Ebola and Non Human primates

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Filoviruses and Non Human Primates

- Source of the first Marburg epidemic in 1967 (Hennessen, 1971)
- Source of the first (and only) description of Ebola Ivory Coast in 1994 (LeGuenno et al, 1995)
- In Gabon, three human Ebola outbreaks occurred in 1994 (Minkouka) and 1996 (Mayibout and Booué). All three were concomitant or associated with primates mortality. In Booué, a dead chimpanzee was found positive for Ebola by immunohistochemistry assay. Molecular analysis confirmed independent events, likely originating from different sources.
- Non-human primates are used as model of infection and human disease
Laboratory Testing

- First studies done by IFA found a lot of antibody positive (Ebola and Marburg) primates. Cross reaction with paramyxoviruses?
- During the original Reston episode, ELISA IgG, IgM, and antigen detection were developed
- In animals rapid death without antibody: antigen detection, RT-PCR, or isolation
- Few or no survivors in laboratory setting, unknown in the wild
Reston Virus (1)

• First described in 1989 in Reston, Va following the importation of Cynomolgus monkeys from the Philippines. Contained in the quarantine facility, but clearly propagation from animals to animals and from quarantine room to room due to poor husbandry procedures (Jahrling et al, 1990)
• Rapid euthanasia of all the monkeys in the quarantine facility and disinfection
• Investigation in the Philippines inconclusive
Reston Virus (2)

- Early 1990, importation of cynomolgus from the same supplier. Ebola found again in some animals. Reluctance to euthanized all. At the end, 450/500 animals died (or euthanized) in all but one quarantine room. Place was shut down
- Investigation in the Philippines pointed to a specific source but not to the origin
- New regulations for shipping and importing leading to a better animal survival during transportation
- New surveillance and testing regulation still in place
Reston Virus (3)

- Importation and isolation of Reston virus in 1992 in Sienna, Italy
- Importation of infected animal in Alice, Tx in 1996
- All from same supplier in the Philippines
- Investigation in the one supplier found very poor husbandry practices and lack of records. Facility was finally closed
- Support of other exporting facilities for testing
- Since, several thousand animals tested and none found positive
Ebola’s Impact on Primates

• Gabon and the Republic of Congo alone are thought to hold roughly 80% of the world’s gorillas and most of the common chimpanzees.

• Ape populations in Gabon declined by more than half between 1983 and 2000. Commercial hunting was the primary cause.

• Surveys in some areas showed little sign of hunting pressure.

Ebola’s Impact on Primates (RC)

- From October 2002 to January 2003, 91% (130/143) of the individually known gorillas had disappeared.
- From October 2003 to January 2004, Ebola spread sequentially from north to south, killing 91 of the 95 individuals (95.8%) in the newly monitored groups.
- The onset of ZEBOV deaths in each group was predicted by the number of home ranges separating it from the first group to experience deaths.
- The estimated time lag between deaths in successive groups (11.2 days) was very similar to the typical length of the ZEBOV disease cycle of about 12 days.
- Group-to-group transmission have played a role in amplifying outbreaks.

Ebola’s Impact on Primates (RC)

- Results strongly support the massive dieoff scenario, with gorilla mortality rates of 90 to 95% indicated both by observations on 238 gorillas in known social groups and by nest surveys covering almost 5000 km².
- Nest encounters in the survey done in 2004/05 in the Lossi sanctuary
- Not explained by hunting
- Estimation of 5500 gorilla deaths

Ebola Transmission among Primates

- No observation among infected wild animals
- Tendency of gorillas to closely approach and inspect dying or dead gorillas from same or other groups
- Gorillas from different groups feed in the same trees in close temporal succession
- Gorilla and chimpanzee groups feed simultaneously in the same tree (potential mechanism for cross-species transmission)

Ebola Transmission among Primates

Figure 1: Overlap frequency for gorilla foraging in Nuclea trees surrounding each clearing. A) Time series of days in which two different social units foraged at any Nuclea tree in the clearing on the same day or separated by one or two days. B) Number of days when members of two different social units foraged in exactly the same Nuclea tree on the same day or separated by one or two days. C) Frequency histogram for number of overlaps per social unit.
Ebola spread among Primates

Is it a spread from one area to the other or a multi-emergence of viruses causing larger outbreaks?
Ebola spread among Primates

- Molecular clock-based analysis in program BEAST
- Based on large part of glycoprotein sequence

Ebola spread among Primates

- A previous study focusing on the most divergent region of the GP gene shows that the EBOV isolates obtained from fatalities and survivors during the Kikwit outbreak in 1995 are genetically stable.
- Similarly, there were no nucleotide sequence variations in the GP and nucleoprotein genes among isolates from fatalities, survivors, and asymptptomatically infected individuals during the 7-month Gabon outbreak in 1996–1997.

### Table 1. Nucleotide differences between sequences of the EBOV GP gene identified during Ebola outbreaks in Gabon and the Republic of Congo between October 2001 and May 2003. The table gives the names of identified epidemic chains and reports the number of viral sequences obtained in each chain. See (12).

<table>
<thead>
<tr>
<th>Epidemic chain</th>
<th>Nucleotide position</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>247</td>
</tr>
<tr>
<td>Mendemba A (Oct 01) 7 available sequences</td>
<td>G</td>
</tr>
<tr>
<td>Mendemba B (Oct 01) 10 available sequences</td>
<td>G</td>
</tr>
<tr>
<td>Ekata (Nov 01)</td>
<td>G</td>
</tr>
<tr>
<td>Olloba (Dec 01) 1 available sequence</td>
<td>G</td>
</tr>
<tr>
<td>Etakangaye (Dec 01) 1 available sequence</td>
<td>G</td>
</tr>
<tr>
<td>Makokou (Dec 01) 2 available sequences</td>
<td>G</td>
</tr>
<tr>
<td>Entsiami (Jan 02) 1 available sequence</td>
<td>G</td>
</tr>
<tr>
<td>Olloba (May 02)</td>
<td>C</td>
</tr>
<tr>
<td>Yembelengoye (Dec 02) 8 available sequences</td>
<td>A</td>
</tr>
</tbody>
</table>
Ebola spread among Primates

- Most recent analysis using BEAST on full-length virus sequences from a larger number of isolates dismissed the wave-like spread theory

- Certainly combination of multi-emergence and group-to-group spread of the virus
Animal Surveillance (Gabon-R.Congo)

- Development, testing, and evaluation of an Animal Mortality Monitoring Network (AMMN) in northeastern Gabon and northwestern Republic of Congo designed to alert human and animal health authorities on emerging epidemics
- Alert network set up by the Ministries of Health in hospitals and clinics in the different regions of Gabon and Republic of Congo
- Wildlife organizations: Wildlife Conservation Society (WCS), Programme de Conservation et Utilisation Rationnelle des Ecosystèmes Forestiers en Afrique Centrale (ECOFAC), and World Wildlife Fund (WWF)
- Ministries of Forestry and Environment of the 2 countries.

Animal Surveillance (Gabon-R.Congo)

Fig. 1. (A) Location of sources of Ebola outbreaks in Gabon and the Republic of Congo between October 2001 and May 2003. Stars indicate the location of the first human case, each infected by an animal source. Red and white stars indicate identified or suspected animal sources. (B) Locations of gorillas, chimpanzees, duikers, and ebolavirus in Gabon and Republic of Congo, 2001–2003. EID 2005

species and the presumed contact date with the human index case. The Mekamba and Mbomo outbreaks during October 2001 to May 2002 involved at least six different animal sources. We found at least one animal source in Entsiami during the 2002 outbreak in RC, another animal source in Olloba during the May 2002 outbreak in RC, and at least two additional animal sources in the Kelle area (Yembelegoye and Mvoula villages) during the 2003 outbreak in RC. (B) Location of animal carcasses in Gabon during the 2001 to 2002 outbreak and in RC during the 2002 to 2003 outbreak. Animal carcasses were precisely located by us or local villagers by using global positioning satellite technology.
Surveillance: Temporal Distribution of Carcasses

Animal Surveillance (Gabon-R.Congo)

- Remote areas, raising major logistic problems, requires on-site people.
- Autopsy on high-risk animal carcasses, Carcasses decompose very rapidly; degraded samples increase the false-negative rate. Rapid sampling is therefore crucial for successful diagnosis.
- Identified wild animal outbreaks before human Ebola outbreaks. Alert to the health authorities of Republic of Congo and Gabon to an imminent risk for human outbreaks, after the discovery of carcasses of Ebola virus–infected animals.

Primate Serological Surveillance


Table 2. Seroprevalence rates of Ebola-specific IgG in wild-born nonhuman primates in central Africa.

<table>
<thead>
<tr>
<th>Family, tribe, genus</th>
<th>Cameroong</th>
<th>Gabon</th>
<th>RC</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of animals seropositive/total no. of animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cercopithecidae</td>
<td>1/74</td>
<td>0/33</td>
<td>...</td>
<td>1/107 (0.9)</td>
</tr>
<tr>
<td>Cercopithecinae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cercopithecus</em></td>
<td>1/74</td>
<td>0/33</td>
<td>...</td>
<td>1/107 (0.9)</td>
</tr>
<tr>
<td>Papionini</td>
<td>1/25</td>
<td>...</td>
<td>...</td>
<td>1/25 (4.0)</td>
</tr>
<tr>
<td><em>Papio</em></td>
<td>1/25</td>
<td>...</td>
<td>...</td>
<td>1/25 (4.0)</td>
</tr>
<tr>
<td><em>Mandrillus</em></td>
<td>6/52</td>
<td>0/163</td>
<td>...</td>
<td>6/215 (2.8)</td>
</tr>
<tr>
<td><em>Cercocebus</em></td>
<td>0/13</td>
<td>0/3</td>
<td>...</td>
<td>0/16 (0.0)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>7/90</td>
<td>0/166</td>
<td>...</td>
<td>7/256 (2.7)</td>
</tr>
<tr>
<td>Pongidae</td>
<td>2/17</td>
<td>0/13</td>
<td>...</td>
<td>2/30 (6.7)</td>
</tr>
<tr>
<td><em>Gorilla</em></td>
<td>2/17</td>
<td>0/13</td>
<td>...</td>
<td>2/30 (6.7)</td>
</tr>
<tr>
<td><em>Pan</em></td>
<td>21/119</td>
<td>3/71</td>
<td>5/35</td>
<td>29/225 (12.9)</td>
</tr>
<tr>
<td>Total</td>
<td>31/300</td>
<td>3/283</td>
<td>5/35</td>
<td>39/618 (6.3)</td>
</tr>
</tbody>
</table>

- Wider distribution
- Other species
- Non-lethal infections
- None of 145 captive-born animals positive
- Positive in Gabon 1985, RC 1996, before die-off descriptions
Ebola/Primates Conclusion

- Huge impact of endangered primates (Central Africa)
- Geographic range linked to the real reservoir
- Origin of human death in endemic area with impact on healthcare structure
- Limited risk of exportation in other countries (animal and human)
- Role as sentinel and intermediate vector but not the reservoir