SARS: THE FIRST PANDEMIC OF THE 21ST CENTURY

- Nov, 2002- Feb 2003- SARS outbreaks in Guangdong China; spread to Hong Kong via doctor from China ("Superspreader"), then to Vietnam, Thailand, Singapore, Canada
- Apr 2003- SARS Coronavirus sequenced: Identified as new betacoronavirus (b)
- July 2003- Global SARS epidemic contained: 8,402 cases; 772 deaths in 29 countries

MERS: Emerged one decade latter in different continent

- Apr 2012- MERS coronavirus outbreak in Jordanian hospital
- June 2012- MERS coronavirus isolated and fully sequenced in Sept 2012: Identified as betacoronavirus (c)
- Feb 2014 - ME origin infections--10 countries, 184 cases, 80 deaths to date
Questions Addressed

Why are coronaviruses so diverse and how do they evolve?

• Coronaviruses have a large RNA genome (27-32Kb) and the polymerase has a high error rate
  – Estimated as $10^{-6}$ per nt per replication cycle for SARS CoV

• Coronaviruses occur as quasispecies or swarm of viruses in a population
  – This may explain origin of intraspecies variants with altered tissue tropisms (TGEV/PRCV; FECV/FIPV, etc)

• Coronaviruses have high recombination frequencies (25%) leading to emergence of new zoonotic strains
  – SARS CoV as a mosaic of avian and mammalian ancestors
What is evidence for interspecies transmission of coronaviruses/zoonoses?

- Swine, feline and canine alpha CoVs appear to be host range mutants of common ancestral CoV
  - Share APN receptors
  - Intraspecies variants with altered cell (FECV/FIPV) or tissue (TGEV/PRCV) tropisms and interspecies recombinants occur frequently
  - Constitute a reservoir community with virus persistence and evolution
- Bats are presumed ancestor for alpha CoVs (NL63, 229E, PEDV) and host reservoir for beta CoVs SARS and MERS
## Coronaviruses resulting from interspecies transmission or tissue tropism changes and associated genomic modifications

<table>
<thead>
<tr>
<th>Suspected original CoV/host</th>
<th>New CoV/host or cell tropism</th>
<th>Genomic modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCoV-I/dog and unknown CoV</td>
<td>CCoV-II/dog</td>
<td>Recombinant S gene (Lorusso et al., unpublished)</td>
</tr>
<tr>
<td>CCoV-II/dog</td>
<td>TGEV/pig</td>
<td>ORF3 insertion (Decaro et al., 2007)</td>
</tr>
<tr>
<td>FECoV</td>
<td>FIPV/cat</td>
<td>Substitutions: M and ORF7b genes (Herrewegh et al., 1998; Brown et al., 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intrahost S gene Mutations (Chang et al 2012)</td>
</tr>
<tr>
<td>TGEV/pig</td>
<td>PRCV/pig</td>
<td>621-681-nt deletion in 5’ end of S gene; deletions in ORF3 (Wesley et al., 1991)</td>
</tr>
<tr>
<td>BCoV/cow (HE)</td>
<td>HCoV-OC43 (HE)/human</td>
<td>290-nt deletion (loss of BCoV nsp 4.9 kDa and nsp 4.8 kDa) (Vijgen et al., 2005)</td>
</tr>
<tr>
<td>BCoV/cow (HE)</td>
<td>CRCoV (HE)</td>
<td>8.8kDa nsp (fusion nsp 4.9 and truncated 4.8) (Erles et al., 2007)</td>
</tr>
<tr>
<td>BCoV/cow (HE)</td>
<td>GiCoV (HE)/giraffe</td>
<td>Deletion in the S1 subunit (aa 543-547) of S protein (Hasoksuz/Saif et al., 2007)</td>
</tr>
<tr>
<td>Bat and civet SARS-CoV</td>
<td>SARS-CoV/human</td>
<td>29-nt deletion in ORF8 and substitutions in S gene (K479N, S487T) and ORF3 (Li et al 2005; Qu et al 2005; Sheahan et al 2008)</td>
</tr>
</tbody>
</table>

Many changes were related to S or accessory genes-- CoVs have evolved multiple mechanisms for interspecies transmission.
**Porcine α coronaviruses**

**Intestinal infections**

**TGEV**: *Transmissible Gastroenteritis Virus (1965)*

- **Epidemic**: Infects seronegative pigs; high mortality in piglets
- **Endemic**: Common in US seropositive herds

**PEDV**: *Porcine Epidemic Diarrhea Virus (1978)*

- **Epidemic**: Infects seronegative pigs; highest mortality in piglets; emerged in *US swine in 2013*
  - Closest to China 2012 strains (>99% nt identity)
- **Endemic**: PEDV common in Asia in seropositive herds

**Pathogenesis**: TGEV infects small intestinal enterocytes
PEDV infects the small and large intestinal enterocytes
- Implications for pathogenesis unknown

**Jung et al EID 2014**
Respiratory Infections

**PRCV**: Porcine Respiratory Coronavirus (1986, Europe; 1989, USA)
- S gene deletion mutant of TGEV (621 – 682 bp, N-terminus)
- PRCV infects upper/lower respiratory tract—atypical pneumonia
- PRCV infections in pigs in Europe and Asia have reduced the severity of TGE due to widespread PRCV herd immunity

- PEDV, TGEV and PRCV share APN receptor, yet tissue tropisms differ due to loss of SA binding (gut mucins) by PRCV Spike *(Schultze et al 1996)*
- PRCV induces only partial immunity to enteric TGEV *(Van Cott/Saif 1994)*
- PEDV and TGEV do not induce cross-neutralizing antibodies and do not cross-protect
Transmissible gastroenteritis coronavirus

**Structural Proteins**
- Spike (S)
- Membrane glycoprotein (M)
- Nucleocapsid phosphoprotein (N)
- Small Envelope glycoprotein (E)

**Genome**
- ORF 1a
- ORF 1b
- S
- 3a
- 3b
- E
- M
- N
- 7

**PRCV: Respiratory tropism**
1. Spike gene deletion (621-682nt)
2. Deletions in ORF3

**TGEV: Attenuation-respiratory**
1. S gene deletion S585A in RBD (*Zhang et al*)
2. Deletions in ORF 3; KO 7 (*Otega et al*)

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Emerging CoVs in US Swine 2013- Now

1. **Virulent PEDV** emerged in Apr 2013 as highly fatal diarrheal disease in baby pigs
   - 2 US clades now identified based on complete genome data

2. **S INDEL PEDV variants** reported Feb 2014 (June 2013)
   - Insertions and deletions in S gene
   - Reportedly milder in field; fewer piglet deaths
   - Introduced with virulent PEDV or mutants of US strains?

**Delta CoV**

3. **SDCV** identified in US swine with diarrhea in Feb 2014; disease like PEDV and TGEV but less severe (*Zhang et al 2014*)
   - Reported in swine in HK (HKU15) in 2012 (2009-10) Disease?
   - Prior reports mainly in avian species, but also leopard (ALC)
   - SDCV, sparrow and ALC in same species (*Woo et al JVI 2012*)
# Emerging Porcine Coronaviruses, Target Tissues and Diseases

<table>
<thead>
<tr>
<th>Genus</th>
<th>Virus</th>
<th>Host</th>
<th>Disease/ Infection Site</th>
<th>Year</th>
<th>Year US</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>TGEV</td>
<td>pig</td>
<td>(X)</td>
<td></td>
<td>1946</td>
</tr>
<tr>
<td></td>
<td>PRCV</td>
<td>pig</td>
<td>X</td>
<td></td>
<td>1989</td>
</tr>
<tr>
<td></td>
<td>PEDV</td>
<td>pig</td>
<td>X</td>
<td>2013</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PEDVv S</td>
<td>pig</td>
<td>X (Mild?)</td>
<td>2013</td>
<td></td>
</tr>
<tr>
<td>Beta (a)</td>
<td>HEV</td>
<td>pig</td>
<td>X</td>
<td></td>
<td>1962</td>
</tr>
<tr>
<td>Delta</td>
<td>SDCV</td>
<td>pig</td>
<td>X ?</td>
<td></td>
<td>2014</td>
</tr>
</tbody>
</table>

**CoV Reservoirs:**
- **Alpha CoV**: Bat
- **Delta CoV**: Bird
Unresolved Questions for PEDV and Human CoVs

1. What was host reservoir for PEDV introduction into European swine in 1970’s?
   - Bats are closest PEDV ancestor - role intermediate host?

2. Why did PEDV disappear from Europe in late 1990’s?
   - No PEDV vaccines were used in Europe
   - Why did SARS disappear from China? Bat host still present

3. Why did more virulent PEDV emerge in China in 2010?
   - Did use of live vaccines select for highly transmissible pig host-adapted PEDV?

4. What was origin of PEDV in US swine?
   - Genetically closest to China 2012 strains, \textit{AH2012,CHZMOZY11}
   - Will S INDEL strains moderate PED in the US?

Did PEDV in pigs and SARS in humans disappear because not host-adapted?
Questions Addressed

◆ What is evidence for interspecies transmission of coronaviruses/zoonoses?

• Bovine beta CoVs share sequence identities to CoVs from dromedary camels, captive wild ruminants and a child
  — The latter cross-infect and induce disease and two-way cross-neutralizing and HI antibodies (Tsunemitsu/Saif et al 1995)
  — They are host range mutants of one species that cross-infects and co-circulates in ruminants as reservoir community

• BCoV are projected ancestor (1890) of human CoV OC43 (lack BCoV ORF for ns4.8, ns4.9) based on sequence data (93.5-98% nt ID) (Vijgen et al 2005. J Virol)

What is role of sialic acid binding (S) and HE acquired from influenza C in BCoV pathogenesis and its broad host range?
Do coronaviruses cross the species barrier?

Example: Oral inoculation of calves with enteric CoVs from captive wild ruminants (CWR) or humans (HECV 4408, Zhang et al, 1994 J.Med Virol.)

<table>
<thead>
<tr>
<th>Coronavirus Calf inoculation</th>
<th>Sambar Deer</th>
<th>WT Deer</th>
<th>Waterbuck</th>
<th>Child</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea:</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Fecal shedding:</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Seroconversion:</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Conclusion: Coronaviruses from wild ruminants or humans can experimentally infect young calves (Tsunemitsu/Saif et al.1995; Han/Saif et al 2006)
Many coronaviruses have restricted host ranges, but Bovine, SARS and MERS? Beta-CoVs are promiscuous.

**BCoV infects:**
1. Wild ruminants+
2. Dogs+
3. Occasionally Humans?+

Experimentally:
4. Young turkeys+ but not chicks*

**SARS CoV infects:**
1. Humans+
2. Civet cats*
3. Raccoon dogs*
4. Horseshoe bats*
5. Swine (secondary)*

Experimentally:
6. Non human primates+
7. Ferrets+
8. Cats*
9. Mice*
10. Guinea pigs/Hamsters*

**MERS CoV infects:**
1. Humans+
2. Bats*
3. Camels

Experimentally:
3. Rhesus macaques*

In Vitro: Human, bat, monkey and pig cells

+ =Clinical Disease  *=Subclinical Disease
WHAT FACTORS INFLUENCE INITIAL INTERSPECIES TRANSMISSION OF ENTERIC/RESPIRATORY VIRUSES?

1. Exposure
   • Close contact with infected animals, infectious secretions or materials
     – Wildlife/domestic animal/ human interface
     – Live animal markets (multiple species, unsanitary conditions)
     – Fecal contaminated feed sources? (SDCW)
   • Stability/dose of agent in infectious material
     – Greater efficiency of combined aerosol and fecal/oral transmission? (pneumoenteric viruses – BCoV, SARS, MERS?)

2. Viral attachment/entry: Receptors/Co-receptors
   BCoV has an influenza C-like HA acquired in recombination event
   Role of highly fusogenic Class I fusion S proteins? (Graham & Baric J Virol 2010)

Do viruses (BCoV, Influenza) that bind to sialic acids or have highly fusogenic (Class I) S proteins have unusually broad host ranges?
What is the host animal reservoir for SARS
- Are there multiple animal reservoirs?
  Horseshoe bats (Host reservoir), Civet cats (intermediate host?); Role of domestic cats, rodents, swine? (Guan et al, 2003 Science; Lau et al 2005 PNAS; Li et al 2005 Science)

Bats are flying mammals and a reservoir for zoonotic diseases (rabies, Hendra, Nipah, etc)

Will SARS re-emerge from an animal reservoir?
What is the host animal reservoir for MERS-- Is there a reservoir community: bats, dromedary camels? (sheep, goats negative)

1. Develop MERS CoV specific antibody ELISA tests to screen sera from animal hosts in MERS epidemic and nonepidemic areas.
   - MERS CoV antibodies have been identified in recent and archived camel sera to 1992 (Alagaili et al mBio 2014)

2. Isolate and sequence MERS-like CoV strains from camels in MERS epidemic and nonepidemic areas.
   - Recent reports of MERS-like CoVs in camels detected in nasal swabs

3. Isolate and sequence new CoV strains from wild and domestic animals to understand their diversity and to study their pathogenesis in the natural host.

One Health approach for zoonotic diseases: Multidisciplinary teams and combined efforts of medical and veterinary scientists are essential
Phylogeny of Complete and Partial MERS Genomes
(Rooted using Egyptian Camel)

Human: Cotton et al 2013, 2014
Camel: Chu et al 2014
Alagaili et al 2014
Haagmans et al 2013

http://epidemic.bio.ed.ac.uk/coronavirus_analysis
UNRESOLVED QUESTIONS ABOUT MERS

Epidemiology
• How was MERS transmitted to humans?
• What circumstances promote interspecies transmission?
• Are bats the only persistent animal reservoir?
• Role of camels—intermediate host or reservoir community?
• Are there subclinical infections in humans?
• Why is there enhanced disease severity in people with co-morbidities or the elderly?

Pathogenesis
• Role of co-factors and treatments (co-infections, antibiotics, corticosteroids) in enhancing the severity of MERS?
• Are there superspreading events like for SARS?
• Role of non-respiratory shedding routes (feces, urine) in transmission and pathogenesis in humans and camels?