A personal journey through coronavirus evolution

The Sir Arnold Theiler Memorial Lecture
Let me introduce myself

My name is Marian (♂)
Horzinek (ž),
I am a Dutchman by choice…
...a Pole by birth (1936)
became a German by annexation (1939)
lived in the “German Democratic Republic” (1945)
studied in the Federal Republic of Germany (1951)
worked in Venezuela (1967)
and finally emigrated to the Netherlands (1971)
I graduated in veterinary medicine, but (fortunately for the animals) never worked as an animal doctor - I went into science and became a virologist.
...but my professional evolution continued: from a laboratory worker, I became a writer and speaker (oratory)

Virology laboratory, Veterinary University Vienna/Austria, 2010

Pythagoras
Chartres, XII century
In Utrecht, it all started with a disease: Feline Infectious Peritonitis (FIP)

which is fatal in most (clinical) cases
its biology was poorly understood
prevention (still) is difficult
It is an enigmatic disease:
a sporadic fatal viral condition is a contradiction in terms
antibodies are of no benefit for the cat
they may even precipitate disease, causing the 'early death' phenomenon
Clinical signs

extended abdomen
undulating, unresponsive fever
anorexia, emaciation
malaise
ocular/neurologic symptoms, icterus
wet form: polyserositis with effusions
dry form: disseminated pyogranulomas
The discovery (1977): FIP is caused by a coronavirus

Feline Infectious Peritonitis Virus

By

Marian C. Horznike, Albert D. M. Osterhaus and Daniel G. Reynolds

With 6 figures and 3 tables

(Received for publication October 13, 1976)

Introduction

Feline infectious peritonitis (FIP) was described as a new disease entity only ten years ago (9); it is a variably progressive, usually fatal disease affecting domestic and wild Felidae, characterized by fever, depression and ascites. Prominent pathologic changes are peritonitis, mesothelial hyperplasia and focal necrosis in mesentery, mesothelial hyperplasia and focal necrosis in peritoneum, peritonitis, mesothelial hyperplasia and focal necrosis in other tissues. The infectious nature of the disease was initially suspected (10). It was then observed that virus particles were observed in tissue sections from pathologic lesions and that the virus could be grown in cell cultures. The virus was found to be transmitted to new hosts by the inoculation of infected cell cultures. The virus was also observed to be present in the feces of infected cats.

Seroepidemiology of Feline Infectious Peritonitis Virus Infections

Using Transmissible Gastroenteritis Virus as Antigen

By

Albert D. M. E. Osterhaus, Marian C. Horznike and Debby J. Reynolds
The result of this discovery was threefold:

1. We started to work on feline viruses
2. We focused on coronaviruses
3. We became fascinated by viral evolution
following the Darwinian adage:

“...nothing in biology makes sense except in the light of evolution...”

Theodosius Dobzhansky

(1900-1975)
I should like to entertain you about coronavirus evolution

as it leads to new* diseases

in individual animals: pathogenesis (FIP)
in the field: epidemiology (TGEV/PEDV; SARS; MERS)

*new in the sense: hitherto unknown to science
Why coronaviruses?
Because they are the largest enveloped, positive-stranded RNA viruses with the largest viral RNA known to science, and thus: the highest probability of making genetic mistakes (errors - mutations) without a proof-reading mechanism to correct them.
Mutation frequencies

$10^{-9}$  $10^{-8}$  $10^{-7}$  $10^{-6}$  $10^{-5}$  $10^{-4}$  $10^{-3}$  $10^{-2}$  $10^{-1}$

Cellular DNA

RNA virus genomes

Suppression of proofreading/repair activities

Low fidelity polymerases
Anatomy of the coronavirion
The genome of a feline coronavirus

Genes:

S – spike
M – membrane
N – nucleocapsid
1a/b – polymerase
3a-c - nonstructural
7a/b - nonstructural
Evolutionary “behaviour” of coronaviruses

Occupation of new ecological niches through change in tropism (deletions; point mutations; recombinations)

- TGEV – gut to lung
- FIPV – enterocyte to macrophage
- SARS-CoV: “species jumping” civet to human
- MERV-CoV: “species jumping” bat to camel to human
Deletions:

Transmissible Gastroenteritis Virus (TGEV) of swine is found in feces of pigs ≤ 8 wk after recovery but has been isolated from lungs > 3 mo p.i. - meaning virus persistence

1984: a “new” respiratory coronavirus was identified in pigs in Belgium, with ≈700nt deletions in the S gene, but conservation of neutralisation-relevant epitopes

The respiratory variant has displaced the enteropathogenic parent virus in all pig populations thereby acting as a “natural vaccine”
Interspecies transmission and genomic recombination:

CCV = canine coronavirus  
FCoV = feline coronavirus
FCoV type 2 strains originate from a double recombination event.
Feline (enteric) coronavirus infections are widespread - seropositive cats in:
catteries: >90%
single-cat households: <25%
cause FIP only rarely:
in 1 – 5% of the seropositive cats
in the young and the very old
The close phylogenetic relationships between FECV/FIPV pairs in isolates from kitten litter mates
Peritonitis-causing feline coronaviruses

are *in vivo* mutants occurring in individual, persistently infected cats e.g. when cell-mediated immunity is suppressed (such as under “crowding” stress, after FeLV- or FIV- infections)

arise stochastically, under conditions that allow expansion of the so-called “quasispecies cloud”
Manfred Eigen: 1967 Nobel Prize in Chemistry

Statistical geometry in sequence space: A method of quantitative comparative sequence analysis

Biophysics: Eigen et al.  

Fig. 3. The iterative buildup of sequence space, starting with one position. Each additional position requires a doubling of the former diagram and to connect corresponding points in both diagrams (which represent nearest neighbors). The final hypercube of dimension \( \nu \) contains as subspaces \((j)2^{n-k}\) hypercubes of dimension \(k\).
The ‘quasispecies’ concept
The ‘quasispecies’ concept

- **PERFECT REPlication OF WILD TYPE**
- **HIGHLY IMPERFECT REPlication**
- **QUASISPECIES**

○ = FIP variants
Crucial for the FECV – FIPV transition:

The A at nucleotide 23531 was 100% conserved in all 183 FECVs in our collection.

Of the 118 FIPVs, 96 (81.4%) had a T and 12 (10.2%) a C at this position; in both cases, this changes the methionine (M) occurring at position 1058 in the FECV S protein into a leucine (L) in FIPV (i.e., mutation M1058L).
Viruses have crossed the host species barrier time and again, and will forever…
Interspecies transmission

severe acute respiratory syndrome (SARS): palm civet – to man

middle East respiratory syndrome (MERS): African bats, camelids – to man

bat coronavirus: Leschenault's rousettes (*Rousettus leschenaultii*, fruit bats Megachiroptera) - to Pomona leaf-nosed bats (*Hipposideros pomona*, insectivorous, Microchiroptera)
SARS – the first human ‘killer’ coronavirus
Severe Acute Respiratory Syndrome (SARS)
ORDER OF THE CENTERS FOR DISEASE CONTROL AND PREVENTION, DEPARTMENT OF HEALTH AND HUMAN SERVICES
Notice of Embargo of Civets

ACTION: Notice of embargo of civets (Family: Viverridae).

SUMMARY: According to published scientific articles, Severe Acute Respiratory Syndrome (SARS)-like virus has been isolated from civets (Family: Viverridae) captured in areas of China where the 2002–2003 SARS outbreak originated. Shipments of civet are being imported into the United States and further distributed. CDC is banning the importation of all civets immediately and until further notice. CDC is taking this action to prevent the importation and spread of SARS, a communicable disease.

DATE: This embargo is effective on January 13, 2004, and will remain in effect until further notice.
Viruses are “the mistletoe on the tree of life”

They have co-evolved with their hosts and continue to do so.

Each milliliter of ocean water contains several million virus particles – a global total of $10^{30}$ virions! If lined up end to end, they would stretch 200 million light years into space…
Sir Arnold Theiler (1867 – 1936)

is the father of veterinary science in South Africa – studied in Zurich, and in 1891 started practicing as a veterinarian,

developed a vaccine against rinderpest (eradicated in 2011) during the Anglo-Boer War of 1899-1902.

was the first Director of the Onderstepoort Veterinary Research Institute and

first Dean of the University of Pretoria Faculty of Veterinary Science (1920).

His son Max Theiler (1899-1972), was awarded the Nobel Prize in Physiology and Medicine (1951) for the development of a Yellow Fever vaccine.

...and then, about a century later:

A flavivirus story:

father Arnold (1919)

son Max (Nobel 1951)

...it runs in the family...
A personal paraphrase:

“...nothing in virology makes sense except in the light of evolution...”

...also of its history – and the role of its protagonists.
The End