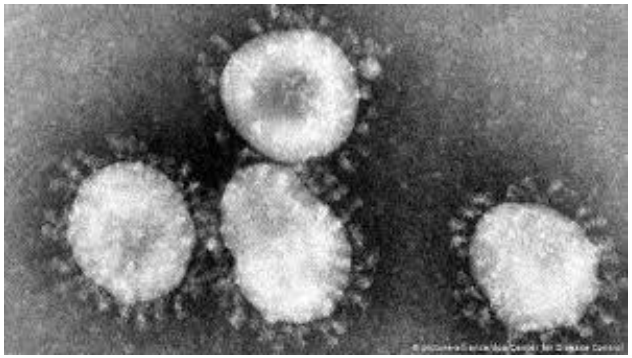
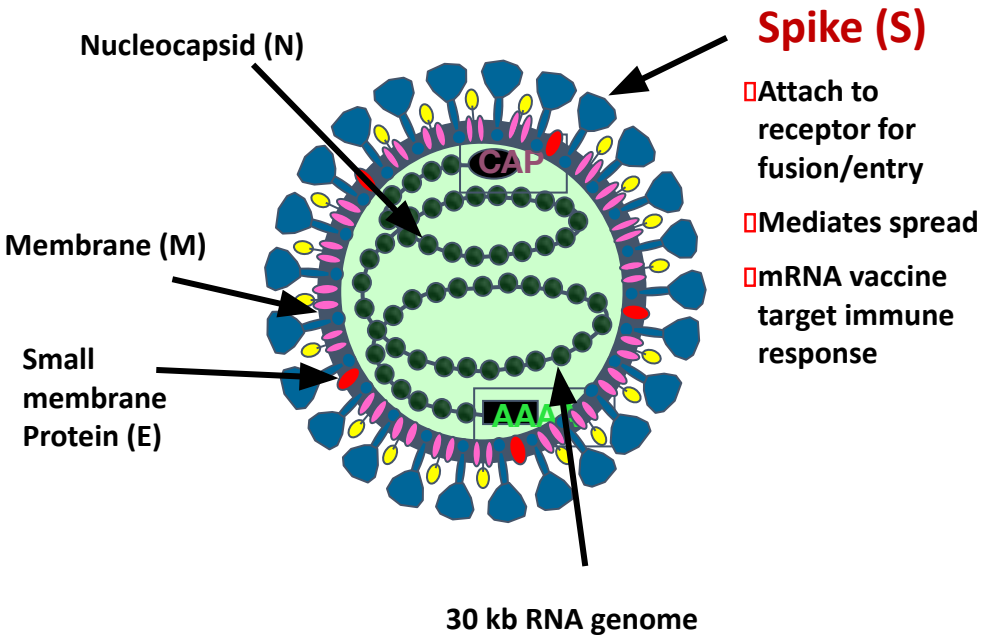
Natural history of coronaviruses, and what they do.

Stanley Perlman, MD, PhD
University of Iowa Distinguished Chair
Department of Microbiology and Immunology
Department of Pediatrics

Coronaviruses

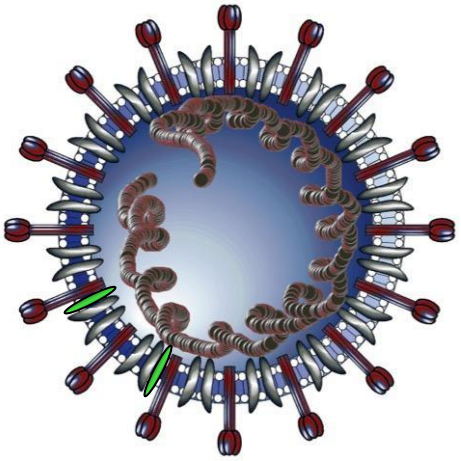




Coronaviruses are a family within the Nidovirus order; named for the nested messenger RNAs generated during infection

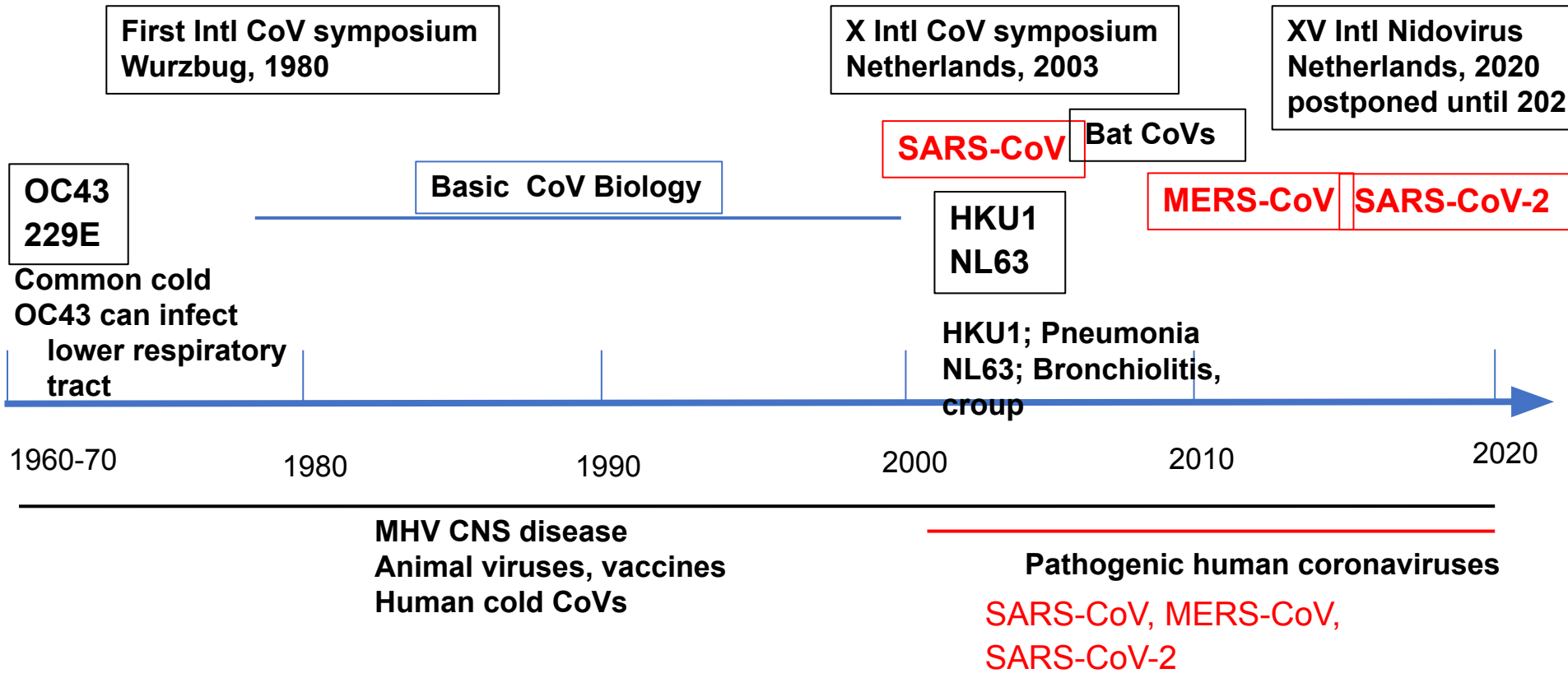
Provided by Dr. Susan Weiss

Coronaviruses

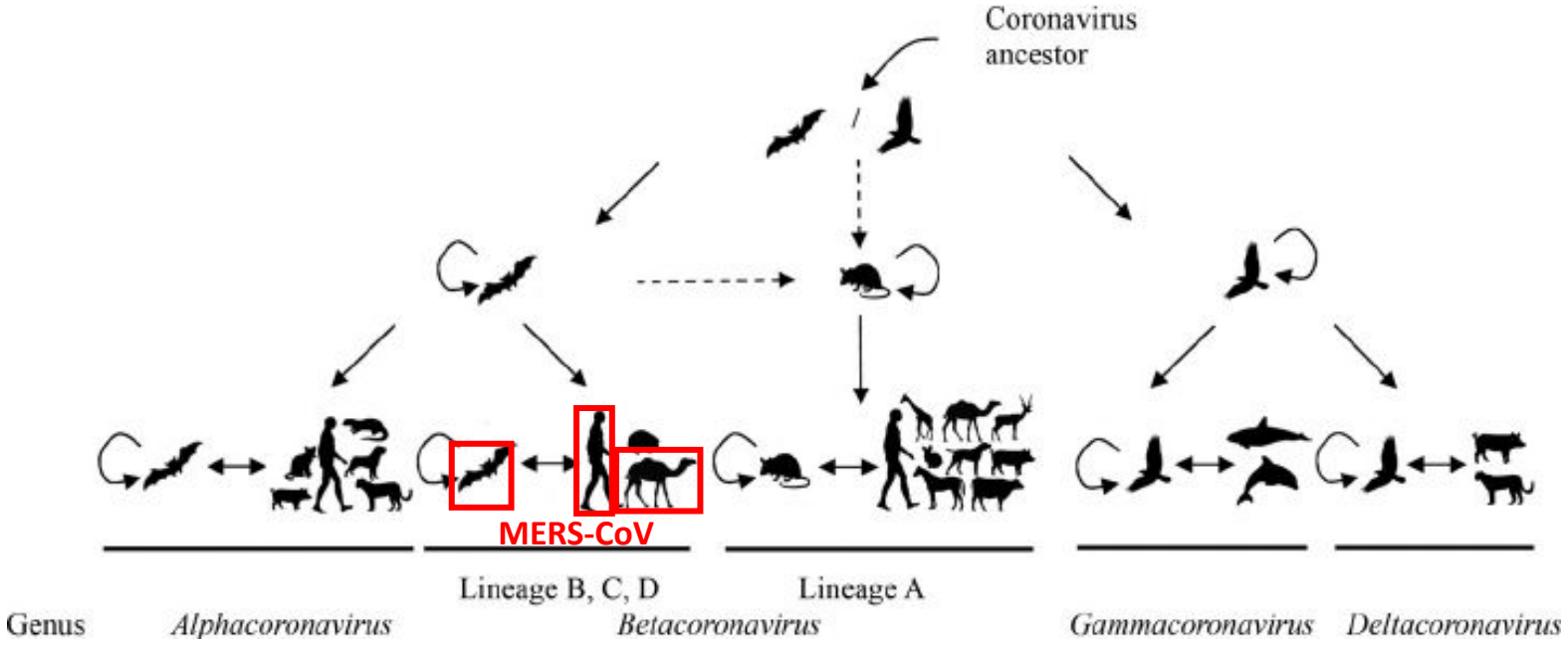


- Enveloped (+) strand RNA viruses
- 31 kB (only other members of Nidovirales are larger)
- Broad diversity across mammalian and avian species.
- Pneumotropic (cows, human), enterotropic (TGEV, PEDV, porcine deltacoronavirus), neurotropic (swine, MHV)
- 7 known human CoVs: SARS-CoV, MERS-CoV, SARS-CoV-2, OC43, 229E, NL63, HKU-1
    - All respiratory viruses

Coronavirus Timeline



Coronaviruses (CoVs) are global zoonotic threats



HCoV-229E - diverged
HCoV-NL63 1000 year ago

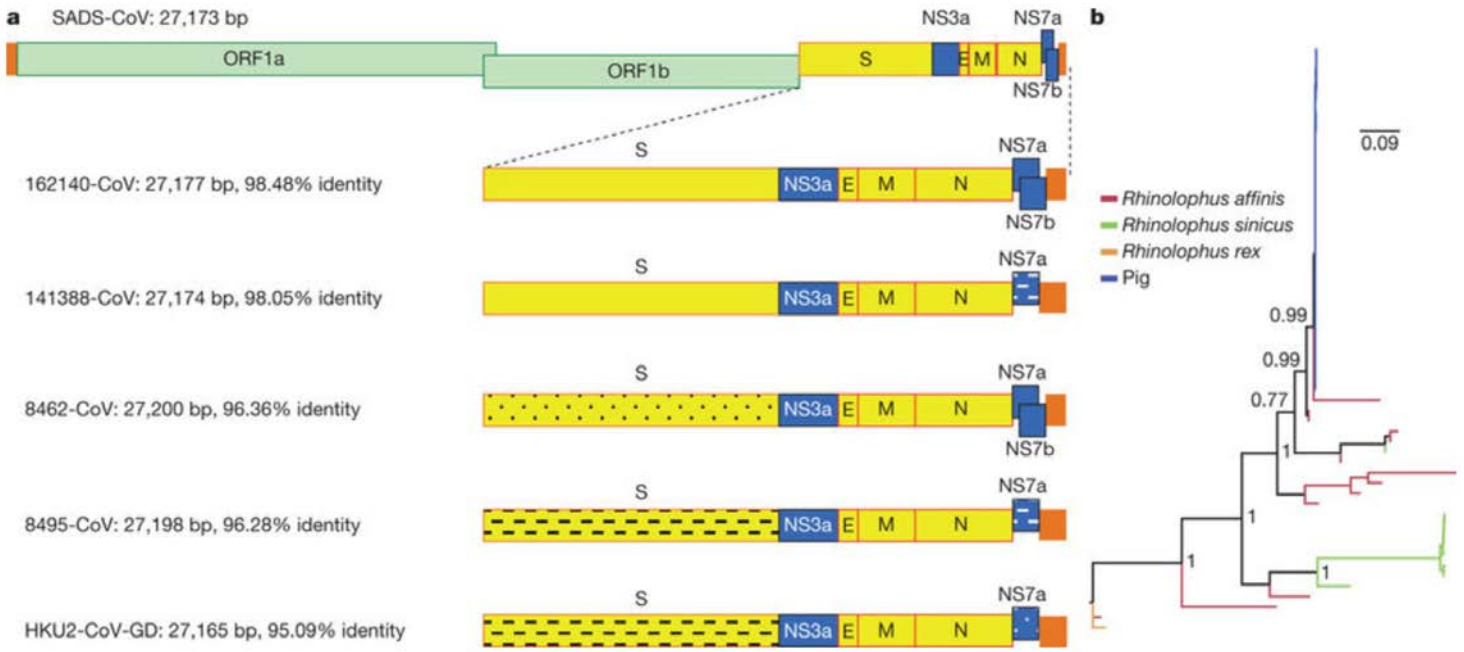
HCoV-OC43 - 120 years infected humans
SARS-CoV - 18 years
MERS-CoV - 9 years

https://www.researchgate.net/figure/Evolution-of-CoVs-from-their-ancestors-in-bat-bird-and-rodent-hosts-to-virus-species_fig4_270343342

Recent human spillovers-2021

- Canine coronavirus spillover – 8 children in Malaysia developed pneumonia. Novel CoV isolated from nasopharyngeal swabs.
  - Backbone-CCoV-II; S1-CCoV-I; S2; FCoV-II.
- Children in Haiti infected with porcine delta coronavirus.
  - RNA found in blood
  - No enteritis or pneumonia.
  - 3 separate introductions into children
  - First delta CoV to infect humans

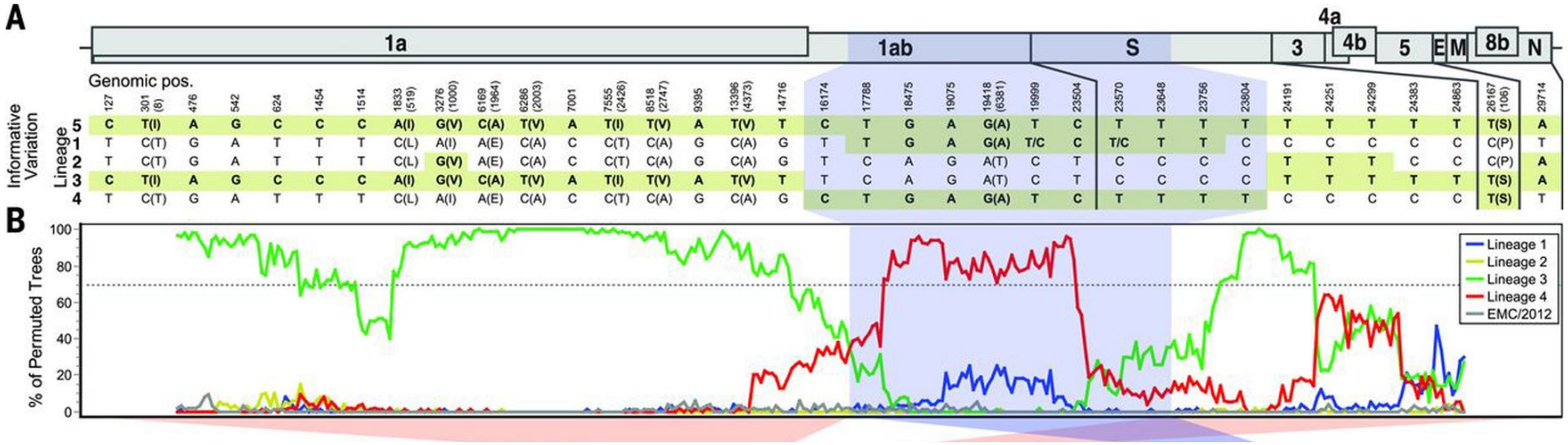
Peng Zhou, Hang Fan, […] Jing-Yun Ma (2018) **Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin** *Nature* **556**:255–258 (2018)



High frequency of CoV Recombination
Lower frequency of mutations than other RNA viruses

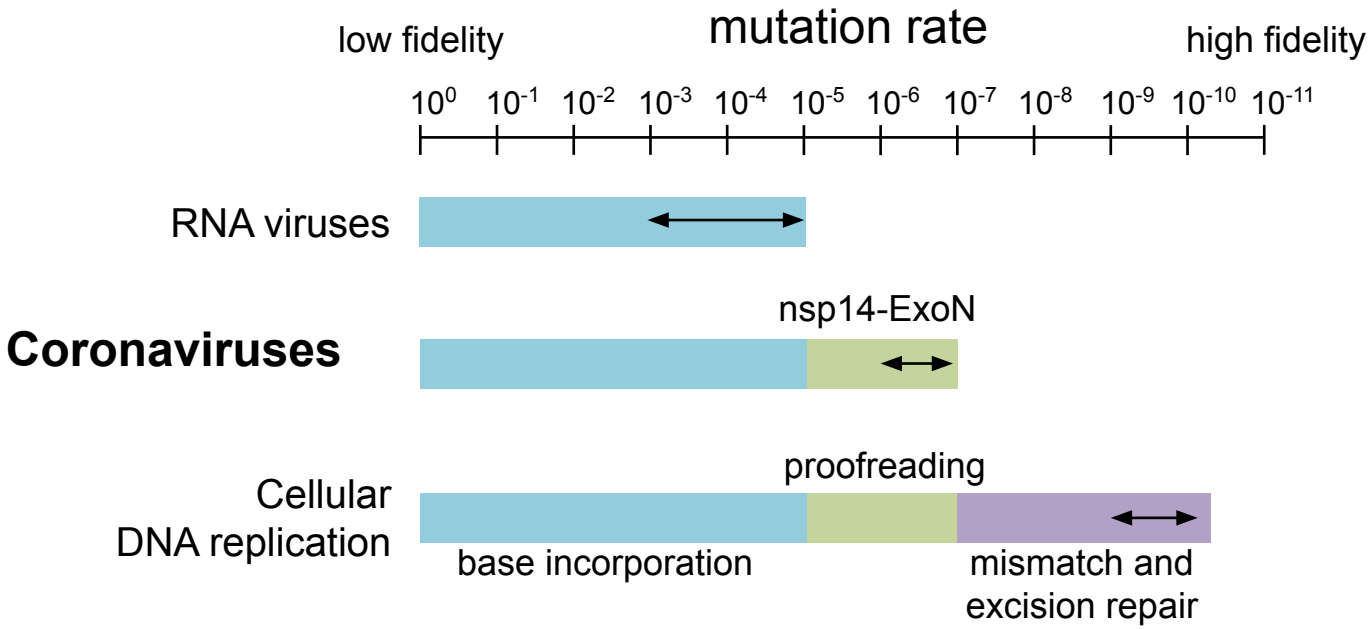
- RNA viruses readily mutate and recombine.
- Unique method of ’leader priming’ facilitates recombination.
- Recombination makes it easier for viruses to cross species.
- In camels, there is evidence for recombination between circulating strains of MERS-CoV.
- Recent work shows that nsp14 is also required for recombination.

High frequency of MERS-CoV Recombination in camels in KSA.



Same recombinant strains are circulating in patients. Are camels reservoir or are they being seeded by virus circulating in another host?

Coronaviruses have higher replication fidelity than other RNA viruses.



Immune protection against CoV-General principles

- In experimentally infected animals, both virus-specific antibody and T cells, in sufficient quantities can mediate virus clearance.
- Both are required for clearance.
- In absence of antibody, virus may recrudesce (brain infection).
- In absence of T cells, virus is never cleared from mice infected with murine coronavirus, SARS-CoV, MERS-CoV.
- If $RAG1^{-/-}$ mice are treated with very small amounts of antibody at time of infection with murine coronavirus, they survive for at least 3-4 months, with high titers of virus present throughout the animal.
  - Performed before RNA sequencing was routine so information about virus mutations not available.
- Supports the notion of immunopathological disease in CoV infections.

Immune protection-Non human CoV

- Vaccines against CoV infections in domestic and companion animals provide imperfect protection.
- Feline coronavirus
  - Mutates to causes feline infectious peritonitis virus (lethal disease)
  - Variable protection
  - **Antibody enhanced infection of macrophages (best (only?) example of ADE in CoV infection**
- Transmissible gastroenteritis virus-usually fatal diarrhea in newborn pigs.
  - Vaccination had variable success, but TGEV no longer a problem.
  - Protective vaccine arose naturally (porcine respiratory virus). Lost ability to infect gastrointestinal tract, but still infected respiratory tract.
  - Shows that live attenuated vaccines results in most effective protection.

Immune protection-infectious bronchitis virus

- First CoV to be identified was IBV (1931, Schalk and Fawn).
  - Cultured in 1937 by Hudson and Beaudette using chicken embryos.
- Caused bronchitis in very young chickens, with high mortality.
  - Death resulted from obstruction of airways.
- Disease in airways is caused by excessive host response to the virus

IBV-vaccine development

- Avian CoV are continuously evolving in nature.
- Live attenuated vaccines are used in chickens
- This requires vaccine modification fairly often.
- Many strains in the wild are now recombinant vaccine strains.

Human Common Cold Coronaviruses (HCoVs)

- 229E, HKU1, NL63, OC43
- Up to 15-30% of human colds
- No durable immunity – frequent cycles of infection
- Upper Respiratory Infections – most common
- Lower Respiratory Infections – Aged and immunocompromised patients
- No vaccines or antivirals licensed or in use

Immune protection-Common Cold CoV

- In non-SARS, non-MERS common cold CoV infections, protection is transient. Waning antibody contributes to susceptibility to reinfection.
- Example:
- 1990 study (Callow et al). 15 volunteers were inoculated with HCoV-229E. 10 with lower antibody titers became infected; 8 developed colds.
- On rechallenge a year later, 9 became reinfected but none developed a cold.
  - Shedding in 6/9 volunteers on reinfection but of shorter duration than observed in primary infection
- Another study tracked common cold CoV infections over 35 years
  - Assayed by serology
  - Reinfections observed 6-105 months after initial infection.
  - Reinfection common after 12 months

Severe Acute Respiratory Syndrome-2002-4

Cases	8437
Deaths	813
Countries	32
Cost	US$20 + Billion

Severe Acute Respiratory Syndrome

- Emerged in late 2002 in Guandong Province, China.
- Wet markets critical in spread to humans.
- Caused by novel coronavirus.
- Animal reservoir found to be bat population in China.
- SARS-like coronaviruses crossed species many times in wet markets.

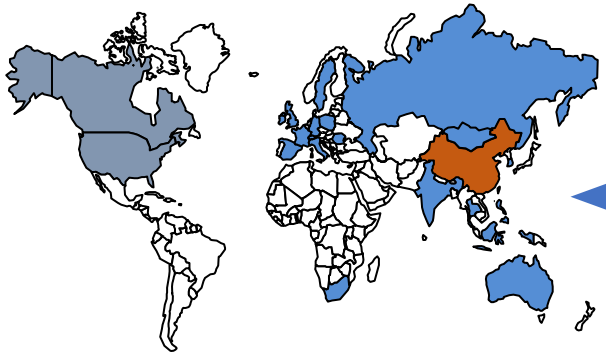
SARS-CoV interspecies transmission (2002)

Horseshoe bat

Civet

Human

Human to human close contact



How many times did this happen?

Ended in eight months,
8098 infections, 9.6% mortality
87% in China and Hong Kong

Images from various internet sites

SARS in East Asia

- Likely transmitted to animal handlers several times since ~35% sero-positivity rate in handlers.
- Worldwide epidemic occurred as result of single set of transmission events, involving a physician in Guangzhou who traveled to Hong Kong.

SARS Epidemiology in Hong Kong

Where the SARS epidemic began

Room 911 is missing

Middle East Respiratory Syndrome

- First documented case was in April 2012.
- Total number of cases: ~2580 (June 2021); 886 deaths (34.4% mortality); majority male; median age ~49 (9m-94y).
  - **About 1 case/day since July 2012**. **14 cases between Jan 1-November 1, 2021 nearly all in Saudi Arabia**
- Infection ranges from asymptomatic to lethal.
- Anti-MERS-CoV antibody response often transitory in mild disease, making epidemiology studies difficult.
  - Increased likelihood of reinfection?
- Human-to-human transmission. **However, at least 50% cases now are primary and not from interhuman contact.**
- Most severe cases are in patients with co-morbidities, including diabetes, chronic lung disease, renal failure, immunocompromised state etc.

MERS-CoV interspecies transmission (2012)

Neoromicia capensis

Camel

Mostly in Arabian peninsula

Camels are a reservoir for MERS-CoV

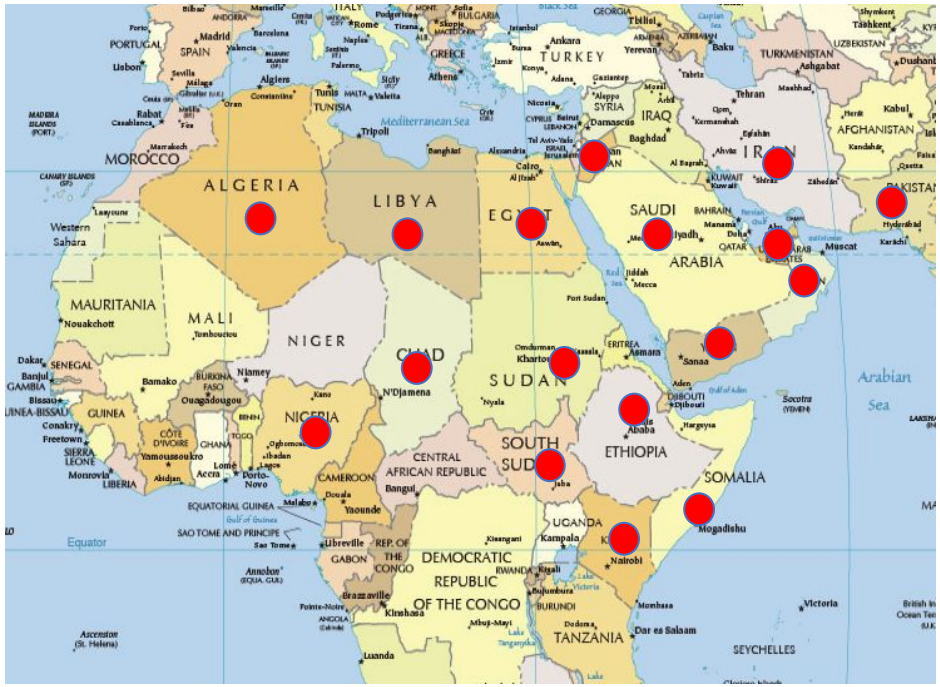
Human

limited human to human spread

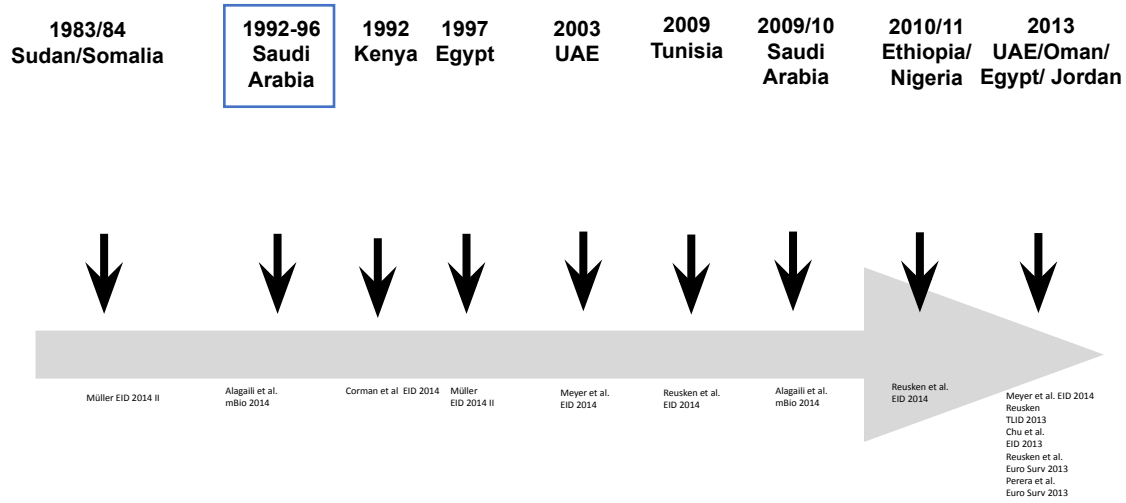
Korea

MERS is a camel disease

Why is there no human MERS in Africa?



Antibodies in dromedaries for at least 30 years



Dr. Christian Drosten

Why did MERS-CoV cross-species only in 2012 and only in the Arabian Peninsula?

Antibody responses in MERS patients

- Neutralizing antibodies critical for protection against rechallenge.
  - Role of mucosal antibody not known but likely important
- Epidemiological studies rely on MERS-CoV antibody measurements.
- In SARS patients, antibody responses were relatively short-lived (not detectable after 6 years).
  - Low levels of SARS-CoV antibody titers persist for 12 years.
  - However, with our Chinese collaborators, we have detected virus-specific neutralizing antibody in 16/18 SARS survivors at 15 years.

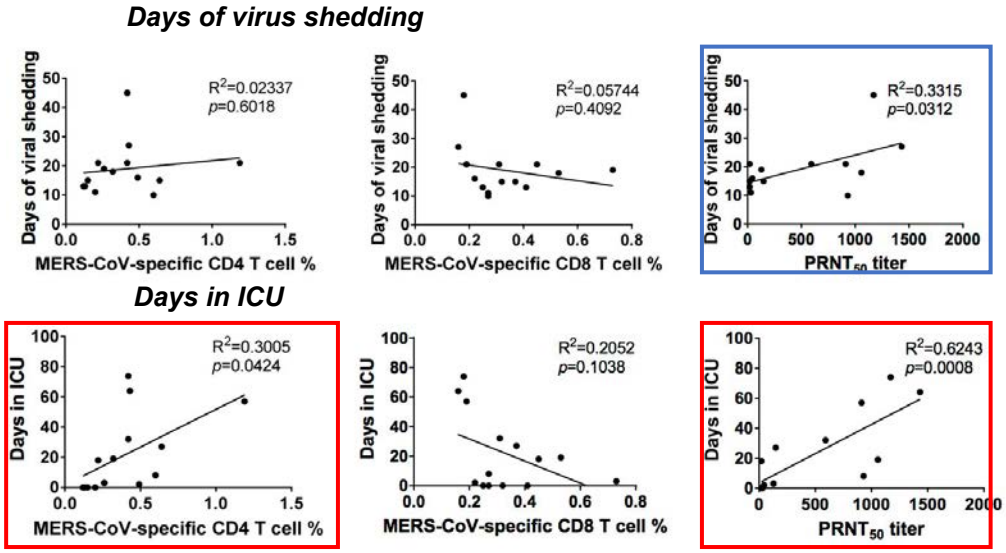
MERS-CoV antibody responses wane rapidly

Patient no.	Age, y/sex	Clinical presentation	PCR, C_t		Serology at 3 mo		Serology at 10 mo		Serology at 18 mo	
			NPS	BAL	ELISA†	IFA‡	ELISA†	IFA‡	ELISA†	IFA‡
1	49/M	Severe pneumonia	28	26	+ (3.17)	+	+ (2.99)	+	+ (3.3)	+
2	33/F	Severe pneumonia	31	26	+ (2.7)	+	+ (2.09)	+	+ (2.9)	+
3	54/F	Pneumonia	34	ND	+ (2.91)	+	+ (1.9)	+	ND	ND
4	40/M	Pneumonia	32	ND	+ (1.29)	+	– (0.65)	–	ND	ND
5	37/M	Pneumonia	35	ND	+ (3.2)	+	+ (1.2)	–	ND	ND
6	36/M	URTI	32	ND	– (0.07)	–	– (0.07)	–	ND	ND
7	27/F	Asymptomatic	33	ND	– (0.046)	–	– (0.04)	–	ND	ND
8	28/F	Asymptomatic	32	ND	– (0.12)	–	– (0.06)	–	ND	ND
9	35/M	Asymptomatic	33	ND	– (0.07)	–	– (0.04)	–	ND	ND

Alshukairi et al, EID, 06/2016

*+, positive; –, negative; BAL, bronchoalveolar lavage; Ct, cycle threshold; IFA, indirect-immunofluorescence assay; MERS-CoV, Middle East respiratory syndrome coronavirus; ND, not done; NPS, nasopharyngeal swab; URTI, upper respiratory tract infection.
†ELISA for MERS-CoV S gene antibody; positive defined as a value > 1.1, negative as <0.8, and borderline as between 0.8 and 1.1.
‡IFA for MERS-CoV IgG; endpoint titers not done.

Relationship between T cell and $PRNT_{50}$ responses and duration of viral shedding and length of ICU stay



T cell responses in MERS patients

- T cell responses detected in all MERS survivors, even in those with low or undetectable antibody titers.
- Length of ICU stay correlates with antibody and CD4 T cell response.
- Robust CD8 T cell responses correlate with fewer days in ICU.

- ***In general, the more severe the illness, the more robust and durable the virus-specific antibody and T cell responses are.***

- **Implications**: Results suggest that we are missing some MERS cases in prevalence studies.

Zhao et al, Science Immunology

Results from three Phase I MERS vaccine studies have been published (ChAd, DNA, Vaccinia virus Ankara)

- Oxford study-ChAdOx1 MERS
- 3 doses tested
- Mild-moderate adverse events.
- 92% ELISA positive at 56 days; 68% positive at one year
- High dose-4/9 developed neutralizing titers (MERS-CoV) at 28 days.
  - Pseudovirus NT assay-79%
- T cell assays-all responded and virus-specific T cells could be detected by 28 days in all recipients.
- T cell response persisted until 1 year.
- **Suggests that vaccination could be protective for at least one year.**

The Present: What have we learned about SARS-CoV-2 that is not new (same as other CoV)?

- CoV readily cross species
- CoV cause disease of variable severity (asymptomatic to death)
- Control of the infection requires coordination of all parts of the immune system (innate and adaptive; memory responses).
- CoV can be controlled by robust neutralizing antibody response
- Host immune responses cause most of the disease manifestations.
- SARS-CoV-2 is most severe in aged individuals and people with co-morbidities-obesity, diabetes, heart disease, pulmonary disease, etc.

The Present: What have we learned about SARS-CoV-2 that is new (not known from other CoV infections)?

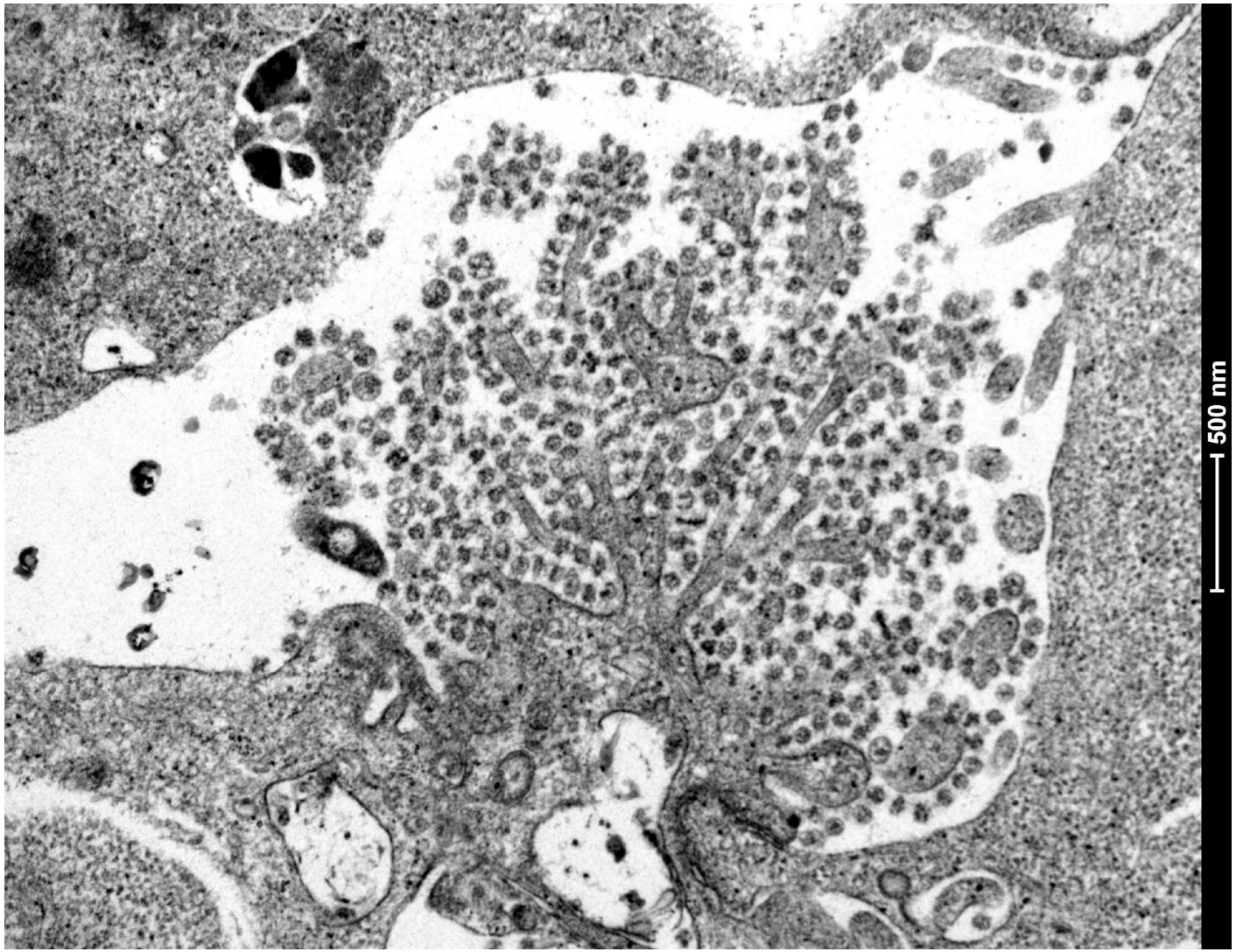
- 1. SARS-CoV-2 is a respiratory tract pathogen with little evidence of virus anywhere else (except the gastrointestinal tract) on autopsy.
  - However, many organs are affected in some survivors (brain, kidney, heart, pancreas).
  - How is disease occurring in these organs?
- 2. Some of these questions can be addressed using animal models.
  - Hamsters, nonhuman primates, minks, cats, dogs, deer are all infectable
- 3. 80% of deer in Iowa (USA) showed evidence of viral RNA in lymph nodes!
  - 36% of deer in Ohio study were positive for virus.
- 4. SARS-CoV-2 is very promiscuous. Most CoV tend to infect single species or group of related species. Bovine CoV, which is similar to HCoV-OC43, is exception.

The Present: What have we learned about SARS-CoV-2 that is new (not known from other CoV infections)? #2

- 5. Goal of virus is to be more transmissible and become dominant in virus population. Virus readily mutates to achieve this goal, even though it encodes proofreading activity
- 6. Factors that enhance transmission include virus intrinsic factors, such as binding to ACE2, as well as others that are not understood.
- 7. Immune evasion may matter increasingly, as most people are vaccinated or naturally infected.
  - Beta variant is immune evasive but never become dominant. Beta variant was detected at times when numbers of immune individuals were low.
  - Omicron variant is immune evasive. However, many of the key mutations were observed in mouse-adapted virus (we reported on BioRxiv in April 2021), so these mutations arose in the absence of immune pressure.

The Present: What have we learned about SARS-CoV-2 that is new (not known from other CoV infections)? #3

- 8. Vaccines are very effective, at least in the short term, and can be readily developed using new (mRNA) technologies.
- 9. For first time, oral anti-viral therapies with activity against a broad array of CoV have been developed.
  - Anti-viral therapies must be delivered early during infection to be efficacious.
- 10. Cocktails of anti-spike antibodies are also effective in preventing disease progression but are also prone to immune escape as virus mutates.
- 11. Anti-viral antibody responses to vaccines and natural infection are quite variable. Boosting increases responses to virus variants.
- 12. Antibody responses waning and waning of vaccine efficacy are more consistent with mucosal rather than systemic infection (where immune responses are expected to be long-lived).
- 13. As pandemic recedes, diagnosing and treating long term effects of COVID-19 (Long COVID-19) will be paramount.



Vero cells infected with SARS-CoV-2

Prof. John Nicholls, HKU