaccination reduces parasite virulence in ancestral and derived parasites (more anaemia).
7. Evolution in the face of imperfect vaccines

Evo. in vaccinated mice increases virulence in naïve mice

Virulence and density in naïve mice of parasites that had previously been serially passaged 21 times through mice that were sham-vaccinated or AMA-1 vaccinated, together with the progenitor parasites (ancestral). Points are individual mice infected with ancestral parasites (black), Control (Sham)-lines (blue), or Vaccinated-lines (red). Horizontal lines indicate means. Vaccinated-line parasites caused more anemia than the Control-lines and ancestral parasites. The Vaccinated-lines also reached higher total parasite densities than the ancestral parasites.

7. Evolution in the face of imperfect vaccines

Malaria vaccine developers are concerned that antigenic escape will erode vaccine efficacy. Evolutionary theorists have raised the possibility that some types of vaccine could also create conditions favoring the evolution of more virulent pathogens. Such evolution would put unvaccinated people at greater risk of severe disease.

Researchers tested the impact of vaccination with a single highly purified antigen on the malaria parasite *Plasmodium chabaudi* evolving in laboratory mice. The antigen used, AMA-1, is a component of several candidate malaria vaccines. *P. chabaudi* is less virulent in vaccinated mice.

Replicated parasites were then serially passaged through control (Sham-vaccinated) or AMA-1 vaccinated mice and evaluated after 21 rounds of selection. The researchers found no evidence of evolution at the *ama-1* locus. Instead, virulence evolved; AMA-1-selected parasites induced greater anemia in naïve mice than both control and ancestral parasites. These data suggest that recombinant blood stage malaria vaccines can drive the evolution of more virulent malaria parasites.

Barclay et al. 2012 PLoS Biology
Imperfect Vaccination Can Enhance the Transmission of Highly Virulent Pathogens

ABSTRACT. Could some vaccines drive the evolution of more virulent pathogens? Conventional wisdom is that natural selection will remove highly lethal pathogens if host death greatly reduces transmission. Vaccines that keep hosts alive but still allow transmission could thus allow very virulent strains to circulate in a population. Here we show experimentally that immunization of chickens against Marek's disease virus enhances the fitness of more virulent strains, making it possible for hyperpathogenic strains to transmit. Immunity elicited by direct vaccination or by maternal vaccination prolongs host survival but does not prevent infection, viral replication or transmission, thus extending the infectious periods of strains otherwise too lethal to persist. Our data show that anti-disease vaccines that do not prevent transmission can create conditions that promote the emergence of pathogen strains that cause more severe disease in unvaccinated hosts.

Read et al. 2015 PLoS Biology
Imperfect Vaccination Can Enhance the Transmission of Highly Virulent Pathogens

Fig 1. Impact of vaccination on mortality and viral shedding of five strains of MDV. Experiment 1. Groups of 20 Rhode Island Red chickens were unvaccinated (dotted lines, light shading) or HVT-vaccinated (solid lines, dark shading) at 1 d of age and challenged with viral strains HPRS-B14 (black), 571 (purple), 595 (green), Md5 (blue), or 675A (red) 8 d later. Viral strains vary in virulence in unvaccinated hosts, and vaccination protects against death (top panels, with strains arranged in order of increasing virulence from left to right.). Vaccination suppresses the concentration of virus in dust, but by keeping hosts alive, prolongs the infectious periods of hyperpathogenic MDV (middle panels). This means that cumulative number of virus genome copies (VCN) shed per bird is suppressed by vaccination for the least virulent strain and enhanced by several orders of magnitude for the most virulent (bottom panels). Error bars and shaded regions indicate 95% confidence interval (c.i.) Raw data can be found at http://dx.doi.org/10.5061/dryad.4tn48.
Part 8. Virulence and the mode of transmission

Asexual hosts

Symbiont transmission

horizontal
vertical

Sexual hosts

Two examples of symbionts with mixed-mode transmission superimposed on 4-generation pedigrees from asexual and sexual host populations. Vertical and horizontal transmission is indicated by solid and broken lines. Circles represent females, squares represent males. Vertical transmission in the sexual host is both paternal and maternal.
Part 8. Virulence and the mode of transmission

With vertical transmission, the symbiont’s fitness is totally dependent on the host fitness.

With horizontal transmission, the symbiont’s fitness is only weakly dependent on the host's fitness.

With 100% vertical transmission lower virulence is favoured (=> evolution of mutualism).
Algae are transmitted horizontally between adult host generations. Female medusae release planula larvae (a) that disperse and settle as uninfected polyps. Uninfected polyps bud to produce clonal offspring (b) ultimately becoming infected by environmental algal symbionts (c). Infected polyps bud, producing clonal offspring that inherit algae via vertical transmission (d). Once infected, polyps undergo metamorphosis (e). Both medusa and infected polyps release algae into the environment (f) and may be the source of new infections (c). The algal symbiont is mixed-mode transmitted.

Sachs & Wilcox, PNAS, 2005, A shift to parasitism
8. Virulence and the mode of transmission
Experimental evolution with enforced transmission

Figure 2. Experimental design. (a) Vertical treatment (V): buds released from infected polyps are saved in a separate flask, where they settle into infected polyps. After seven weeks of infection, 30 polyps are randomly selected from the newly settled pool. These settled polyps represent the next generation. (b) Horizontal treatment (H): buds are discarded. After seven weeks, infected polyps are put into a new flask with ASW for 48 h of algal expulsion. Thirty isoclonal uninfected polyps are infected with the expelled algae. The newly infected polyps represent the next generation. In both treatments there are two rounds of transmission after initial infection.
Experimental evolution with enforced transmission

*Cassiopea–Symbiodinium* symbiosis

<table>
<thead>
<tr>
<th>fitness measure</th>
<th>treatment</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>polyp growth (mg/day)</td>
<td>horizontal</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>vertical</td>
<td>0.090</td>
</tr>
<tr>
<td>polyp budding rate (buds/day)</td>
<td>horizontal</td>
<td>1.440</td>
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<tr>
<td></td>
<td>vertical</td>
<td>2.235</td>
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<td>algae expulsion</td>
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<td>996.7</td>
</tr>
<tr>
<td></td>
<td>vertical</td>
<td>353.0</td>
</tr>
</tbody>
</table>

For all traits, significant differences were seen among the treatments (p<0.05).
Part 8. Virulence and mode of transmission

With vertical transmission, the symbiont’s fitness is totally dependent on the host fitness. Therefore, lower virulence is favoured (⇒ evolution of mutualism).

Several experimental evolution studies confirmed this idea, using viruses, bacteria or algae as symbionts with vertical and horizontal transmission.

Mixed mode transmission (vertical + horizontal) does only allow to predict virulence if they are traded-off.

![Diagram showing virulence and transmission modes]

<table>
<thead>
<tr>
<th>Vertical Transmission</th>
<th>Horizontal Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

Summing up

100% vertical
100% horizontal
Part 9. Condition-dependent virulence

Virulence may depend on the environment where it is expressed:

- Some bacteriophages are virulent when they transmit horizontally and avirulent when they transmit vertically.
- Trypanosomes infecting bumble-bees and gut microsporidia of Daphnia are more virulent when the host is food stressed.

Virulence may also depend on the host life-stage:

- Many human infectious diseases are less virulent when they infect children (e.g. polio virus).
- Microsporidia infecting mosquito larvae either kill the larvae and transmit horizontally, or allow the host to pupate and to reproduce, which results in vertical transmission.
9. Condition-dependent virulence

Yellow fever mosquito *Aedes aegypti* and the microsporidian parasite *Vavraia culicis*.

Bedhomme et al. (PLoS Biology, 2005) kept infected and uninfected mosquito larvae in the presence of infected or uninfected competitors. Infected focal larvae take longer to develop. This effect is smaller when the competitor is infected. Thus, virulence is higher under conditions of more severe competition. Intraspecific competition exerted by infected individuals is less intense than for uninfected individuals. Longer development increases the pre-adult mortality rate and thus enhances parasite transmission.
9. Condition-dependent virulence
Other parasites influence condition as well
(Multiple infections are more virulent)

Number of parasites per adult *Daphnia* female

Stirnadel & Ebert 1997
The Evolution of disease and its consequences for virulence

- **Accidental association**
  - Harmful, but no benefit for parasite!
  - Evol. theory has little to say here.

- **Parasite adaptation**
  - Parasite adapts to new host!
  - reduction of unnecessary harm
  - rapid evolution
  - specialization

- **Established association**
  - Long lasting association
  - optimal virulence
  - local adaptation

- **Novel association**
  - Parasite transmits!
  - Potential for establishment!
  - Evol. theory has little to say yet. Some rules of thumb apply.

**Summing up**

- **Within host evolution** increases virulence, but may block transmission

- **Coevolution**
  - Hosts may evolve to reduce virulence. Evolution of resistance/immune response.