Animal models to study zoonotic infections

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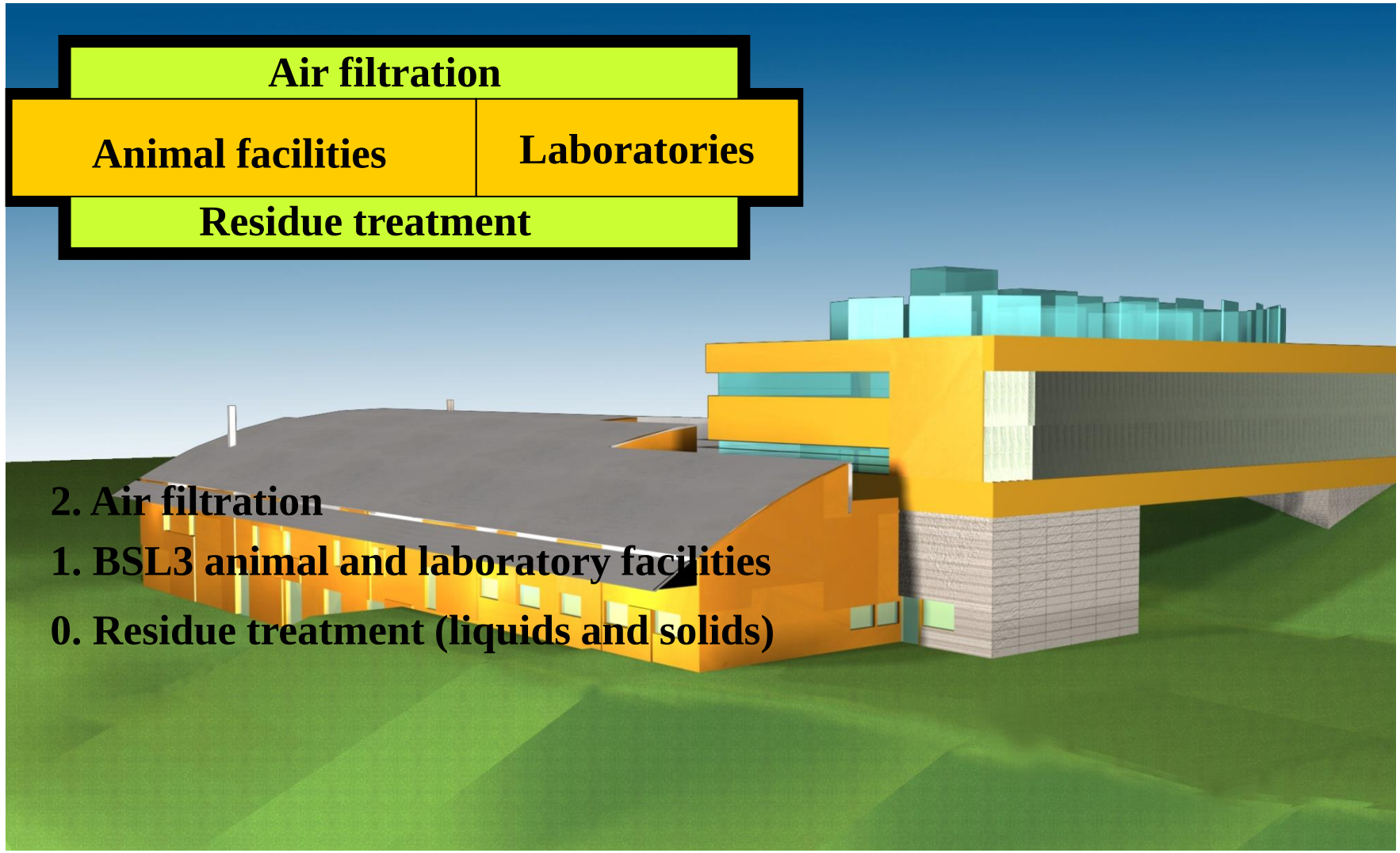
CReSA IRTA RECERCA I TECNOLOGIA AGROALIMENTÀRIES

Biosafety level 3 animal and laboratory facilities

Campus UAB (Universitat Autònoma de Barcelona, Spain)

General design of IRTA-CReSA – “Sandwich system”



Technical details

- Gradient of negative pressure
- Entrance of people
- Protecting people (Personal Protective Equipments)
- Entrance of animals and other materials
- Boxes for animals
- Residue treament
- Air filtration
- ... and many more...

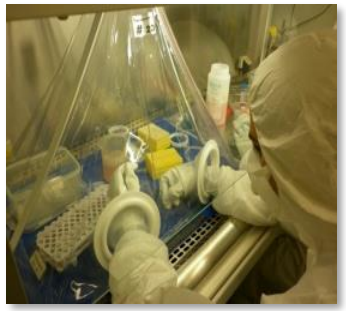





Animal facilities at IRTA-CReSA

Cages for small mammals

Climatic chamber for entomology



Boxes for livestock and wildlife

Animal species we have been working with

- Pig
- Wild boar
- Sheep
- Cattle
- Goat
- Horse (pony)
- Dromedary camel
- Alpaca
- Llama
- Deer
- Chamois
- Ferret
- Golden Syrian hamster
- Mice (different types)
- Rat
- Voles
- Chicken
- Duck
- Turkey
- Pigeon
- Quail
- Falcon
- Partridge
- Goose

Occasionally, plant BSL3 pathogens

… But we are here today to talk about animal models and zoonotic diseases…

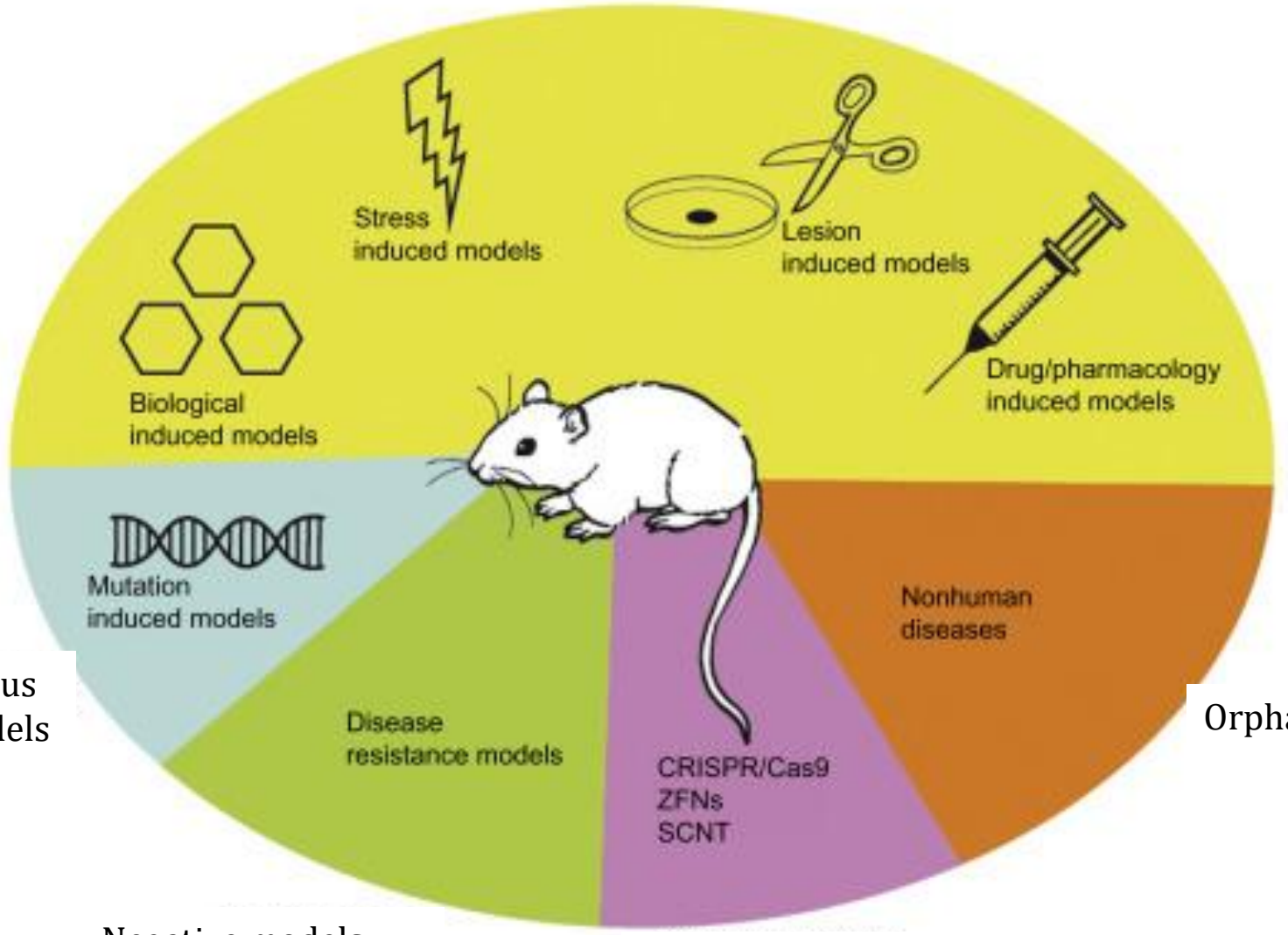
Translational vaccinology/medicinal products

- Interdisciplinary approach built on basic research advances used to develop new vaccine/medicinal products:
  - Study of biological *in vitro* processes
  - Animal models → **Highly regulated and must obey to the lack of other substitutive methodologies**

↓

Vaccinology is based on the immune response of humans and animals as well as its measurement, which limits the scope of models to work with

Induced animal models



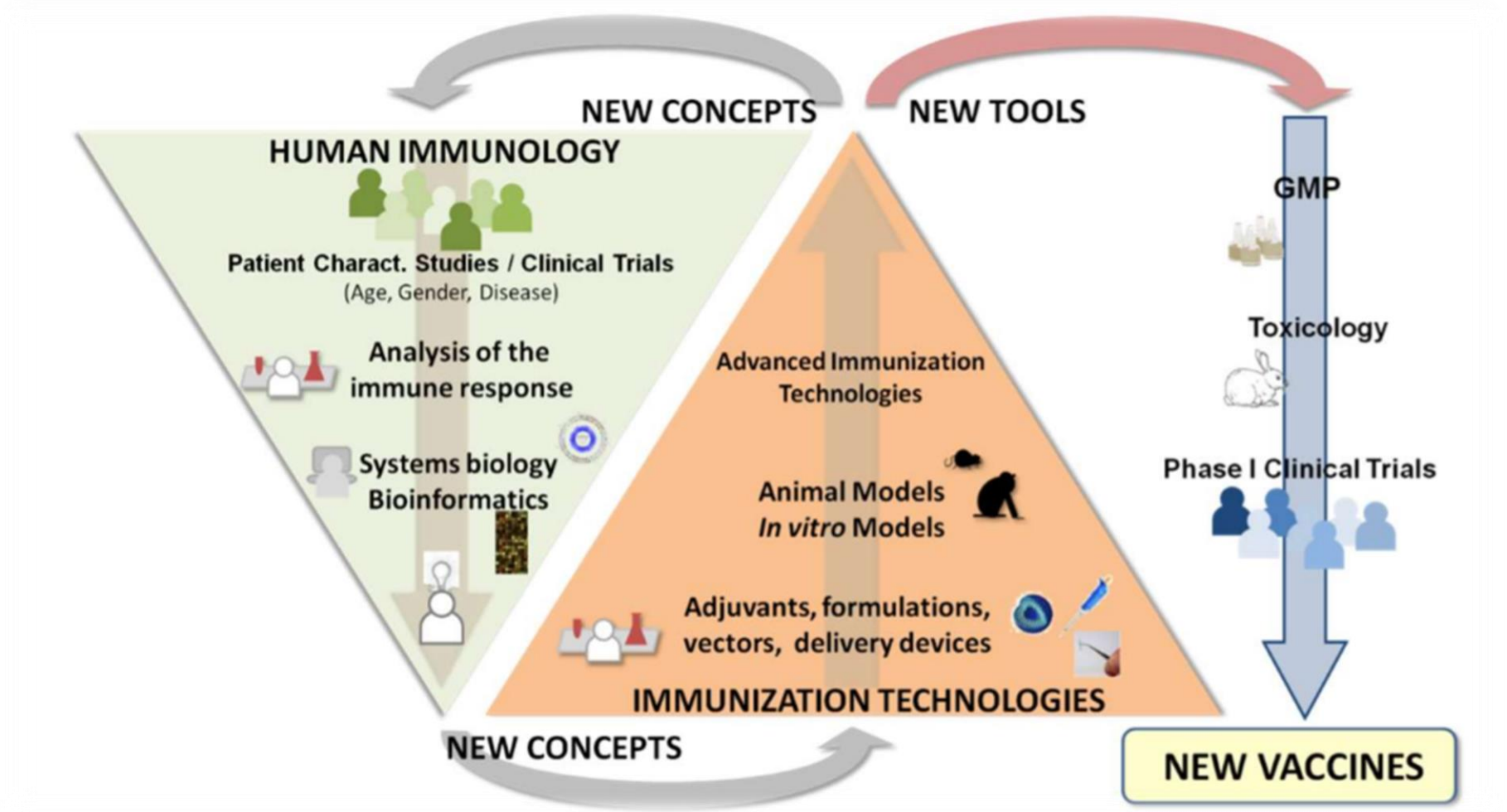
Spontaneous animal models

Orphan models

Negative models

Genetically modified

https://www.sciencedirect.com/science/article/pii/B9780128163528000084



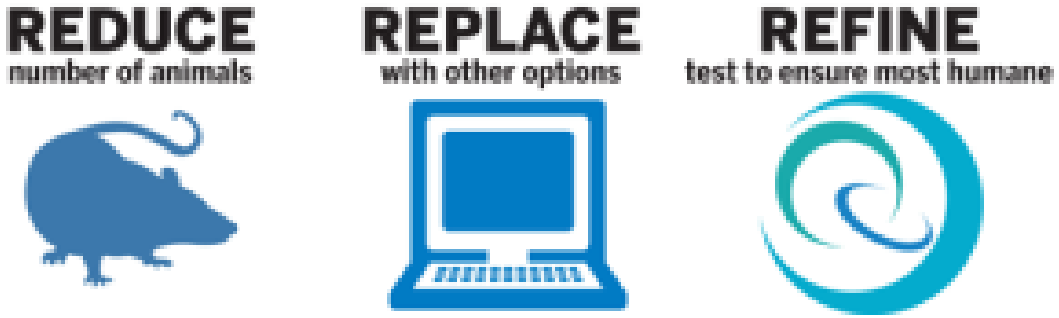
Grant Agreement number: 280873 Project acronym: ADITEC
Project title: Advanced Immunization Technologies

Usefulness of animal models

- The development of pharmaceutical and vaccine products for humans has a number of limitations: **some efficacy studies are not ethical or feasible!!**
- The advantage for developing animal vaccines is that you can do the research in the target species

Essential!!! (preclinical development for human vaccines, and preclinical and clinical development for animal vaccines)

Animal models and ethics



https://healthculturesociety2015.wikispaces.com

“The Animal Rule” (FDA, 2015)

- **“Product Development Under the Animal Rule Guidance for Industry”**
- FDA “may grant marketing approval based on **adequate and well-controlled animal efficacy studies** when the results of those studies establish that **the drug is reasonably likely to produce clinical benefit in humans**”.

Usefulness of animal models for the development of products for humans

- “Mice lie and monkeys exaggerate…”

http://www.keyword-suggestions.com

Sentence attributed toDr. David B. Weiner, University of Pennsylvania, USA

Usefulness of animal models for the development of products for humans

http://www.mitearrest.com

- Easy management
- Quick time of generation
- Genetically modifiable
- Availability of laboratory reagents

How representative of human diseases?; possibility of “humanization”, and “knock-out” and transgenic mice

https://en.wikipedia.org/wiki/Rhesus_macaque

- More similar to human diseases

High legal regulations and animal welfare and ethic considerations; difficult to work in environments BSL3 and BSL4

Usefulness of animal models for the development of products for humans

- Models with **large animals** may represent **better models** for some human diseases (Levast et al., 2013; Seok et al., 2013)

  - Similarity with human diseases
  - Access to different tissue compartments
  - Similarity with the immune response
  - Multiple “readouts” availability for vaccine safety and efficacy

Gerdts et al., 2015

Usefulness of animal models for the development of products for humans

- Some of the best animal models for certain human diseases are not easy to be expected:
  - Human influenza viruses ⇒ **ferret**
  - Human rotaviruses ⇒ **gnotobiotic pig**
  - Hespes simplex virus 1 ⇒ Aujeszky's disease virus in **pigs**
- Pigs, ruminants and horeses may represent good animal models for human diseases

The pig as a model for infectious diseases

Pig models for human infectious diseases

Pathogen	**Comments**
Influenza virus	Various ages (Influenza C and Influenza A subtypes H1N1, H1N2, H2N1, H3N1, H3N2 and H2N3)
Rotavirus	Neonatal gnotobiotic pigs
Norovirus	Neonatal gnotobiotic pigs
Herpes simplex 1	Host-virus interaction
Nipah virus	Young pigs
Bordetella pertussis	Neonatal pigs
Escherichia coli	Various ages, including neonatal pigs
Salmonella spp.	Neonatal gnotobiotic pigs
Shigella spp.	Neonatal gnotobiotic pigs
Clostridium spp.	Neonatal gnotobiotic pigs
Brucella (suis)	Various ages
Staphylococcus aureus	Young pigs
Pseudomona aeruginosa	Various ages
Helicobacter pylori	Neonatal gnotobiotic pigs
Acanthamoeba keratitis	Adult Yucatan micropigs
Chlamydia trachomantis	Adult female (genital model)

Ruminants as a model for infectious diseases

Sheep and cattle models for human infectious diseases

Pathogen	Sheep	Cattle
Parainfluenza virus	Neonatal	Various ages, including neonatal calves
Papilloma virus	-	Adult
Mycobacterium tuberculosis	-	Various ages, including neonatal calves
Escherichia coli	Various ages	Various ages, including neonatal calves
Schistosomiasis	--	Various ages
Brucellosis	Various ages	Various ages
Respiratory syncytial virus	Various ages, including neonatal lamb (models for human and bovine viruses)	Various ages, including neonatal calves (bovine virus only as a model)
Rotavirus	-	Neonatal gnotobiotic calves
Salmonella spp.	Various ages	-

Animal models and zoonoses

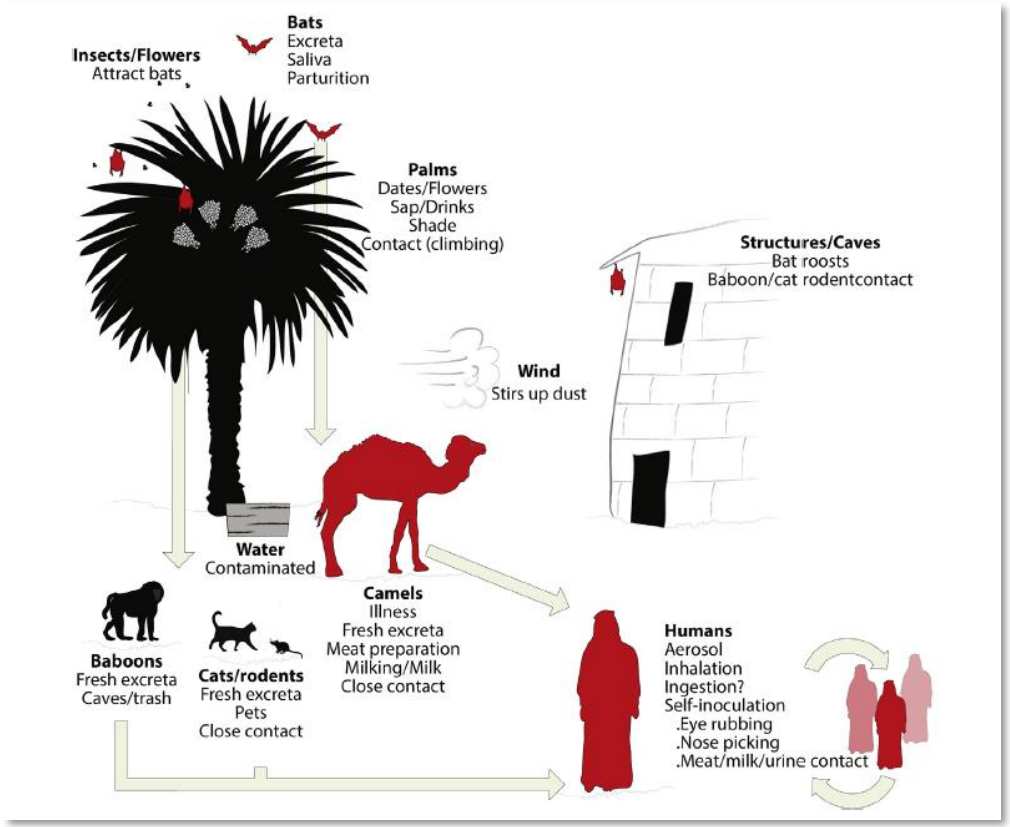
- Livestock species may be excellent models to study some zoonotic diseases, since the vaccines are tested in the own animal host
- Sometimes the animal host is able to display a **clinical disease very similar to that of humans**, but in a number of cases, probably as a result of **host-pathogen co-evolution**, hosts are just experiencing subclinical infections and act as **reservoirs**

Building up an animal model

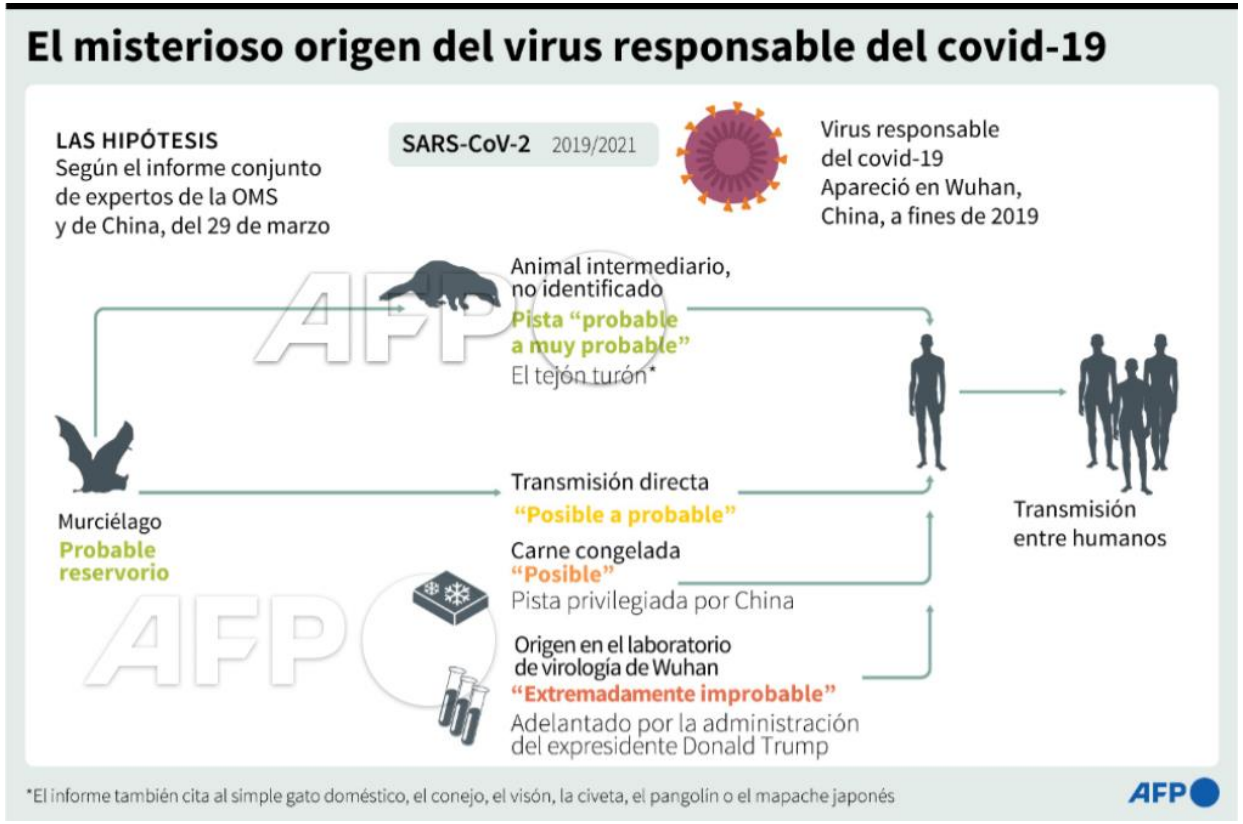
- Do we have the right animal species to work with? - **ANIMAL SPECIES SELECTION**
- Do we have the right health status of the animal species selected? – **CLINICAL AND LABORATORY DIAGNOSIS/MONITORING**
- Do we have the right facilities/animal caretakers to work on the animal model? – **WHICH TYPE OF PATHOGEN SHOULD WE WORK WITH? BSL2? BSL3? OTHER?**

A couple of examples… zoonòtic coronaviruses

MERS-CoV

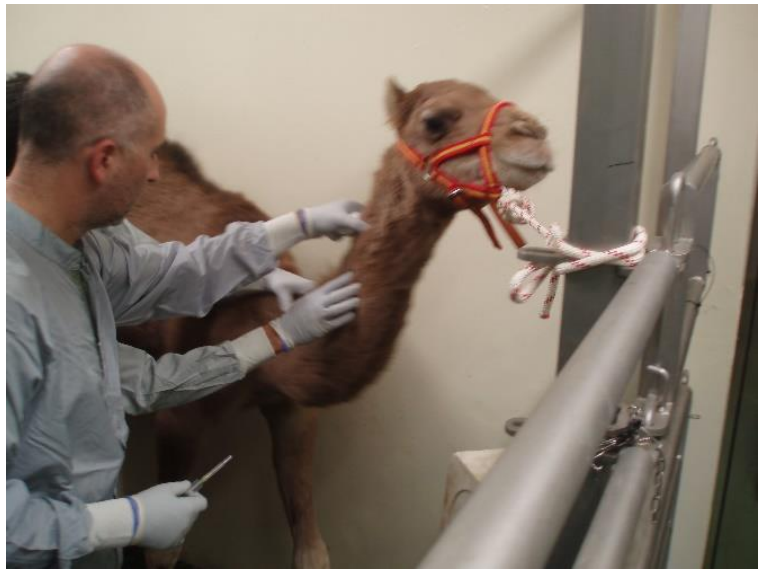


SARS-CoV-2



Working with zoonotic coronaviruses at IRTA-CReSA

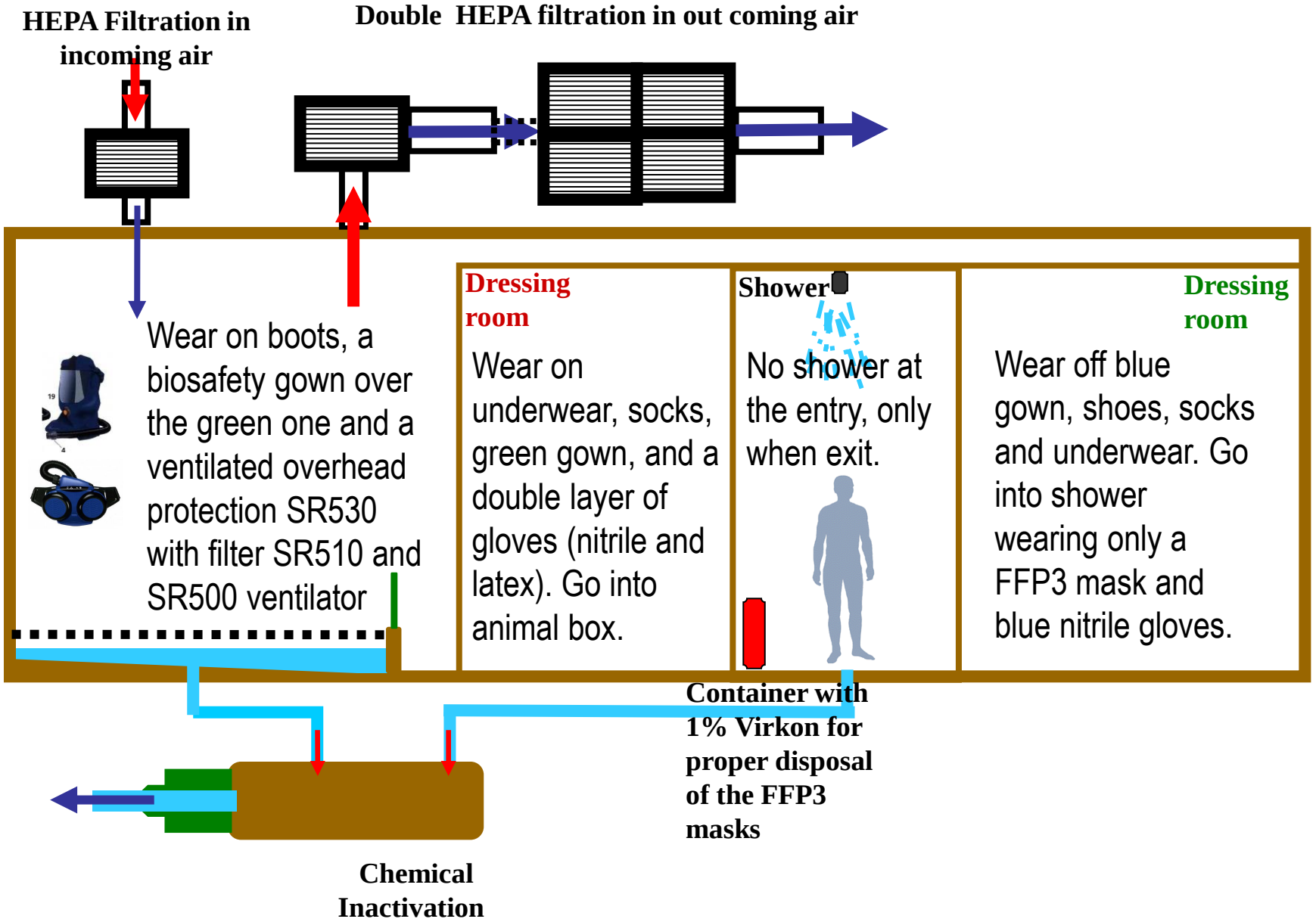
- Working with MERS-CoV animal models since 2014
- Working with SARS-CoV-2 animal models since 2020

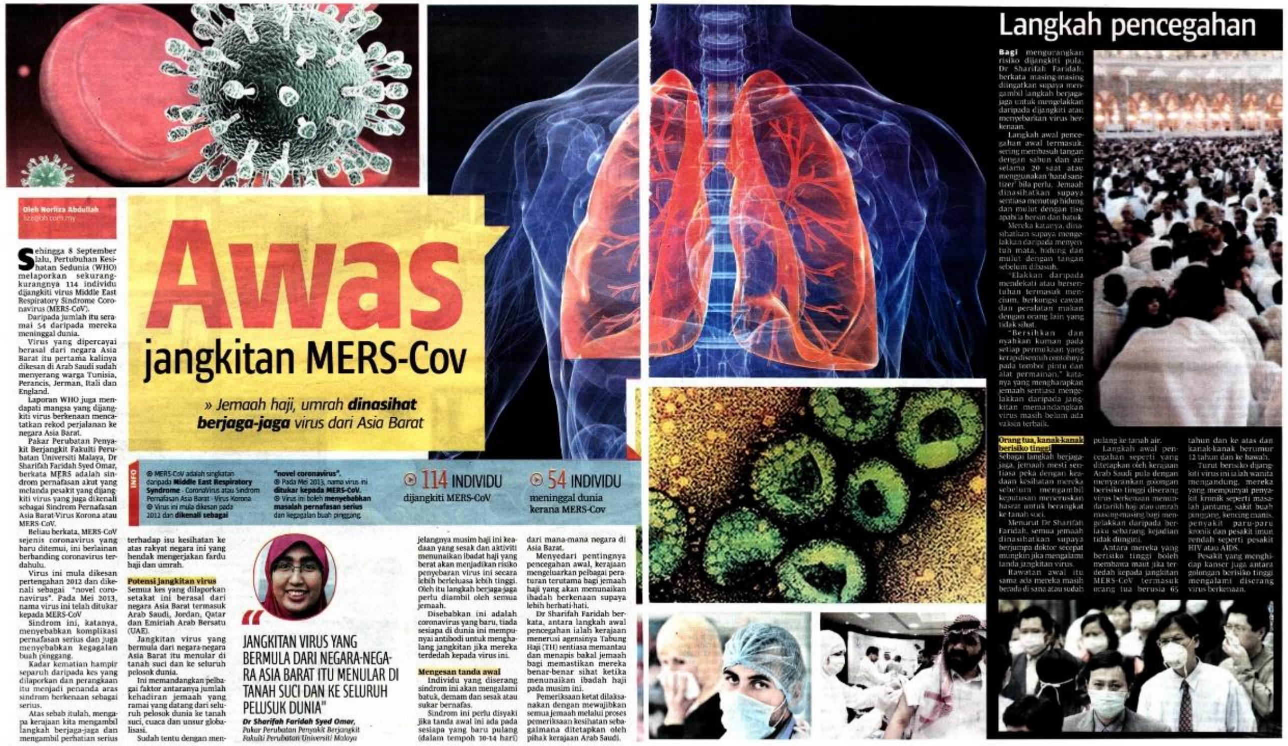


BSL3 laboratory facilities: PPE for MERS-CoV or SARS-CoV-2

- FPP3 mask (North)
- Sundström ventilated (positive pressure) overhead protection SR530 with filter SR510 and ventilator SR500
- Two pairs of gloves
- Second cover for working on box or laboratory (Tyvek®).

Biosafety measures for MERS-CoV or SARS-CoV-2 in animal box





Oleh Norliza Abdullah
liza@bh.com.my

Awas jangkitan MERS-Cov

» *Jemaah haji, umrah* ***dinasihat berjaga-jaga*** *virus dari Asia Barat*

Sehingga 8 September lalu, Pertubuhan Kesihatan Sedunia (WHO) melaporkan sekurang-kurangnya 114 individu dijangkiti virus Middle East Respiratory Sindrome Coronavirus (MERS-CoV).

Daripada jumlah itu seramai 54 daripada mereka meninggal dunia.

Virus yang dipercayai berasal dari negara Asia Barat itu pertama kalinya dikesan di Arab Saudi sudah menyerang warga Tunisia, Perancis, Jerman, Itali dan England.

Laporan WHO juga mendapati mangsa yang dijangkiti virus berkenaan mencatatkan rekod perjalanan ke negara Asia Barat.

Pakar Perubatan Penyakit Berjangkit Fakulti Perubatan Universiti Malaya, Dr Sharifah Faridah Syed Omar, berkata MERS adalah sindrom pernafasan akut yang melanda pesakit yang dijangkiti virus yang juga dikenali sebagai Sindrom Pernafasan Asia Barat-Virus Korona atau MERS-CoV.

Beliau berkata, MERS-CoV sejenis coronavirus yang baru ditemui, ini berlainan berbanding coronavirus terdahulu.

Virus ini mula dikesan pertengahan 2012 dan dikenali sebagai "novel coronavirus". Pada Mei 2013, nama virus ini telah ditukar kepada MERS-CoV.

Sindrom ini, katanya, menyebabkan komplikasi pernafasan serius dan juga menyebabkan kegagalan buah pinggang.

Kadar kematian hampir separuh daripada kes yang dilaporkan dan perangkaan itu menjadi penanda aras sindrom berkenaan sebagai serius.

Atas sebab itulah, mengapa kerajaan kita mengambil langkah berjaga-jaga dan mengambil perhatian serius terhadap isu kesihatan ke atas rakyat negara ini yang hendak mengerjakan fardu haji dan umrah.

INFO

- MERS-CoV adalah singkatan daripada **Middle East Respiratory Syndrome** - CoronaVirus atau Sindrom Pernafasan Asia Barat - Virus Korona
- Virus ini mula dikesan pada 2012 dan **dikenali sebagai "novel coronavirus".**
- Pada Mei 2013, nama virus ini **ditukar kepada MERS-CoV.**
- Virus ini boleh **menyebabkan masalah pernafasan serius** dan kegagalan buah pinggang.

114 INDIVIDU dijangkiti MERS-CoV

54 INDIVIDU meninggal dunia kerana MERS-Cov

Potensi jangkitan virus

Semua kes yang dilaporkan setakat ini berasal dari negara Asia Barat termasuk Arab Saudi, Jordan, Qatar dan Emiriah Arab Bersatu (UAE).

Jangkitan virus yang bermula dari negara-negara Asia Barat itu menular di tanah suci dan ke seluruh pelosok dunia.

Ini memandangkan pelbagai faktor antaranya jumlah kehadiran jemaah yang ramai yang datang dari seluruh pelosok dunia ke tanah suci, cuaca dan unsur globalisasi.

Sudah tentu dengan menjelangnya musim haji ini keadaan yang sesak dan aktiviti menunaikan ibadat haji yang berat akan menjadikan risiko penyebaran virus ini secara lebih berleluasa lebih tinggi. Oleh itu langkah berjaga-jaga perlu diambil oleh semua jemaah.

Disebabkan ini adalah coronavirus yang baru, tiada sesiapa di dunia ini mempunyai antibodi untuk menghalang jangkitan jika mereka terdedah kepada virus ini.

> "JANGKITAN VIRUS YANG BERMULA DARI NEGARA-NEGARA ASIA BARAT ITU MENULAR DI TANAH SUCI DAN KE SELURUH PELUSUK DUNIA"
>
> ***Dr Sharifah Faridah Syed Omar,*** *Pakar Perubatan Penyakit Berjangkit Fakulti Perubatan Universiti Malaya*

Mengesan tanda awal

Individu yang diserang sindrom ini akan mengalami batuk, demam dan sesak atau sukar bernafas.

Sindrom ini perlu disyaki jika tanda awal ini ada pada sesiapa yang baru pulang (dalam tempoh 10-14 hari) dari mana-mana negara di Asia Barat.

Menyedari pentingnya pencegahan awal, kerajaan mengeluarkan pelbagai peraturan terutama bagi jemaah haji yang akan menunaikan ibadah berkenaan supaya lebih berhati-hati.

Dr Sharifah Faridah berkata, antara langkah awal pencegahan ialah kerajaan menerusi agensinya Tabung Haji (TH) sentiasa memantau dan menapis bakal jemaah bagi memastikan mereka benar-benar sihat ketika menunaikan ibadah haji pada musim ini.

Pemeriksaan ketat dilaksanakan dengan mewajibkan semua jemaah melalui proses pemeriksaan kesihatan sebagaimana ditetapkan oleh pihak kerajaan Arab Saudi.

Langkah pencegahan

Bagi mengurangkan risiko dijangkiti pula, Dr Sharifah Faridah, berkata masing-masing diingatkan supaya mengambil langkah berjaga-jaga untuk mengelakkan daripada dijangkiti atau menyebarkan virus berkenaan.

Langkah awal pencegahan awal termasuk, sering membasuh tangan dengan sabun dan air selama 20 saat atau menggunakan 'hand sanitizer' bila perlu. Jemaah dinasihatkan supaya sentiasa menutup hidung dan mulut dengan tisu apabila bersin dan batuk.

Mereka katanya, dinasihatkan supaya mengelakkan daripada menyentuh mata, hidung dan mulut dengan tangan sebelum dibasuh.

"Elakkan daripada mendekati atau bersentuhan termasuk mencium, berkongsi cawan dan peralatan makan dengan orang lain yang tidak sihat.

"Bersihkan dan nyahkan kuman pada setiap permukaan yang kerap disentuh contohnya pada tombol pintu dan alat permainan," katanya yang mengharapkan jemaah sentiasa mengelakkan daripada jangkitan memandangkan virus masih belum ada vaksin terbaik.

Orang tua, kanak-kanak berisiko tinggi

Sebagai langkah berjaga-jaga, jemaah mesti sentiasa peka dengan keadaan kesihatan mereka sebelum mengambil keputusan meneruskan hasrat untuk berangkat ke tanah suci.

Menurut Dr Sharifah Faridah, semua jemaah dinasihatkan supaya berjumpa doktor secepat mungkin jika mengalami tanda jangkitan virus.

Rawatan awal itu sama ada mereka masih berada di sana atau sudah pulang ke tanah air.

Langkah awal pencegahan seperti yang ditetapkan oleh kerajaan Arab Saudi pula dengan menyarankan golongan berisiko tinggi diserang virus berkenaan menunda tarikh haji atau umrah masing-masing bagi mengelakkan daripada berlaku sebarang kejadian tidak diingini.

Antara mereka yang berisiko tinggi boleh membawa maut jika terdedah kepada jangkitan MERS-CoV termasuk orang tua berusia 65 tahun dan ke atas dan kanak-kanak berumur 12 tahun dan ke bawah.

Turut berisiko dijangkiti virus ini ialah wanita mengandung, mereka yang mempunyai penyakit kronik seperti masalah jantung, sakit buah pinggang, kencing manis, penyakit paru-paru kronik dan pesakit imun rendah seperti pesakit HIV atau AIDS.

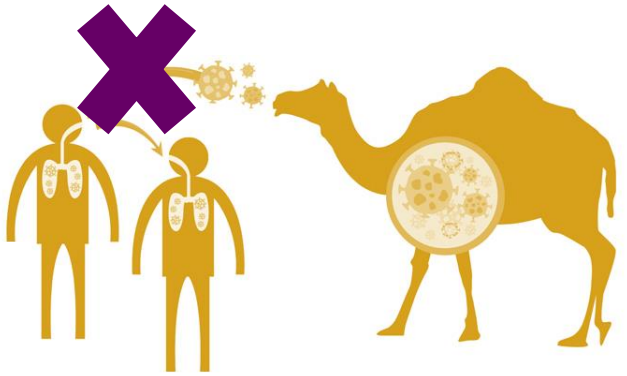
Pesakit yang menghidap kanser juga antara golongan berisiko tinggi mengalami diserang virus berkenaan.

MERS OUTBREAK

MERS-CoV: vaccination of camelids as a first option to control the transmission of the virus to humans

High rate of infection in camelid species

Zoonotic infection



Limited transmission of the virus among people

Isolation and quarantine

Vaccination of the reservoir: camelids

Science
AAAS

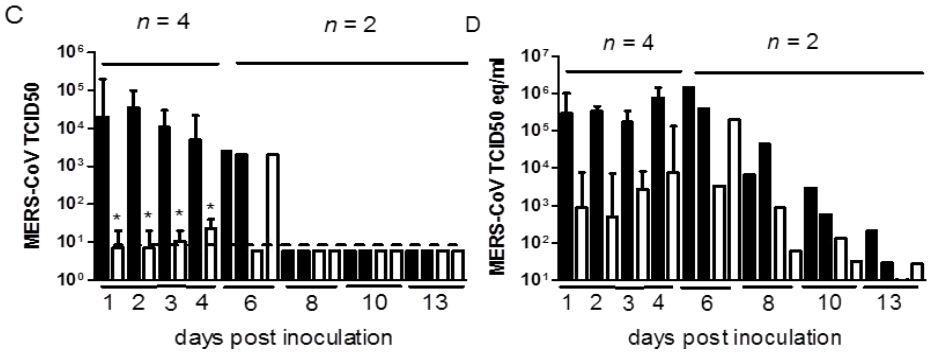
VIROLOGY

An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels

Bart L. Haagmans,[1*] Judith M. A. van den Brand,[1] V. Stalin Raj,[1] Asisa Volz,[2] Peter Wohlsein,[3] Saskia L. Smits,[1] Debby Schipper,[1] Theo M. Bestebroer,[1] Nisreen Okba,[1] Robert Fux,[2] Albert Bensaid,[4] David Solanes Foz,[4] Thijs Kuiken,[1] Wolfgang Baumgärtner,[3] Joaquim Segalés,[5,6] Gerd Sutter,[2*] Albert D. M. E. Osterhaus[1,7,8*]

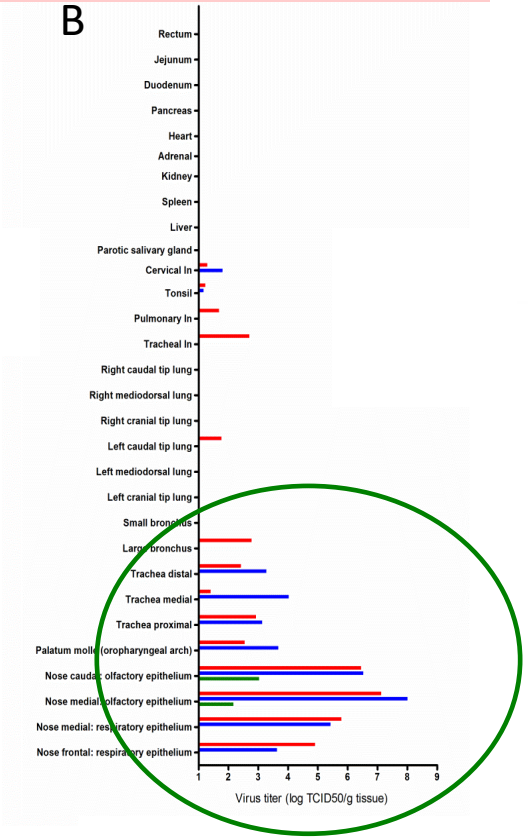
Mild clinical signs in camelids

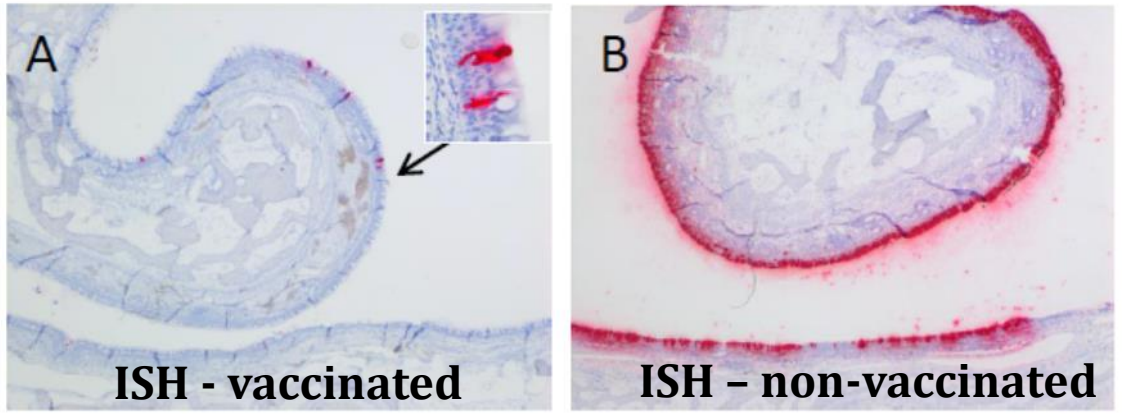
Detection of MERS-CoV RNA and infectious virus in nasal swabs

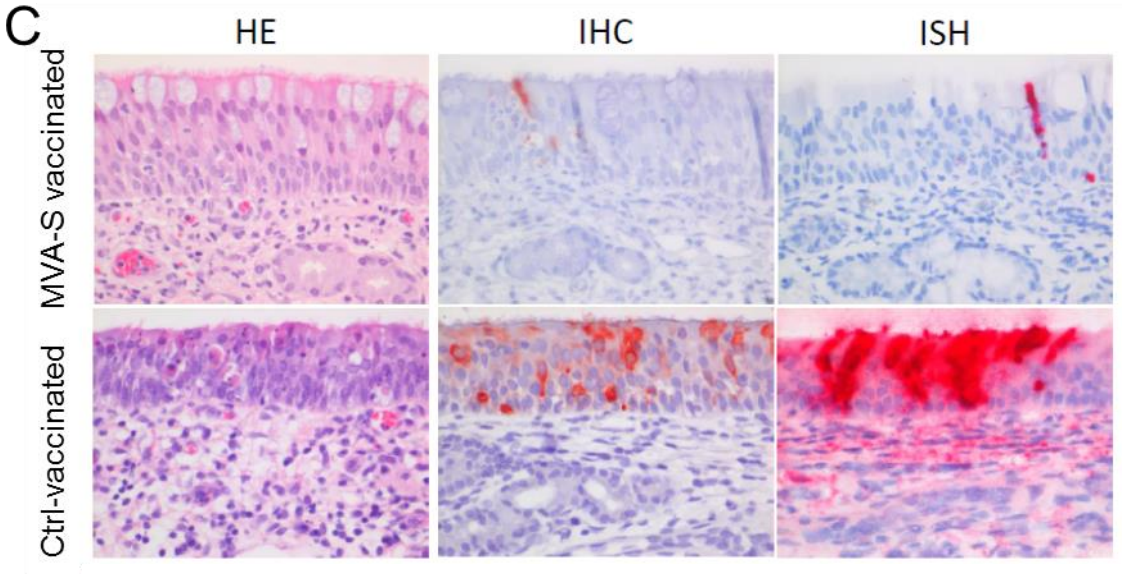


Histopathology and expression of viral antigen and viral RNA in nasal respiratory epithelium

At 4 dpi, infectious MERS-CoV in **noses and tracheas**





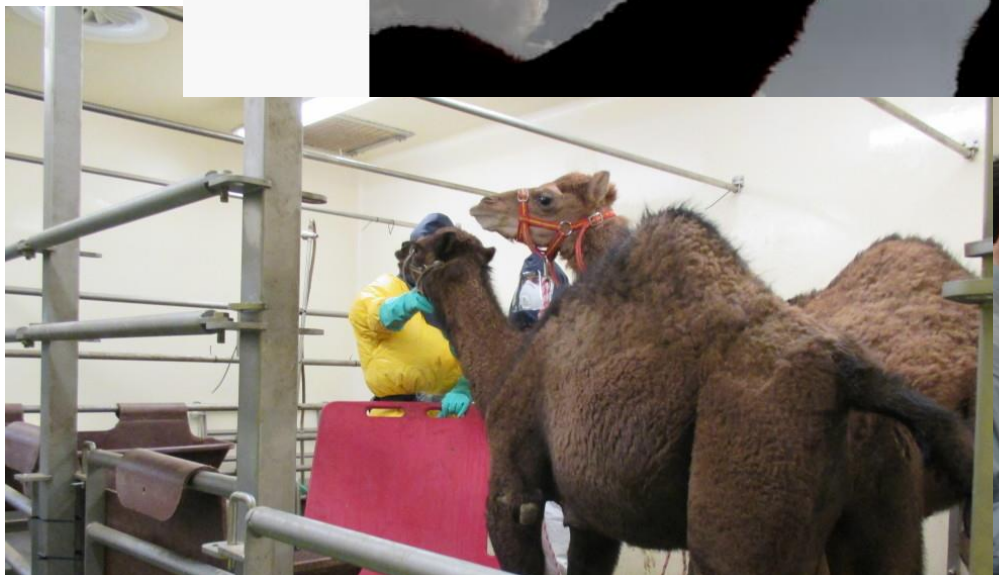


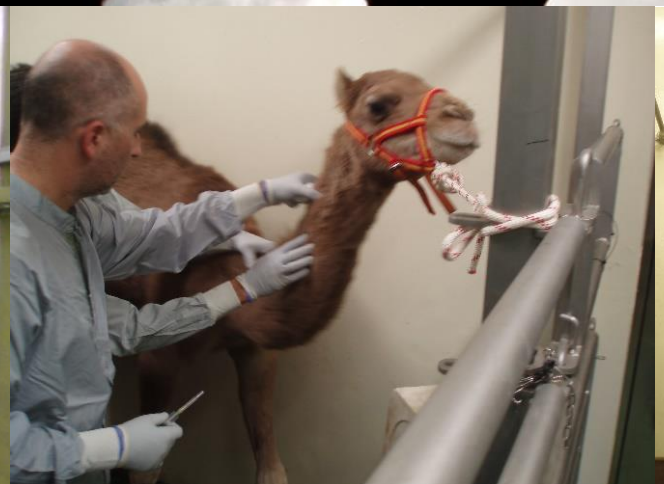
Not easy to work with dromedary camels…

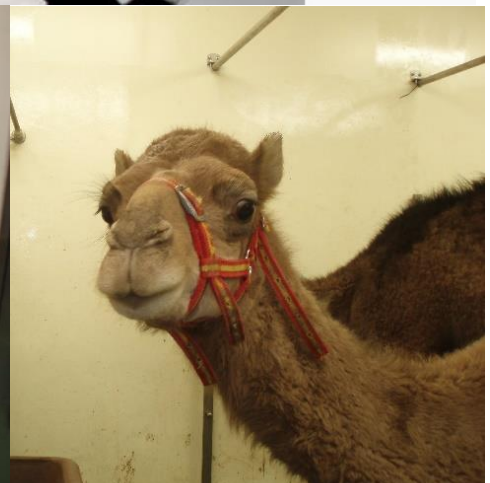
STAT | Reporting from the frontiers of health and medicine

IN THE LAB

Think your job is hard? Try squirting a vaccine up a camel's nostrils







Potential surrogates for camels?

Blocking transmission of Middle East respiratory syndrome coronavirus (MERS-CoV) in llamas by vaccination with a recombinant spike protein

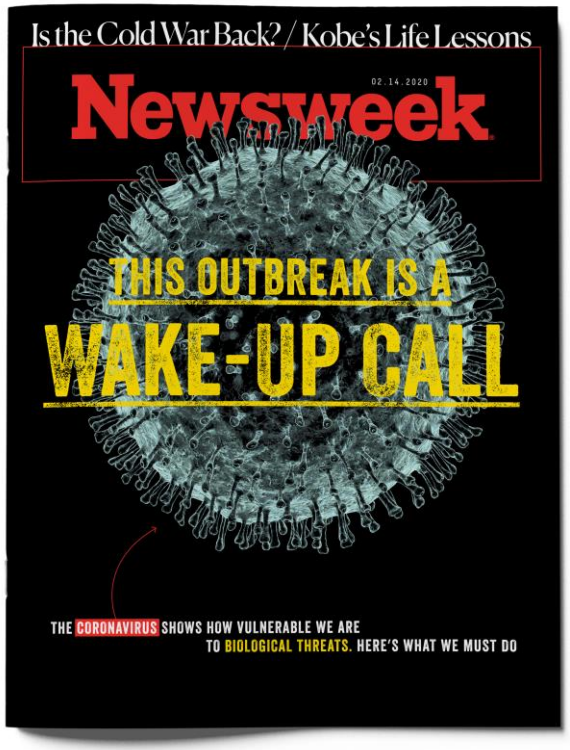
Jordi Rodon, Nisreen M. A. Okba, Nigeer Te, Brenda van Dieren, Berend-Jan Bosch, Albert Bensaid, Joaquim Segalés, Bart L. Haagmans & Júlia Vergara-Alert

EMi

Type I and III IFNs produced by the nasal epithelia and dimmed inflammation are features of alpacas resolving MERS-CoV infection

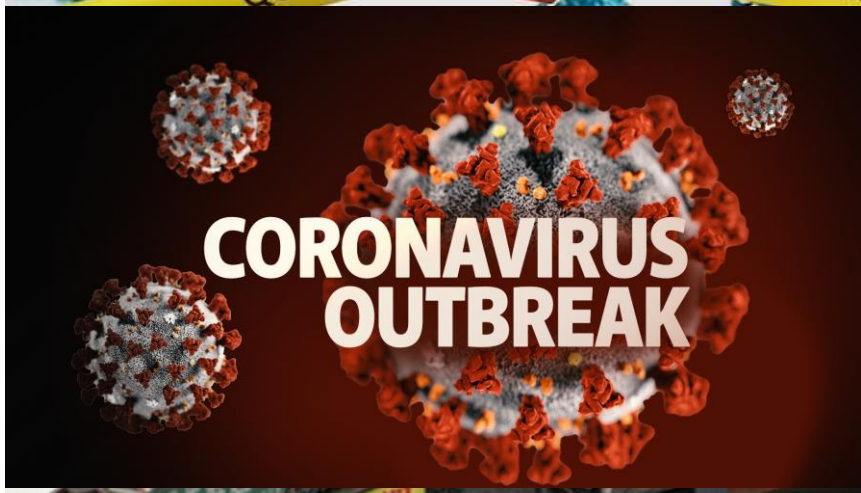
Nigeer Te[1☯], Jordi Rodon[1☯], Maria Ballester[2], Mónica Pérez[1], Lola Pailler-García[1], Joaquim Segalés[3,4], Júlia Vergara-Alert[1*], Albert Bensaid[1]

PLOS PATHOGENS


Is the Cold War Back? / Kobe's Life Lessons
Newsweek
02.14.2020
THIS OUTBREAK IS A
WAKE-UP CALL
THE CORONAVIRUS SHOWS HOW VULNERABLE WE ARE
TO BIOLOGICAL THREATS. HERE'S WHAT WE MUST DO

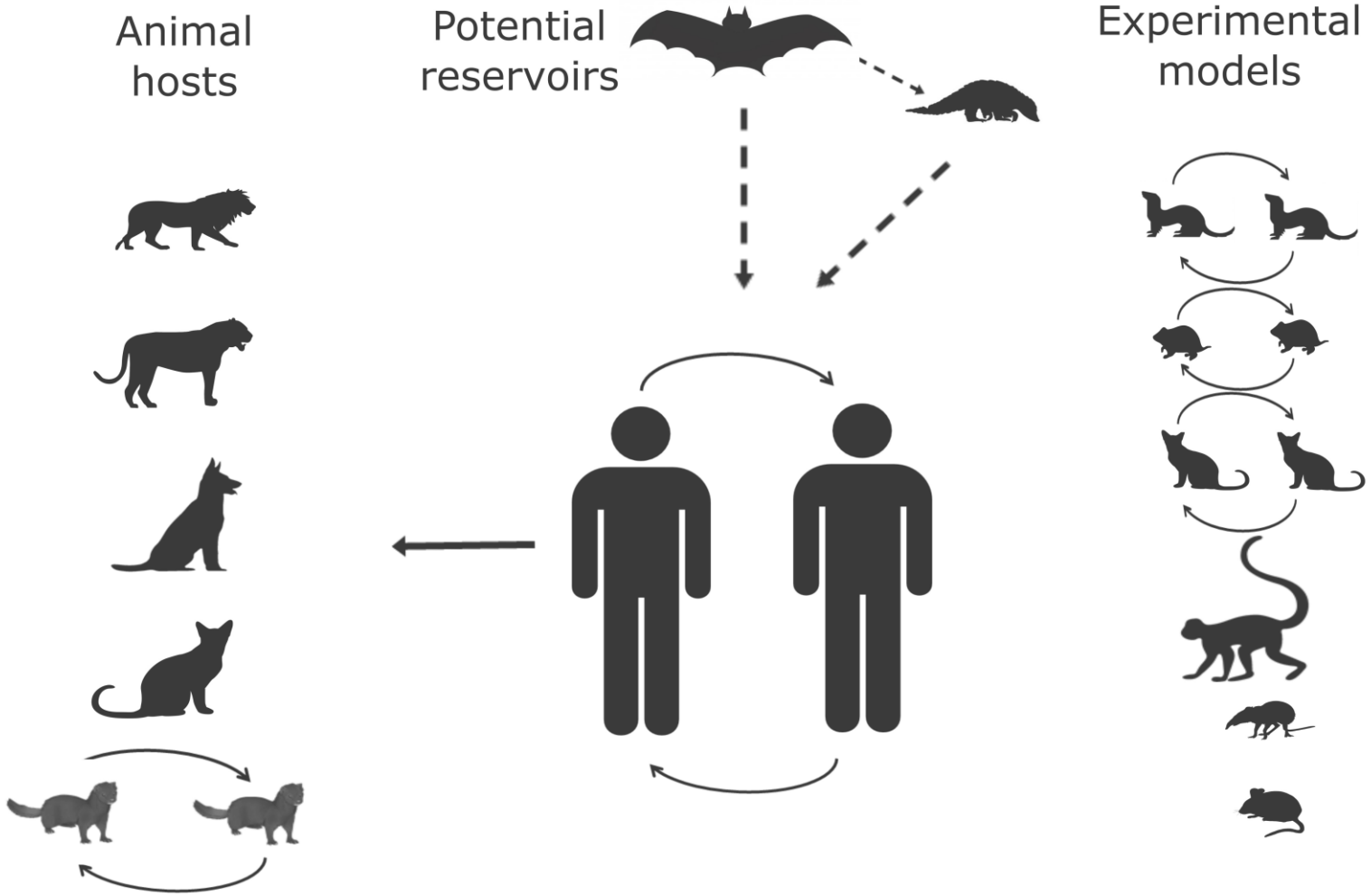

MARCH 30, 2020
TIME
CORONAVIRUS
SPECIAL REPORT
WHAT TO KNOW AND DO ABOUT THE GLOBAL PANDEMIC
LESSONS FROM ASIA'S RESPONSE
WHAT AMERICA MUST DO NOW
time.com


QUARANTINE
COVID-19


CORONAVIRUS
OUTBREAK


COVID-19
FAKE NEWS
BBC NEWS

SARS-CoV-2: Vaccination of people; preclinical studies in animals



Abdel-Moneim y Abdelwhab, 2020

Experimental infections in animals: which ones have been tested?

Cynomolgus macaque and other NHP

hACE2 transgenic mice

Ferret

Golden Syrian hamster

Egyptian fruit bat

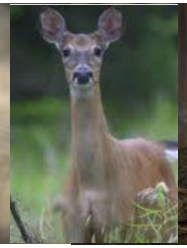
Racoon dog

Bank vole

Rabbit

Cat

Dog (Beagle)



White-tailed deer

Chinese tree shrews

Cattle

Pig

Chicken

Duck

Bao et al., 2020; Chan et al., 2020; Hallmann et al., 2020; Richard et al., 2020; Rockx et al., 2020; Shi et al., 2020; Schlottau et al., 2020, Ulrich et al., 2020; Freuling et al., 2020; Mykytyn et al., 2020; Xu et al., 2020; FLI 2021; Palmer et al., 2021

Experimental infections in animals: which ones are susceptible?

Cynomolgus macaque and other NHP

hACE2 transgenic mice

Ferret

Golden Syrian hamster

Egyptian fruit bat

Racoon dog

Bank vole

Rabbit

White-tailed deer

Chinese tree shrews

Cat

Dog (Beagle)

Cattle

Pig

Chicken

Duck

Bao et al., 2020; Chan et al., 2020; Hallmann et al., 2020; Richard et al., 2020; Rockx et al., 2020; Shi et al., 2020; Schlottau et al., 2020, Ulrich et al., 2020; Freuling et al., 2020; Mykytyn et al., 2020; Xu et al., 2020; FLI 2021, Palmer et al., 2021

nature

Review

Animal models for COVID-19

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Check for updates

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PLOS PATHOGENS

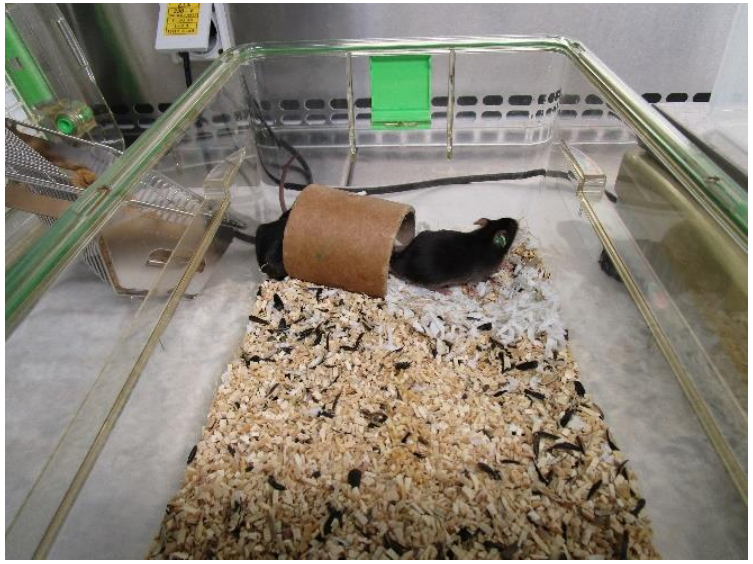
REVIEW

Advances and gaps in SARS-CoV-2 infection models

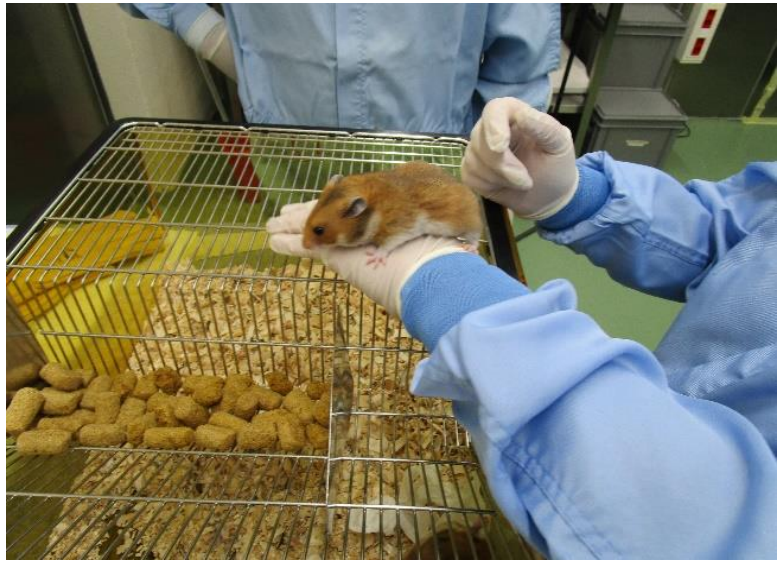
César Muñoz-Fontela[1,2], Lina Widerspick[1,2], Randy A. Albrecht[3], Martin Beer[4], Miles W. Carroll[5,6], Emmie de Wit[7], Michael S. Diamond[8], William E. Dowling[9], Simon G. P. Funnell[5], Adolfo García-Sastre[3], Nora M. Gerhards[10], Rineke de Jong[10], Vincent J. Munster[7], Johan Neyts[11], Stanley Perlman[12], Douglas S. Reed[13], Juergen A. Richt[14], Ximena Riveros-Balta[15], Chad J. Roy[16], Francisco J. Salguero[5], Michael Schotsaert[3], Lauren M. Schwartz[15], Robert A. Seder[17], Joaquim Segalés[18], Seshadri S. Vasan[19], Ana María Henao-Restrepo[15], Dan H. Barouch[20]*

Use of different animal models

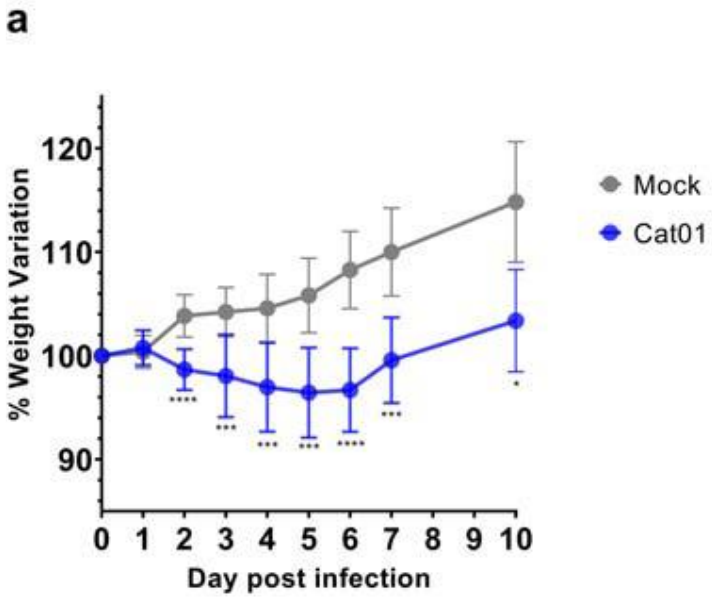
K18-hACE2 transgenic mice

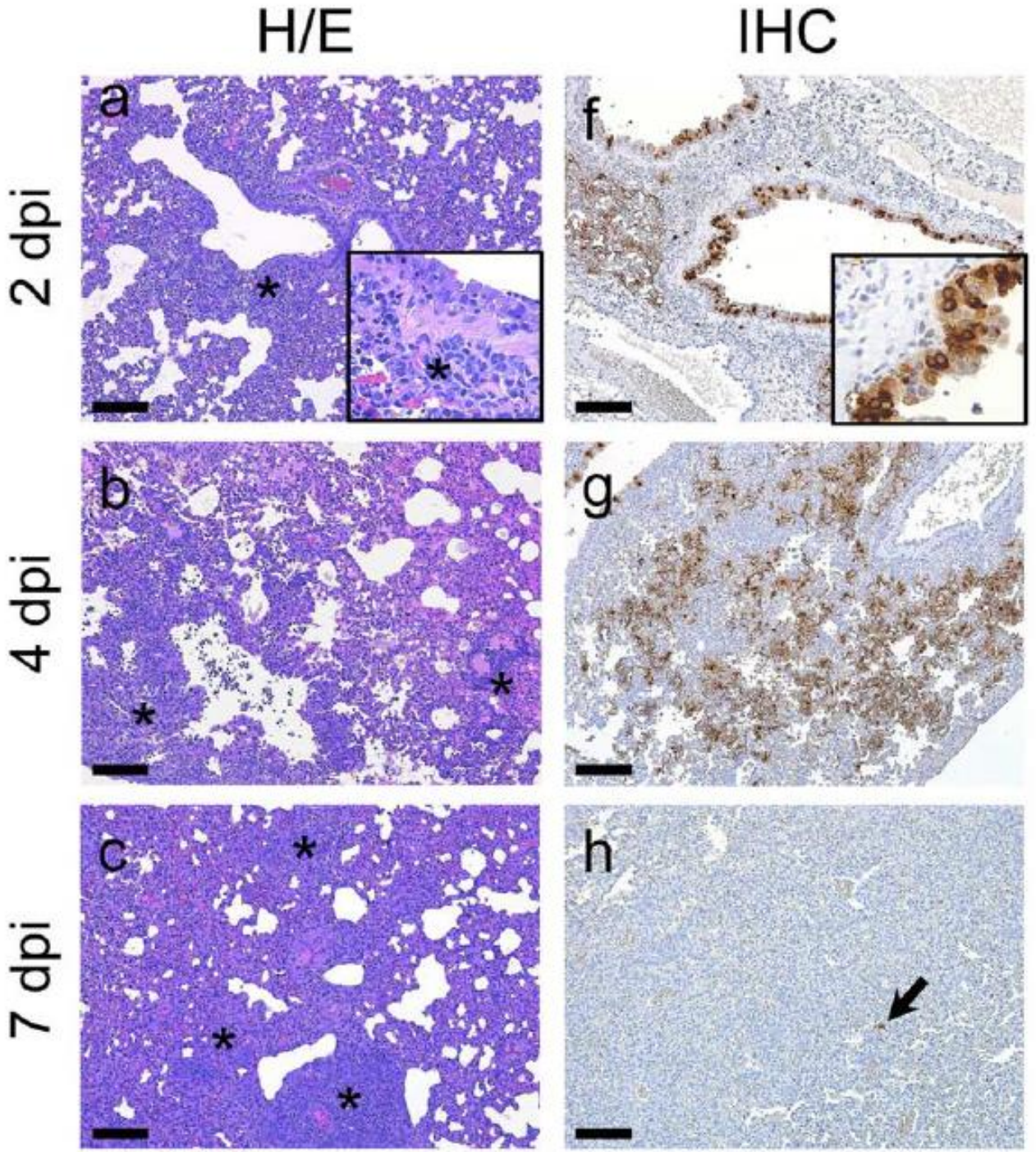


Golden Syrian hamster



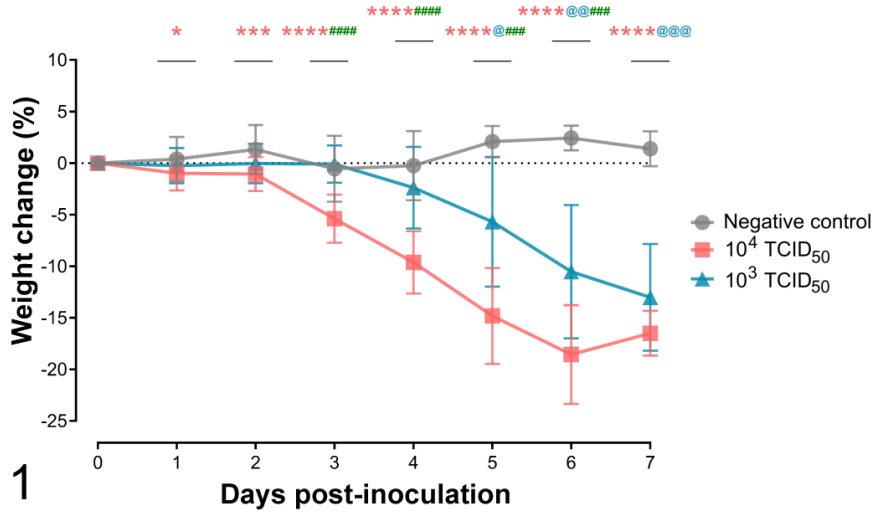
Golden Syrian hamster model





Brustolin et al., 2021

hACE2 transgenic mice model



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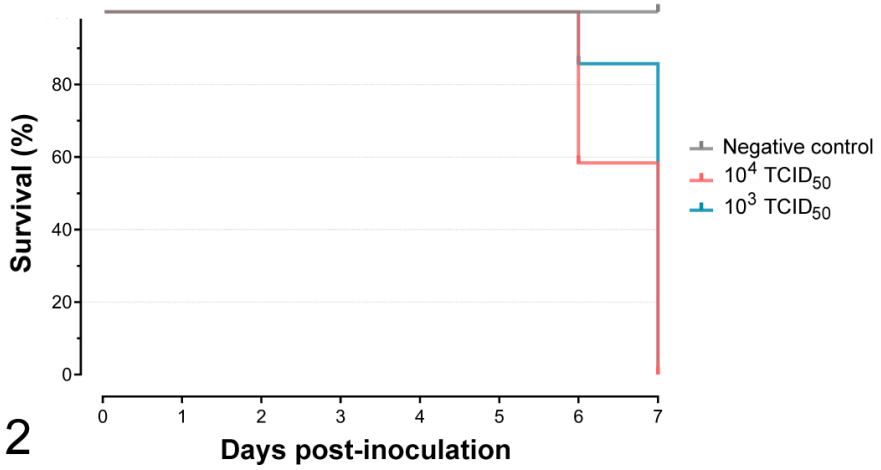
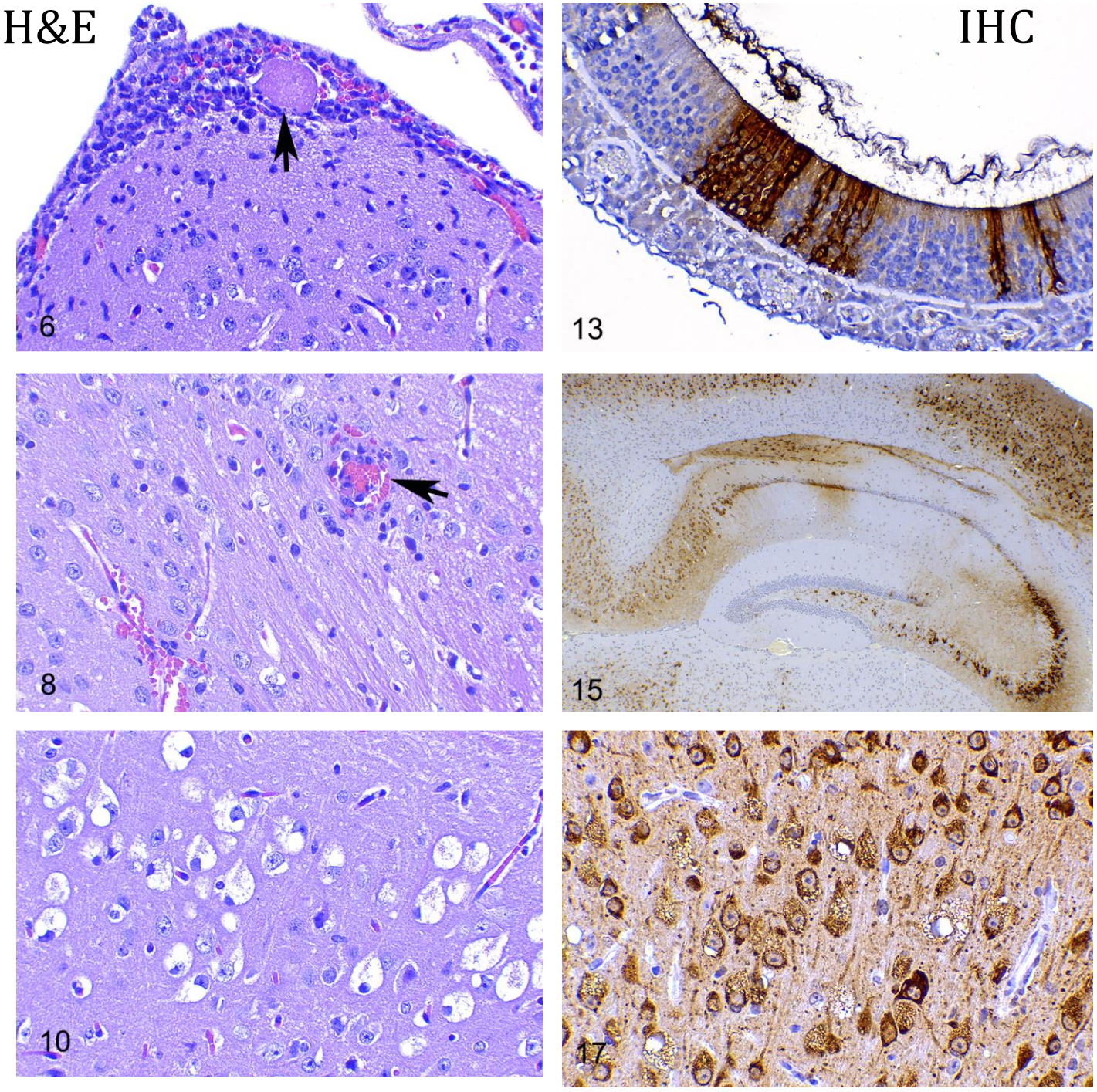


2

Vidal et al., 2021



General thoughts

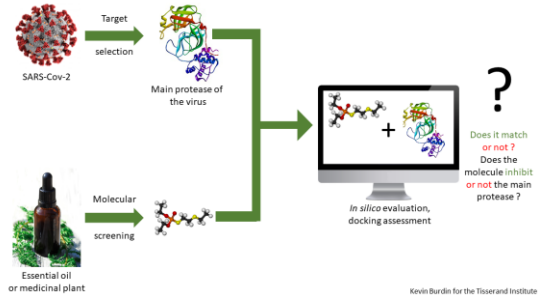
- Permanent need to develop pharmaceutical and vaccine products
- Need to develop new platforms to design and produce products

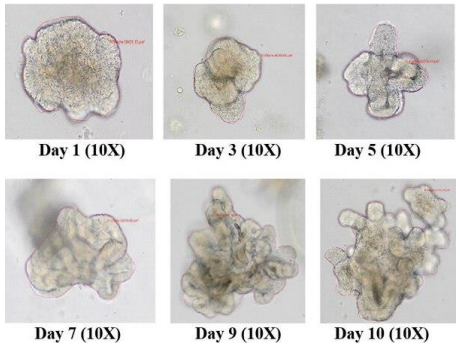
  Necesidad de desarrollo de nuevos modelos animales
- Need to expand the espectre of preclinical possibilities:
  - To reduce the number of animals in the experiment
  - Use of alternative methods to animal experimentation
  - How to reproduce the biological variability

Alternative methods for animal experimentation

- *In silico* methods or computer models
- *In vitro* cell-based models
  - Immortalized cell lines
  - Primary cells
- Three-dimensional (3D) models
  - Organoids
  - Organ-on-a-chip

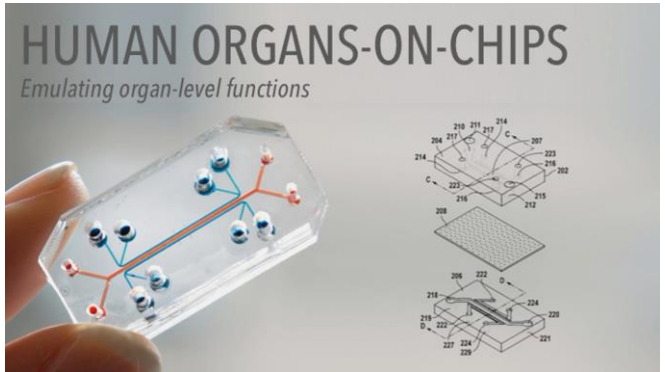
https://tisserandinstitute.org/sars-cov-2-essential-oils-in-silico-studies/





https://www.merckmillipore.com/

https://kosheeka.com/how-do-primary-cells-differ-from-continuous-cell-lines/



https://wyss.harvard.edu/technology/human-organs-on-chips/

PERSPECTIVES

Reproducibility of animal research in light of biological variation

Bernhard Voelkl, Naomi S. Altman, Anders Forsman, Wolfgang Forstmeier, Jessica Gurevitch, Ivana Jaric, Natasha A. Karp, Martien J. Kas, Holger Schielzeth, Tom Van de Casteele and Hanno Würbel

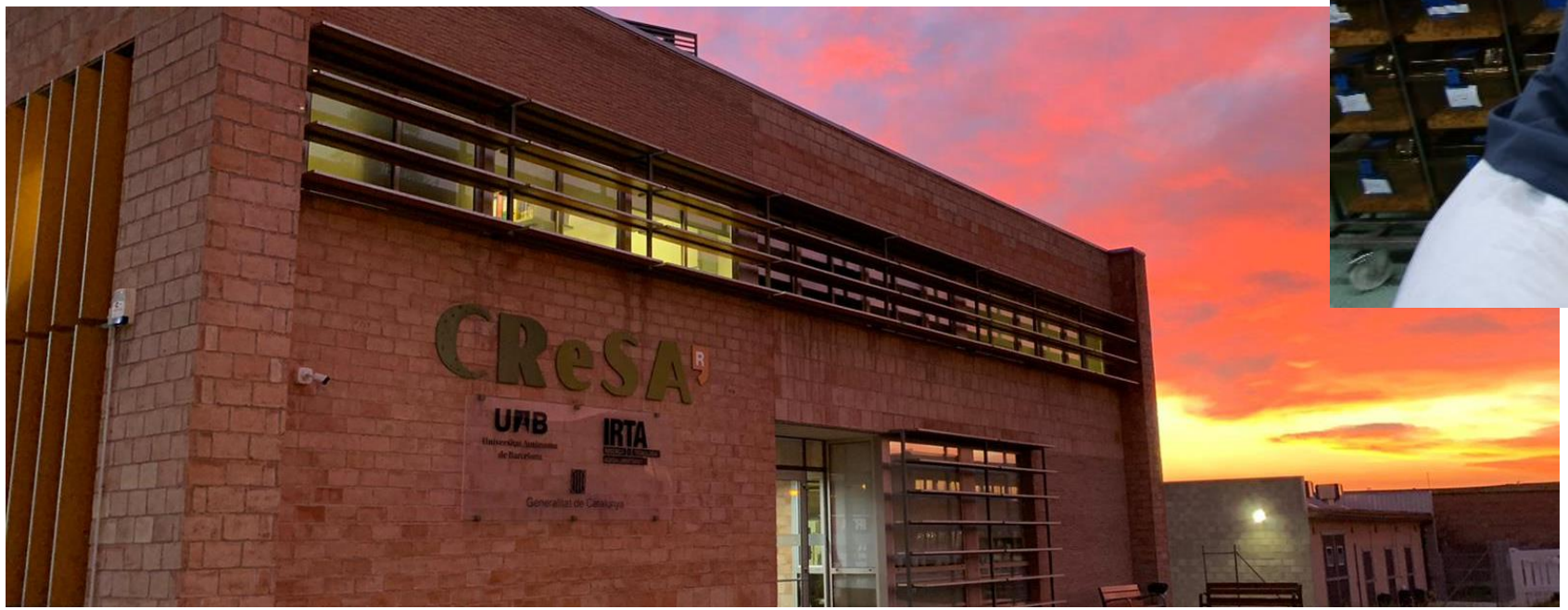
"In contrast to the current gold standard of rigorous standardization in experimental animal research, we recommend the use of systematic heterogenization of study samples and conditions by actively incorporating biological variation into study design through diversifying study samples and conditions."

OIE Animal Health @OIEAnimalHealth · 54m

Preventing future pandemics will likely cost between $18-$27 billion/year over 10 years. That may seem like a lot, but it's less than 1% of the cost of the current pandemic & avoids the additional suffering & disruption.

It's time to invest in pandemic prevention! #OneHealth

8 12



Many thanks for your attention!