The ecology, epidemiology and zoonotic potential of MERS-CoV

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One Health

335 EID events (1940-2004):
60% are zoonotic origin (Jones et al., Nature 2008)

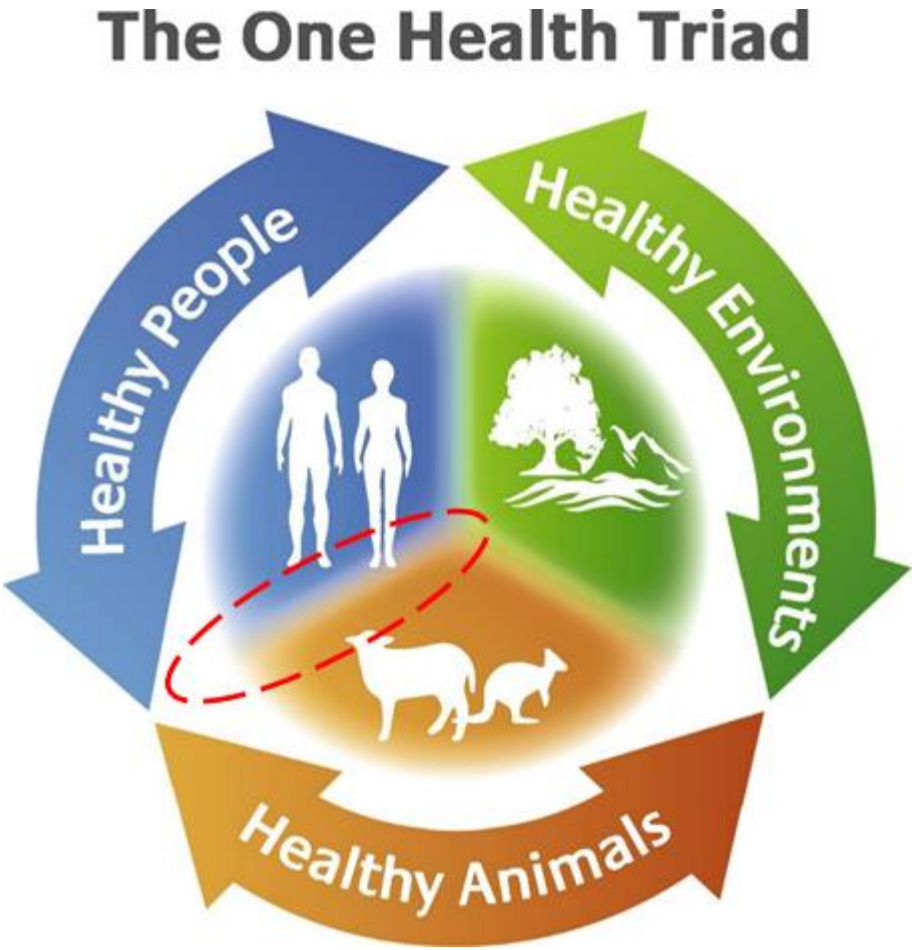
Examples:

Animal coronavirus

- SARS
- MERS

Animal influenza virus

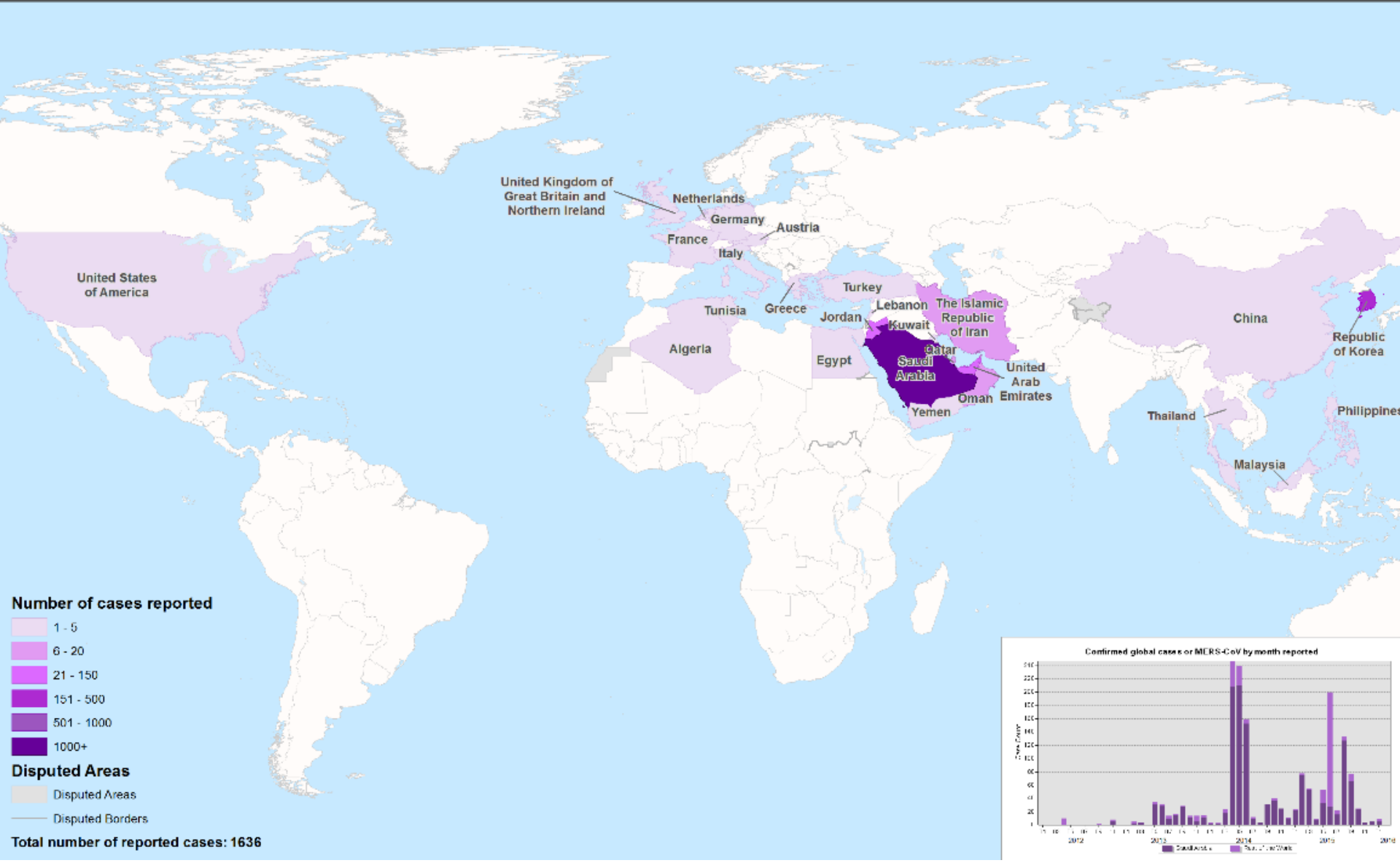
- Pandemic H1N1
- H5N1
- H7N9



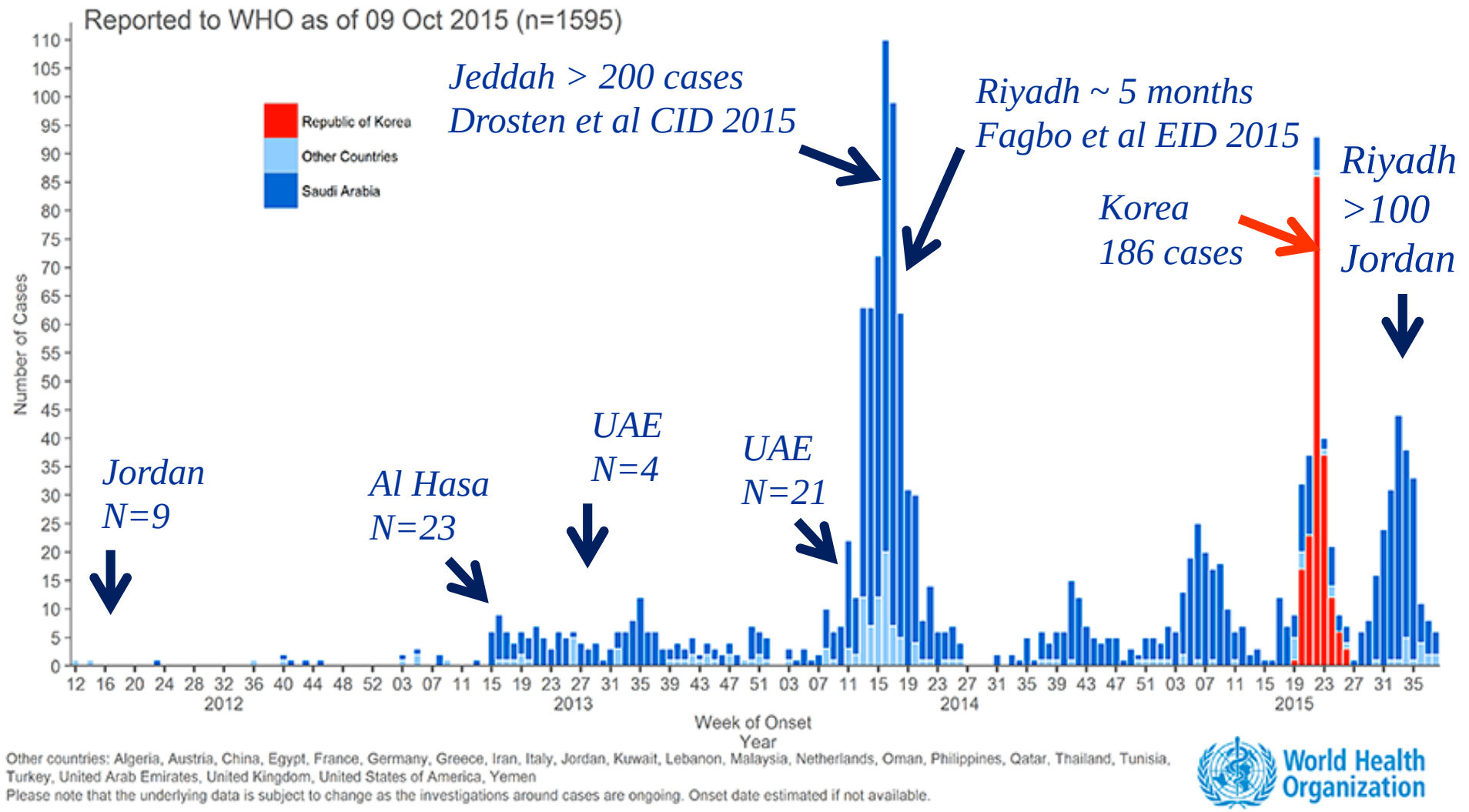
Outline

- Animal hosts / source of zoonotic infection
- MERS-CoV genetic and antigenic diversity
- Therapy/Vaccine?

CONFIRMED GLOBAL CASES OF MERS-COV 2012 - 2016



Epidemic Curve of MERS: Up to 09 October 2015



Other countries: Algeria, Austria, China, Egypt, France, Germany, Greece, Iran, Italy, Jordan, Kuwait, Lebanon, Malaysia, Netherlands, Oman, Philippines, Qatar, Thailand, Tunisia, Turkey, United Arab Emirates, United Kingdom, United States of America, Yemen

Please note that the underlying data is subject to change as the investigations around cases are ongoing. Onset date estimated if not available.

World Health Organization

Animal source of MERS-CoV?

Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study

Lancet Infect Dis 2013

Chantal B E M Reusken*, Bart L Haagmans*, Marcel A Müller*, Carlos Gutierrez, Gert-Jan Godeke, Benjamin Meyer, Doreen Muth, V Stalin Raj, Laura Smits-De Vries, Victor M Corman, Jan-Felix Drexler, Saskia L Smits, Yasmin E El Tahir, Rita De Sousa, Janko van Beek, Norbert Nowotny, Kees van Maanen, Ezequiel Hidalgo-Hermoso, Berend-Jan Bosch, Peter Rottier, Albert Osterhaus, Christian Gortázar-Schmidt, Christian Drosten, Marion P G Koopmans

Eurosurveillance 2013

Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt, June 2013

R A Perera[1,2], P Wang[2,3,4], M R Gomaa[5], R El-Shesheny[5], A Kandeil[5], O Bagato[5], L Y Siu[3], M M Shehata[5], A S Kayed[5], Y Moatasim[5], M Li[3], L L Poon[1], Y Guan[1], R J Webby[6], M A Ali[5], J S Peiris (malik@hku.hk)[1], G Kayali (ghazi.kayali@stjude.org)[6]

Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation

Lancet Infect Dis 2014

Bart L Haagmans*, Said H S Al Dhahiry*, Chantal B E M Reusken*, V Stalin Raj*, Monica Galiano, Richard Myers, Gert-Jan God Elmoubasher Farag, Ayman Diab, Hazem Ghobashy, Farhoud Alhajri, Mohamed Al-Thani, Salih A Al-Marri, Hamad E Al Roma Abdullatif Al Khal, Alison Bermingham, Albert D M E Osterhaus, Mohd M AlHajri, Marion P G Koopmans

Emerg Infect Dis 2014

MERS Coronavirus in Dromedary Camel Herd, Saudi Arabia

Maged G. Hemida,[1] Daniel K.W. Chu,[1] Leo L.M. Poon, Ranawaka A.P.M. Perera, Mohammad A. Alhammadi, Hoi-yee Ng, Lewis Y. Siu, Yi Guan, Abdelmohsen Alnaeem, and Malik Peiris

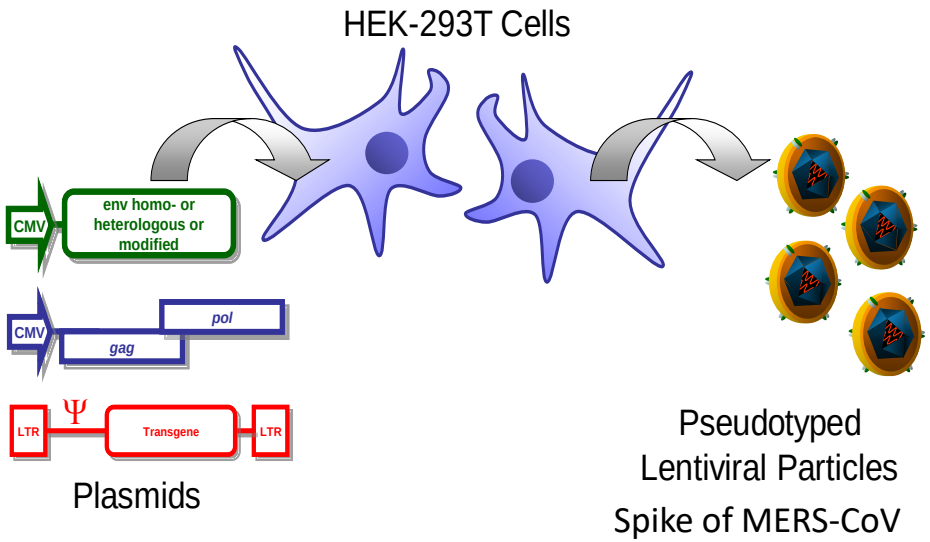
Evidence for Camel-to-Human Transmission of MERS Coronavirus

Esam I. Azhar, Ph.D., Sherif A. El-Kafrawy, Ph.D., Suha A. Farraj, M.Sc., Ahmed M. Hassan, M.Sc., Muneera S. Al-Saeed, B.Sc., Anwar M. Hashem, Ph.D., and Tariq A. Madani, M.D.

NEJM 2014

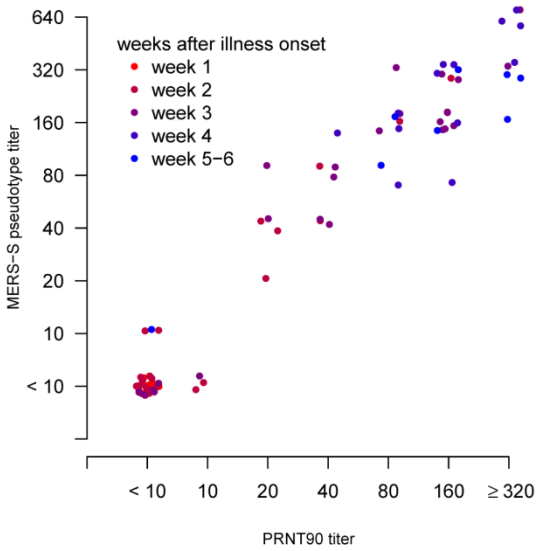
Serological surveillance of MERS-CoV using pseudotyped virus in a BSL2 facility

H5N1 (J Clin Virol, 2010)
MERS (Euro Surveill. 2013)
Ebola (Clin Chem, 2015)

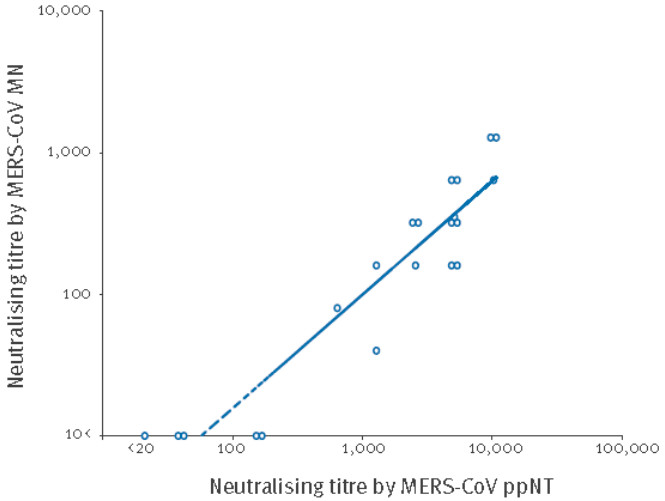


Human sera from the MERS outbreak in Seoul

EID 2015



- **A good correlation between MN and ppNT**



- **Good specificity**

Control serum positive for:

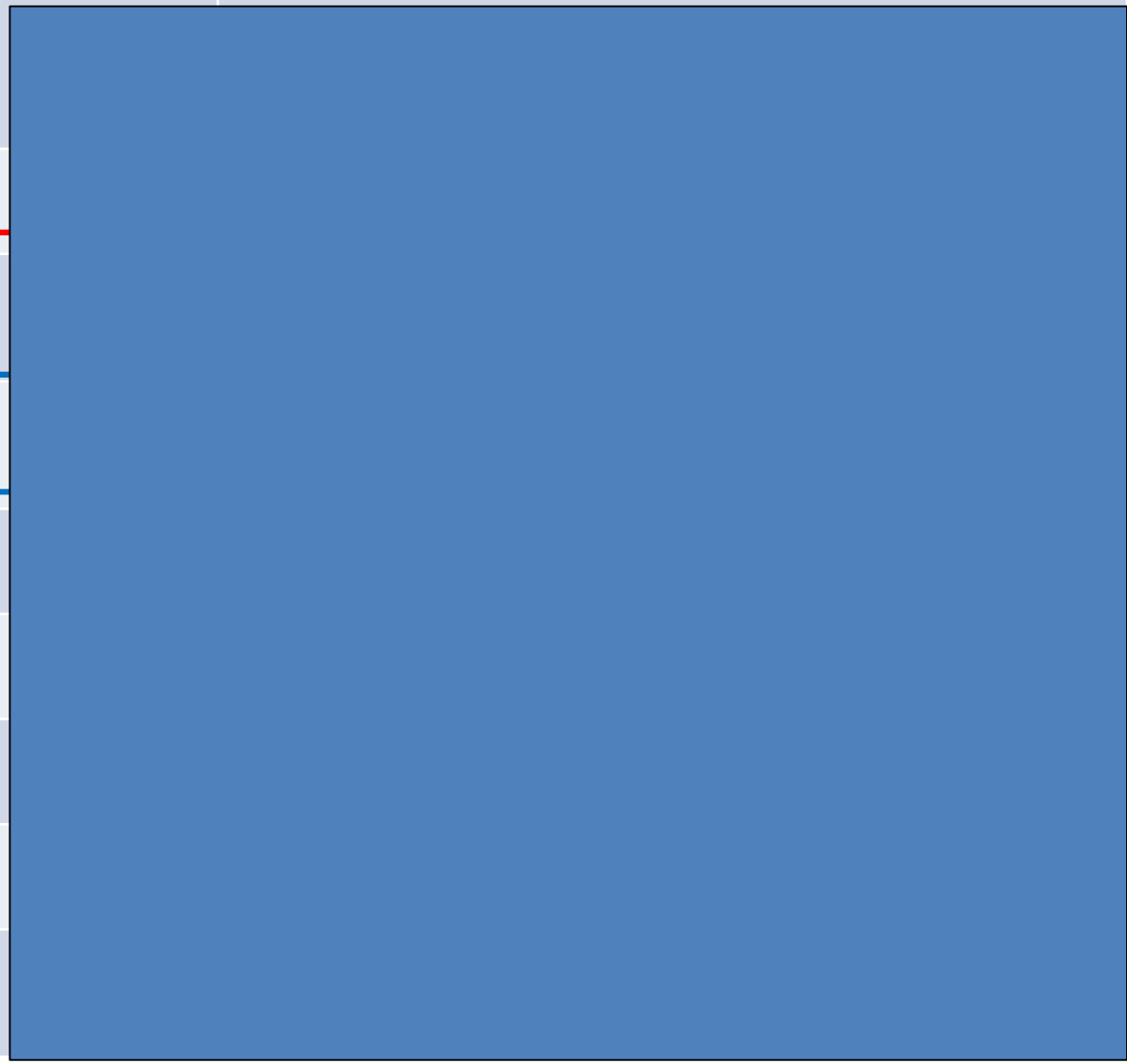
CCoV BCoV TCoV
TGEV SARS-CoV
PRCoV MHV
FIPV

All negative in the assay

Euro Surveill. 2014; Euro Survieill. 2015

Sero-prevalence studies in animals and humans (ppNT/MN)

Animal	KSA	Egypt
Camels	310 (90%)	110 (94%)
Camels (1993)	131 (96%)	
Goats	144 (0%)	13 (0%)
Sheep	199 (0%)	5 (0%)
Cattle	101 (0%)	8 (0%)
Chicken	351 (0%)	
Human	350 (0%)	815 (0%)



Perera et al Eurosurveill 2013; Hemida et al Eurosurv 2013; Chu et al EID 2014; Chan et al EID 2015; Miguel et al EID – submitted; Unpublished data

MERS CoV: Geographic range and species

MERS-CoV active

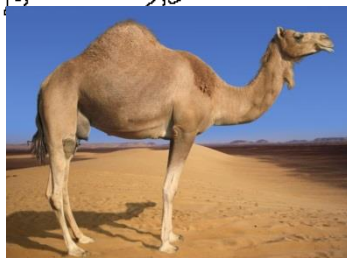
No MERS-CoV

Chan et al EID 2015

77% of global dromedary population

Wherever seropositive camels are found, virus can be detected by RT-PCR (at much lower prevalence)

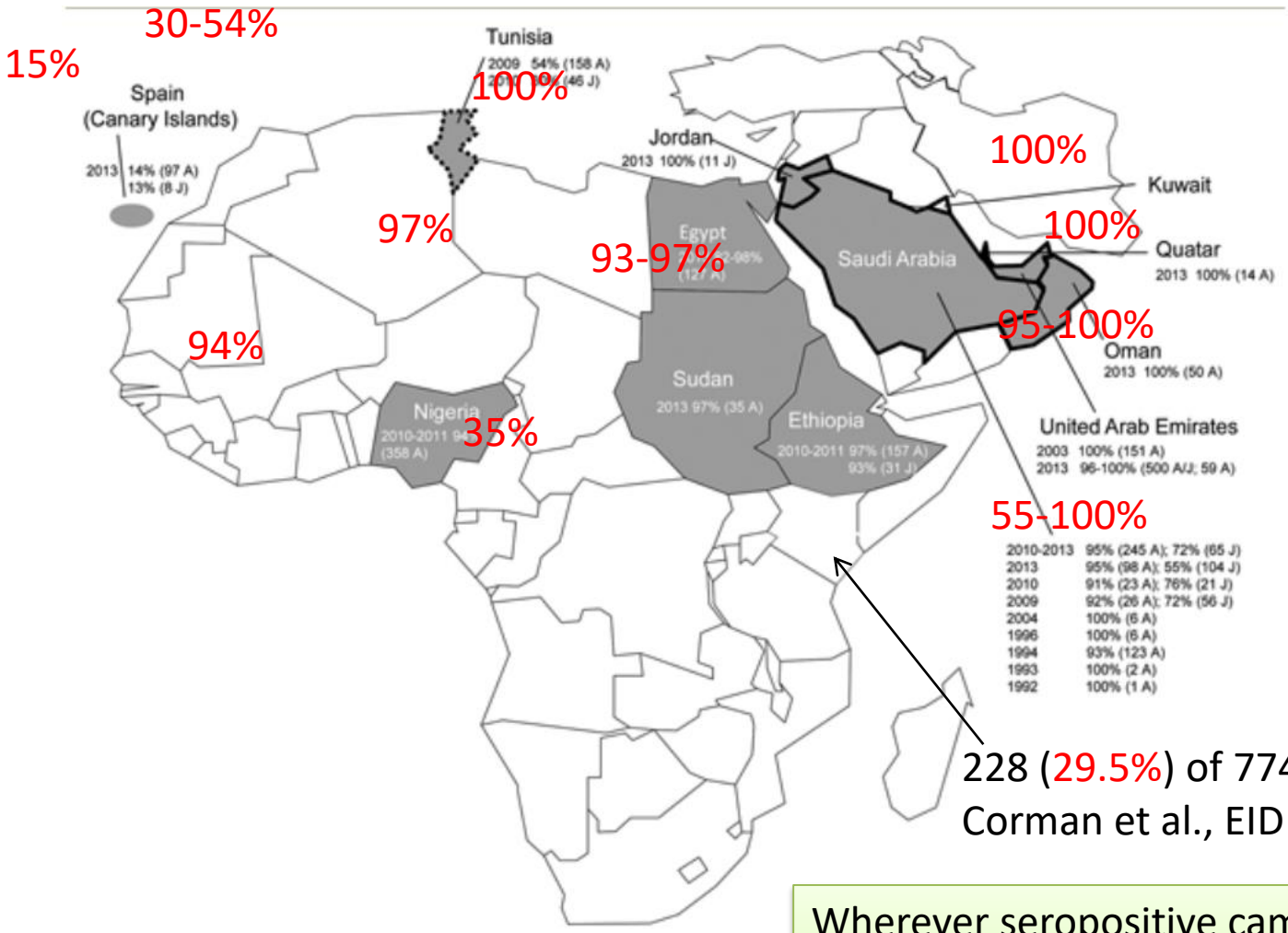
4%



Reusken et al EID 2014; Perera et al EID 2013; Hemida et al 2014; Chan et al 2015; Chu et al., Euro Surveill 2015; Unpublished data

How widespread of MERS-CoV in dromedary camels?

Summarized by Reusken et al. (EID 2014)



228 (29.5%) of 774
Corman et al., EID 2014

Camel sera sampled in Sudan in1983 are seropositive for MERS-CoV (Muller et al., EID 2014)

Wherever seropositive camels are found, virus can be detected by RT-PCR (at much lower prevalence)

Full genome sequence phylogenetic tree of MERS-CoV transmission within a camel herd over 1 month

November 30

December 30

Identical over 30,100 nt

Hemida MG, et al Emerg Infect Dis. 2014 Jul;20(7):1231-4.

Full genome sequence of Vero cell isolate differed from direct sequencing from clinical specimen in 3 nucleotides:
Synonymous: ORF1b;
Non-synonymous:
Spike (S1251F);
Membrane (T8I) proteins

A dromedary herd with virus transmission for 1 month

Table 1. RT-PCR of dromedary camel samples for MERS-CoV, Al-Hasa, Saudi Arabia*

Farm, sampling date	Age†/no. sampled	No. specimens positive/no. tested		
		Nasal	Oral	Fecal
Farm A				
2013 Nov 30	Calf, 0	ND	ND	ND
	Adult, 4	1/1	0/2	0/4
2013 Dec 30	Calf, 8	7/8	0/1	0/6
	Adult, 3	1/3‡	0	1/3‡
2014 Feb 14	Calf, 7	0/7	ND	0/7
	Adult, 2	0/2	ND	0/2

Hemida et al EID 2014

- *Nasal > faecal*
- *Duration of shedding < 1 month*

Contacts:

Herdsman n=4

Other staff in contact with herd n=8

Staff in camel hospital n=30

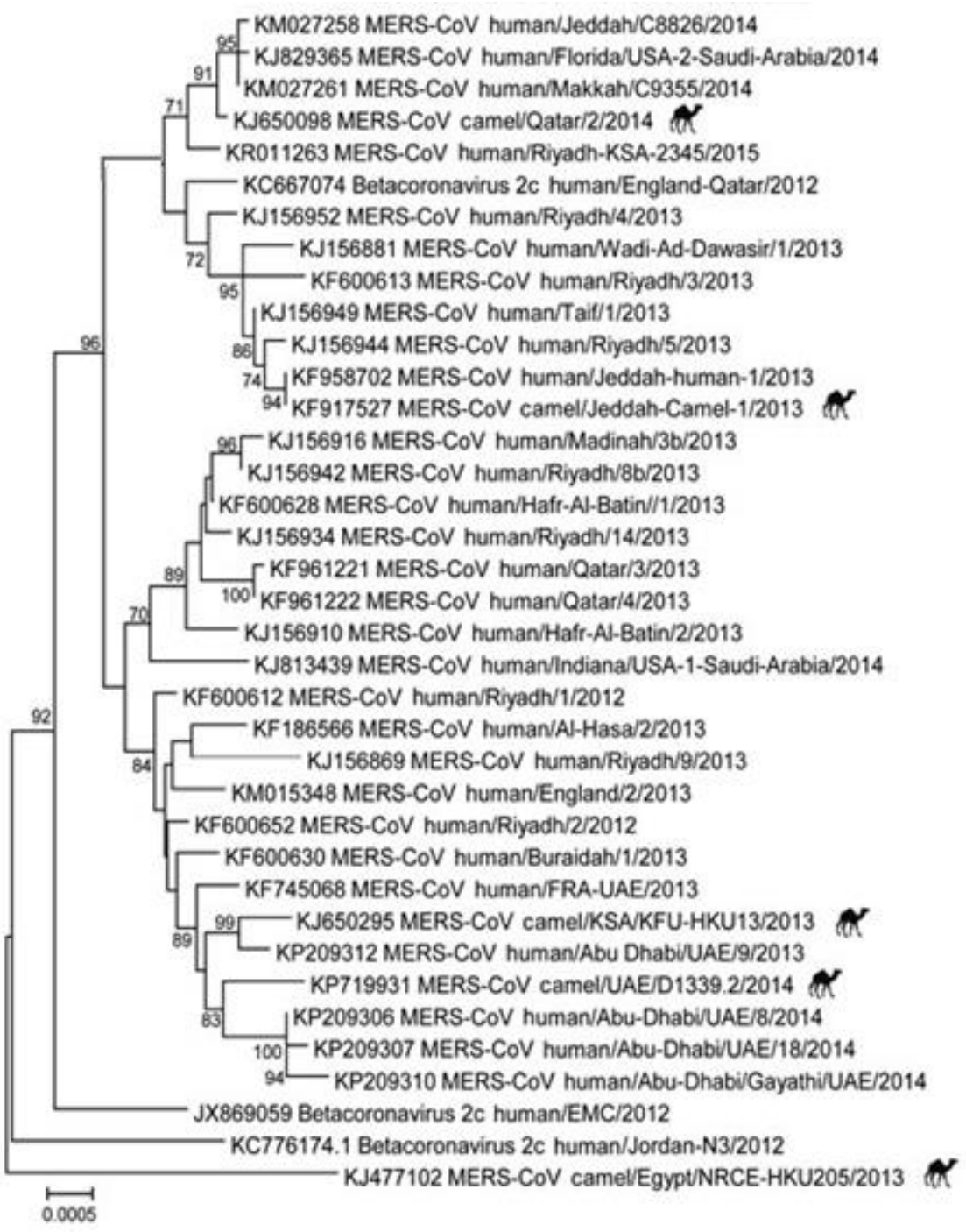
General population = n=146

All sero-negative

Transmission to humans is inefficient

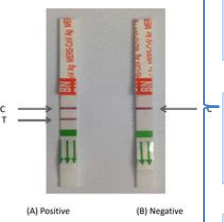
Hemida et al EID 2015

Multiple introduction of MERS-CoV from camel to human



Asymptomatic MERS-CoV Infection in Humans Linked to Infected Dromedaries

Rapid antigen test



PPNT test

Camels

7/5/2015: Dromedary camels transported from Oman to UAE. Sampled at border.
8/8 RT-PCR pos for MERS-CoV

14/5/2015: Dromedary camels still RT-PCR positive

25/5/2015: Dromedary camels RT-PCR negative

Contacts 1 and 2

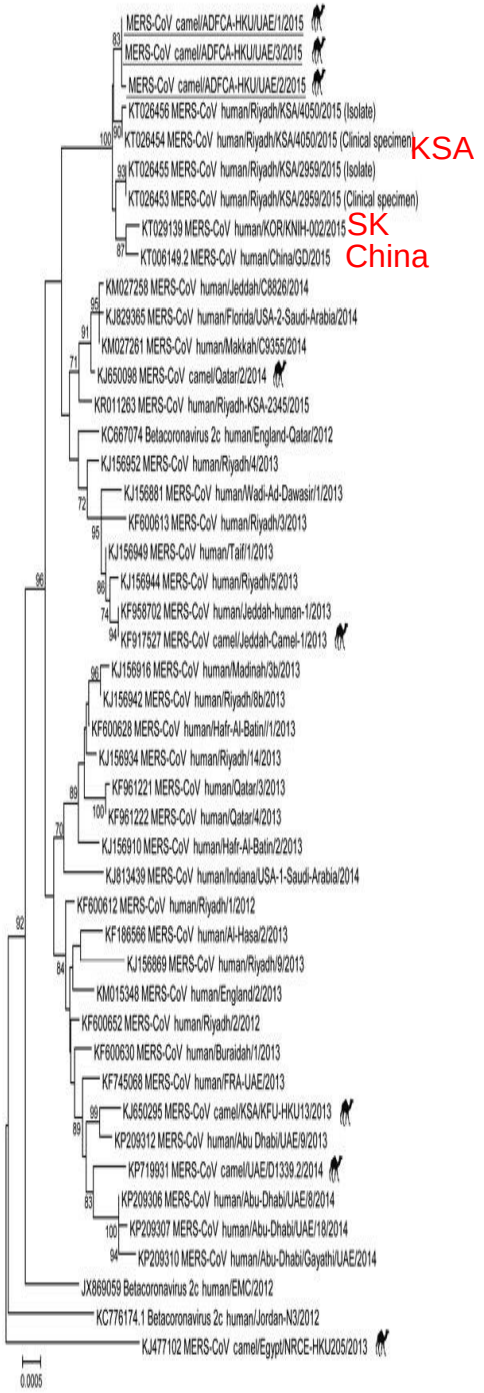
Contact 1: Driver of truck; asymptomatic
10/5/2015: RTPCR +ve
12/5/2015: RT-PCR +ve, Hospitalized
13/5/2015: RT-PCR +ve
14/5/2015: RT-PCR +ve
18/5?2015: RT-PCR -ve

Contact 2: Cleaner in quarantine station, had helped sample dromedaries; asymptomatic
14/5/2015: RTPCR +ve
18/5/2015: RT-PCR -ve, Hospitalized
21/5/2015: RT-PCR -ve

32 other contacts of dromedaries

All RT-PCR -ve

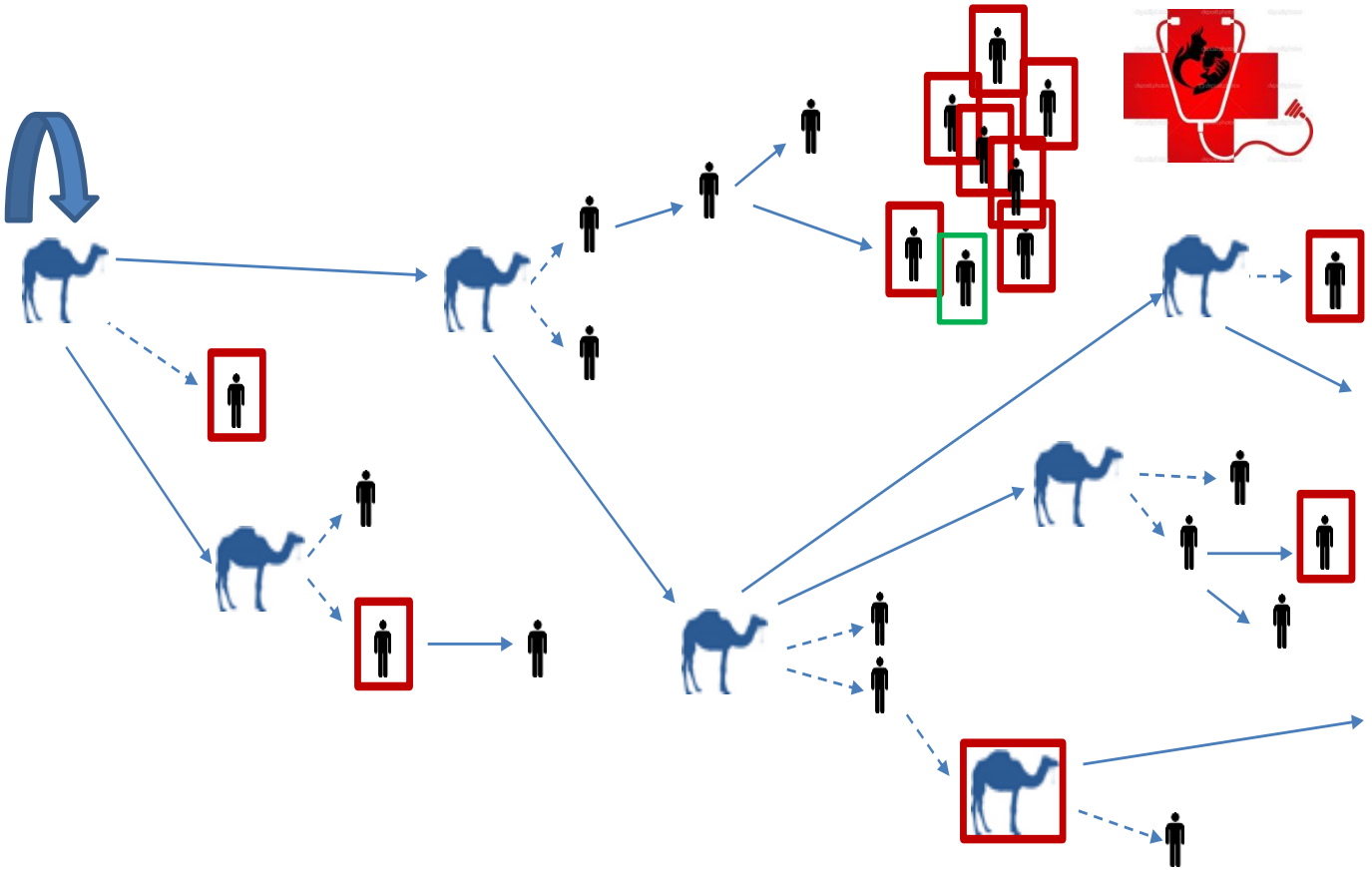
Viral RNA sequence from dromedaries were identical with each other. Human sequences were also identical but only short fragments were available.



Al Hammadi et al., EID 2015

Epidemiology of MERS-CoV: What we know

Sustained transmission in animals, not sustained in humans



Modified from Ferguson & Van Kerkhove 2014

Countrywide cross-sectional sero-epidemiological survey for MERS-CoV antibodies in Saudi Arabia

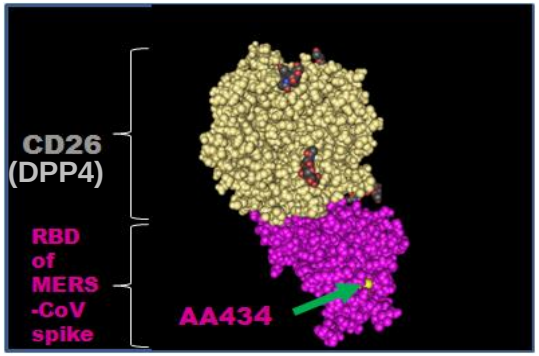
Population sampled	Sero-positives (NT test)		
	N (%)	95% CI	P value
General population (>15 years of age) (n=10,009)	15 (0.2%)	(0.1-0.2%)	Reference
Camel shepherds (n-87)	2 (2.3%)	(0.3-7.4%)	0.0004
Slaughterhouse workers (n=140)	5 (3.6%)	(1.3-7.7%)	0.0001

Muller et al Lancet Infect Dis 2015

- **Estimated 44,951 *(95% CI: 26,971-71,922)* people > 15 years old have been infected with MERS-CoV in Saudi Arabia over their lifetimes**
  - ***Total Saudi population is 29.8 million***
- **Median age of sero-positives younger than confirmed MERS cases** (43.4 yrs vs. 53.8 yrs (p=0.008)
- **Sero-prevalence of Men > Women** (Men 0.25% > Women 0.05%) (p=0.28)
- Higher in central vs costal provinces

Subclinical infected human cases as the viral source in communities ?

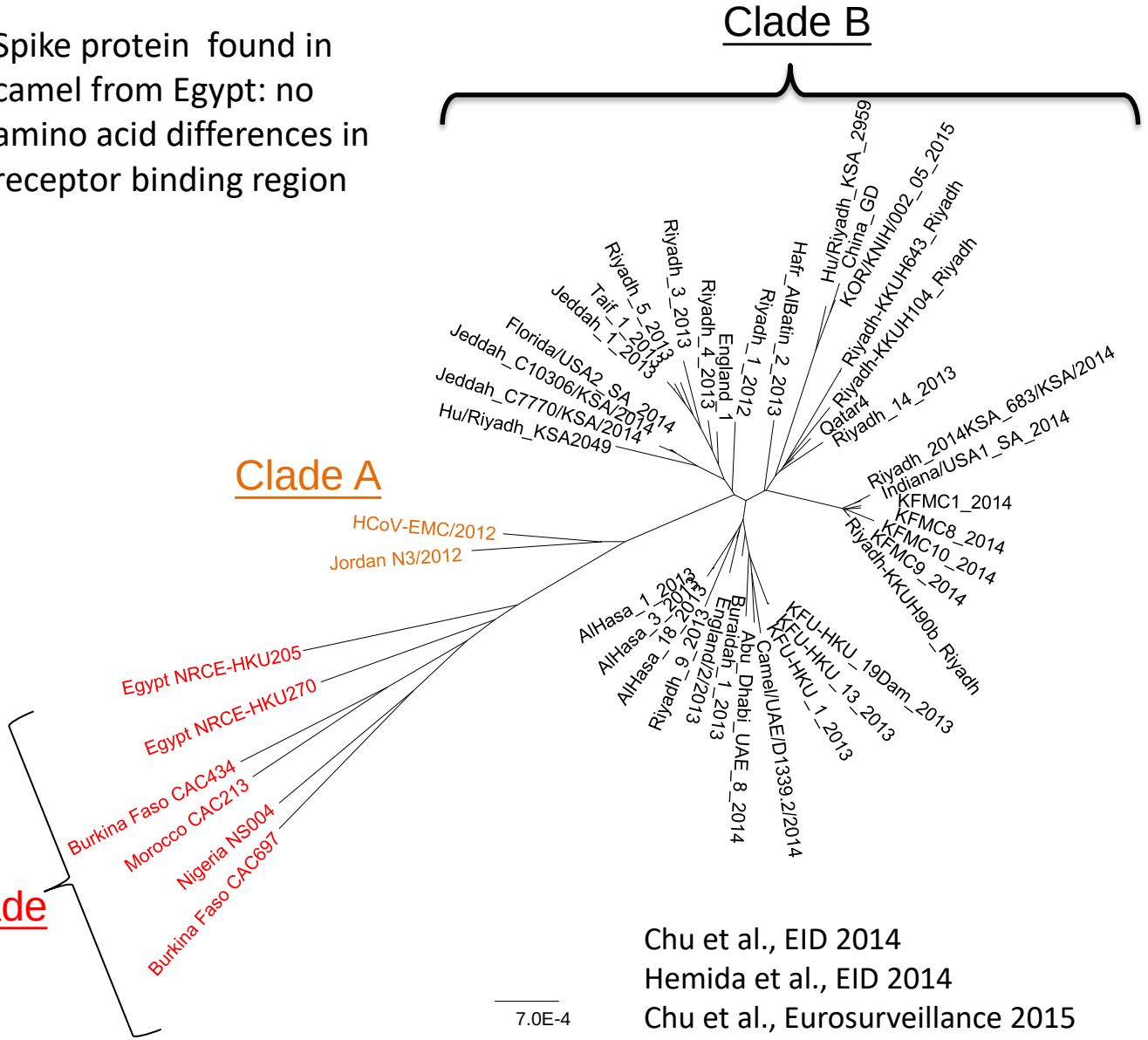
MERS-CoV genetic diversity:
Does camel MERS-CoV from Africa have capacity to infect humans?



Spike protein found in camel from Egypt: no amino acid differences in receptor binding region

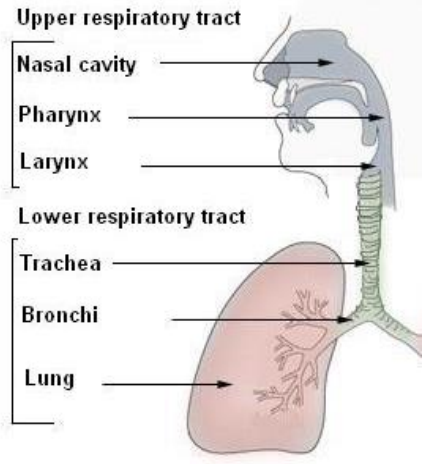
Top 10 countries for camel populations (FAO, 2012)

	Country	species	Number	Unit
*	Somalia	Camels	7000000	Heads
	Sudan	Camels	4751000	Heads
	Kenya	Camels	3065400	Heads
	Niger	Camels	1676567	Heads
	Chad	Camels	1450000	Heads
	Mauritania	Camels	1425000	Heads
	Pakistan	Camels	1000000	Heads
	Mali	Camels	959783	Heads
*	Ethiopia	Camels	915518	Heads
	India	Camels	438000	Heads



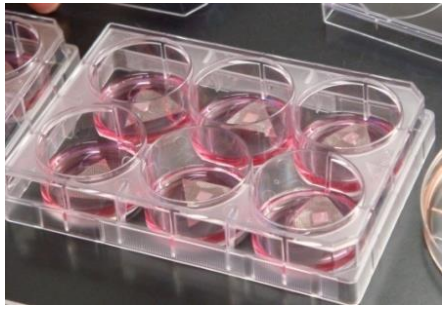
Chu et al., EID 2014
Hemida et al., EID 2014
Chu et al., Eurosurveillance 2015

Ex vivo cultures - Human respiratory tract



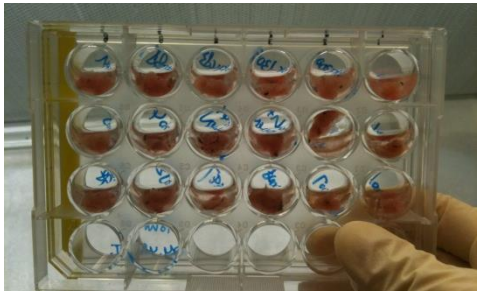
ALI culture = Air liquid interface culture; F12K medium

Nasopharyngeal

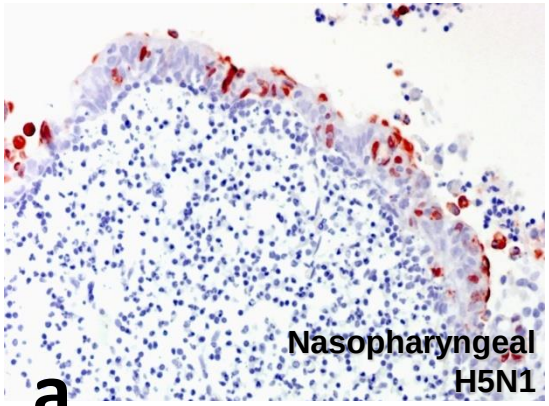


Bronchi

Lung

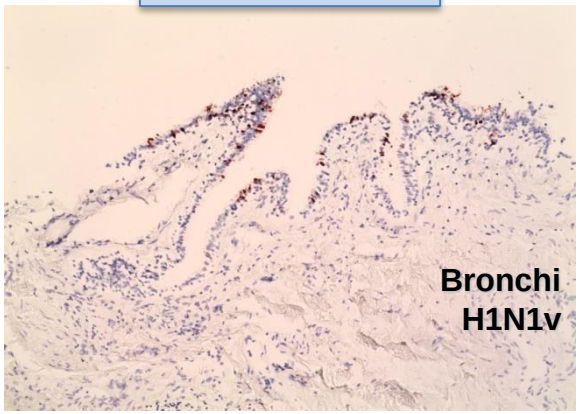


ALI culture – sponge

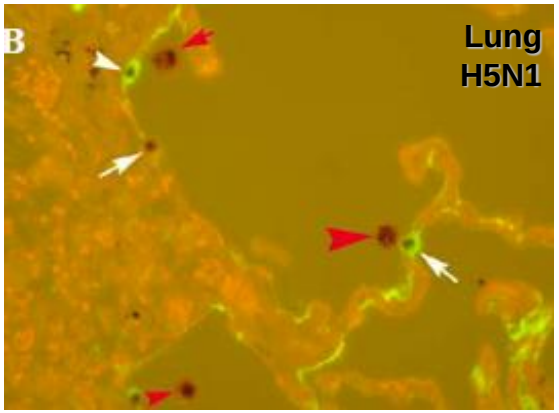


a

ALI culture – Rotor / sponge

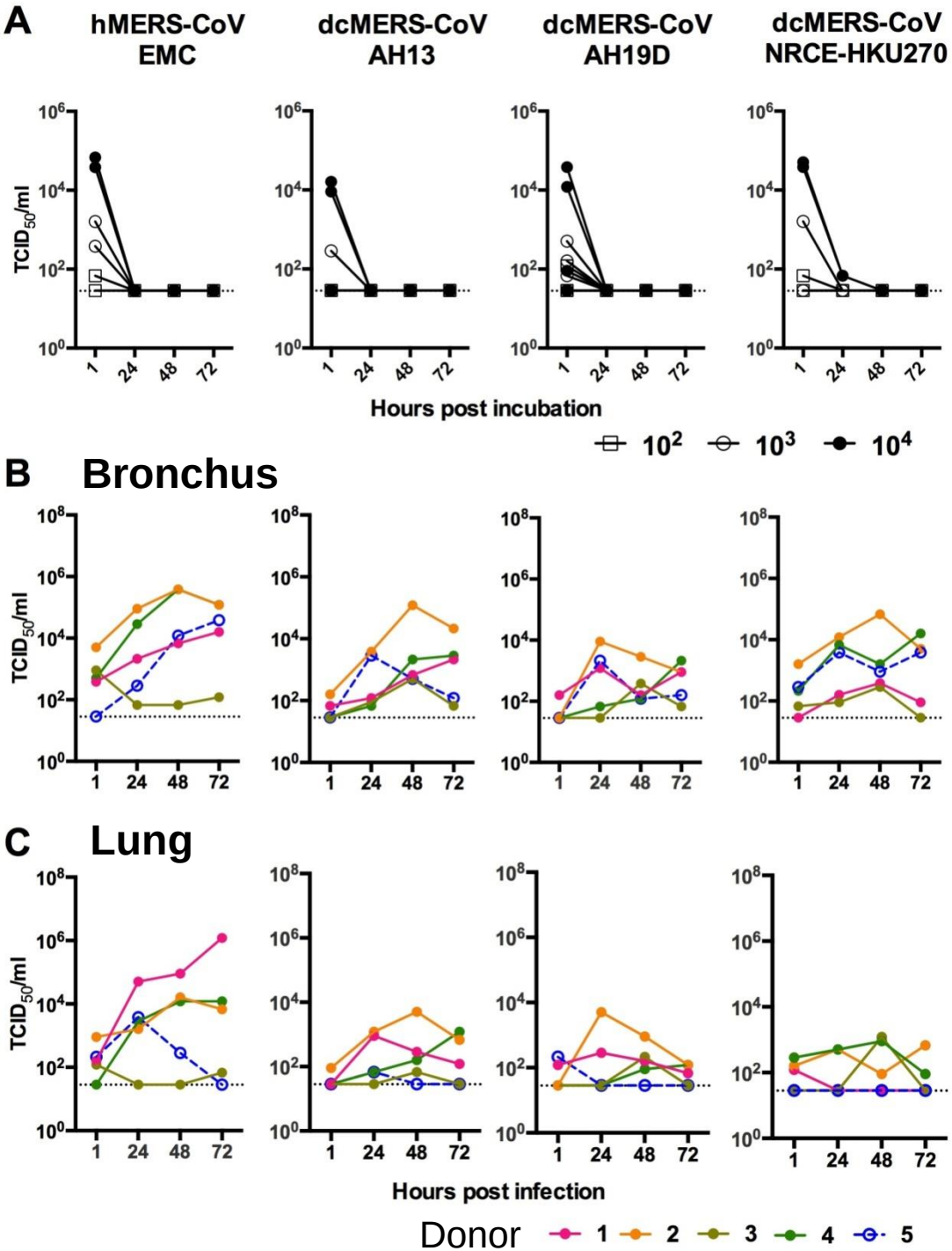


Lung slice culture



Nicholls et al Nat Med 2007; 13: 147-9; Chan et al Virus Res 2013; 178: 133-45

Replication competence of of human and dromedary MERS-CoV in human bronchus and lung



Chan et al Lancet Resp Med 2014

Genetically and phenotypically, Saudi and Egyptian camel MERS-CoV appears able to infect the human respiratory tract

Why no zoonotic MERS in Africa?

Dromedary camels

Camel milk:
taken fresh without boiling

Camel racing

Camel slaughterhouse

Genetically diverse MERS-CoV are antigenically homogenous

Patient ID	Day of illness	Reciprocal $PRNT_{90}$ antibody titer to MERS-CoV		
		EMC (clade A)	Camel Al-Hasa KFU-HKU13 (clade B)	Camel Egypt NRCE-HKU 270 (clade non A/B)
B	12	320	160	160
B	39	320	320	640
G	17	40	40	80
G	35	160	80	160

Collaboration with Seoul National University College of Medicine
Park SW, Perera RAPM, -- Peiris M, Oh MD – Eurosurveillance – on line

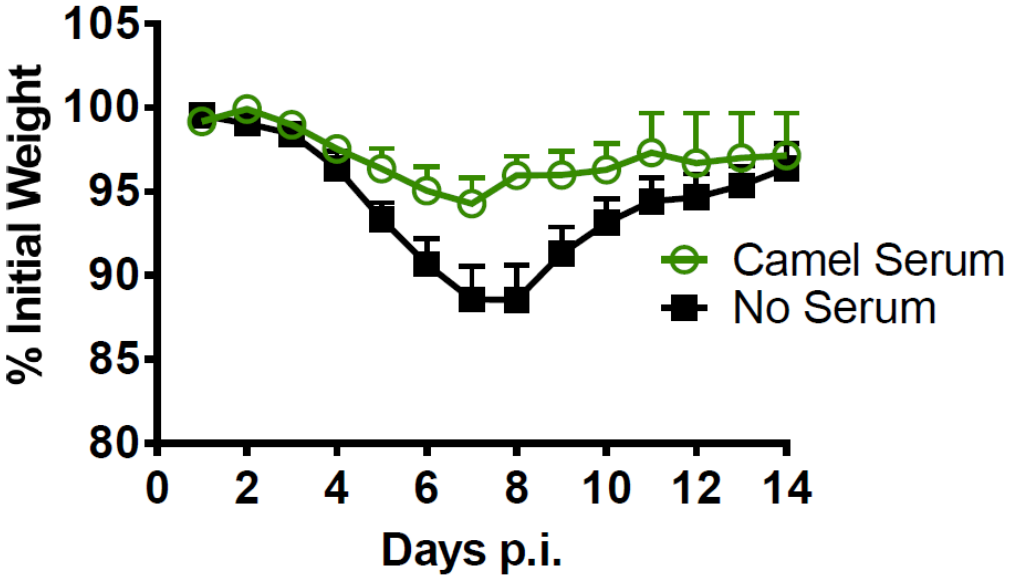
Also demonstrated with naturally infected camel sera

Hemida MG, et al Emerg Infect Dis. 2014 Jul;20(7):1231-4.

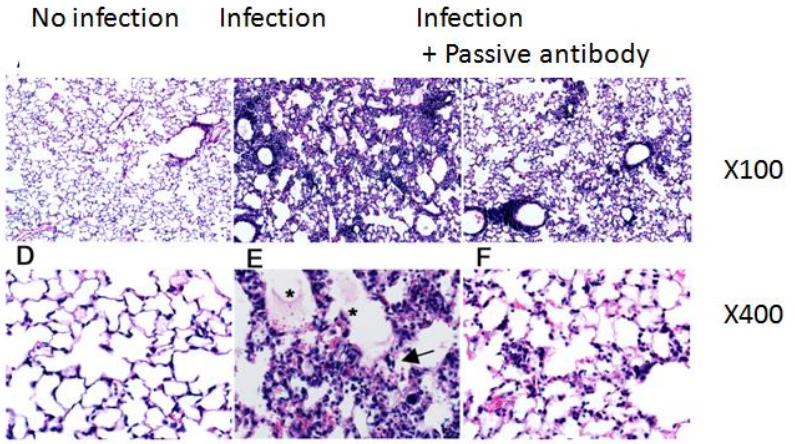
Can we treat MERS with camel immune serum?

Weight loss of IFNAR-/- hDPP4 transfected mice challenged with MERS-CoV followed by passive therapy with MERS-CoV immune serum

Ad5-hDPP4 transfection of IFNAR-/- mice 5 days before challenge
→ Challenge (i.n.) with 10^5 MERS CoV
→ Treat with 200ul camel serum (i.p.) neut Ab tier 1:1280 1 day later



N=5 mice per group



Immune sera could be also used for prophylactic purposes in experimental conditions

Zhao et al. J Virol 2015

Science AAAS

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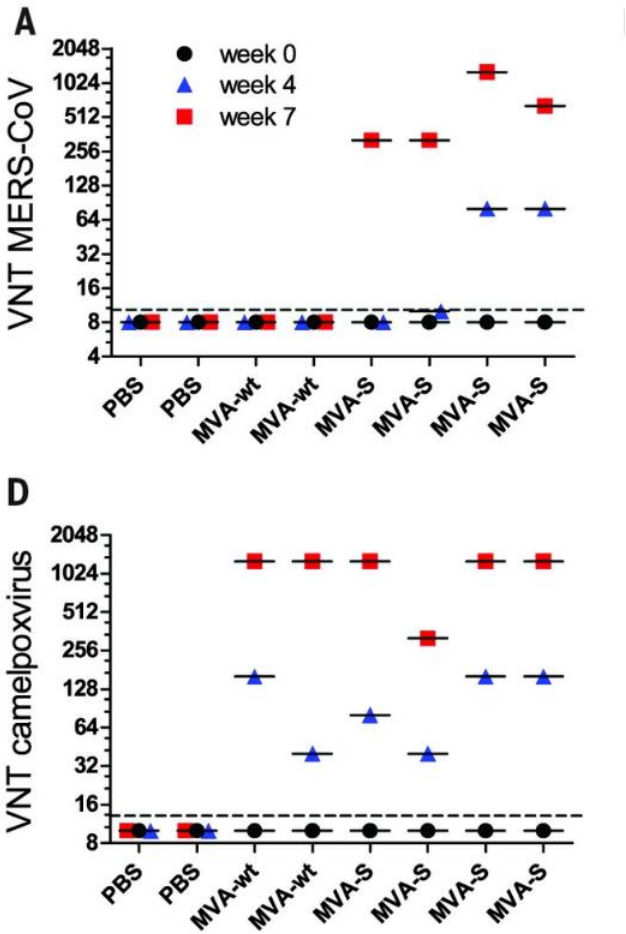
REPORT

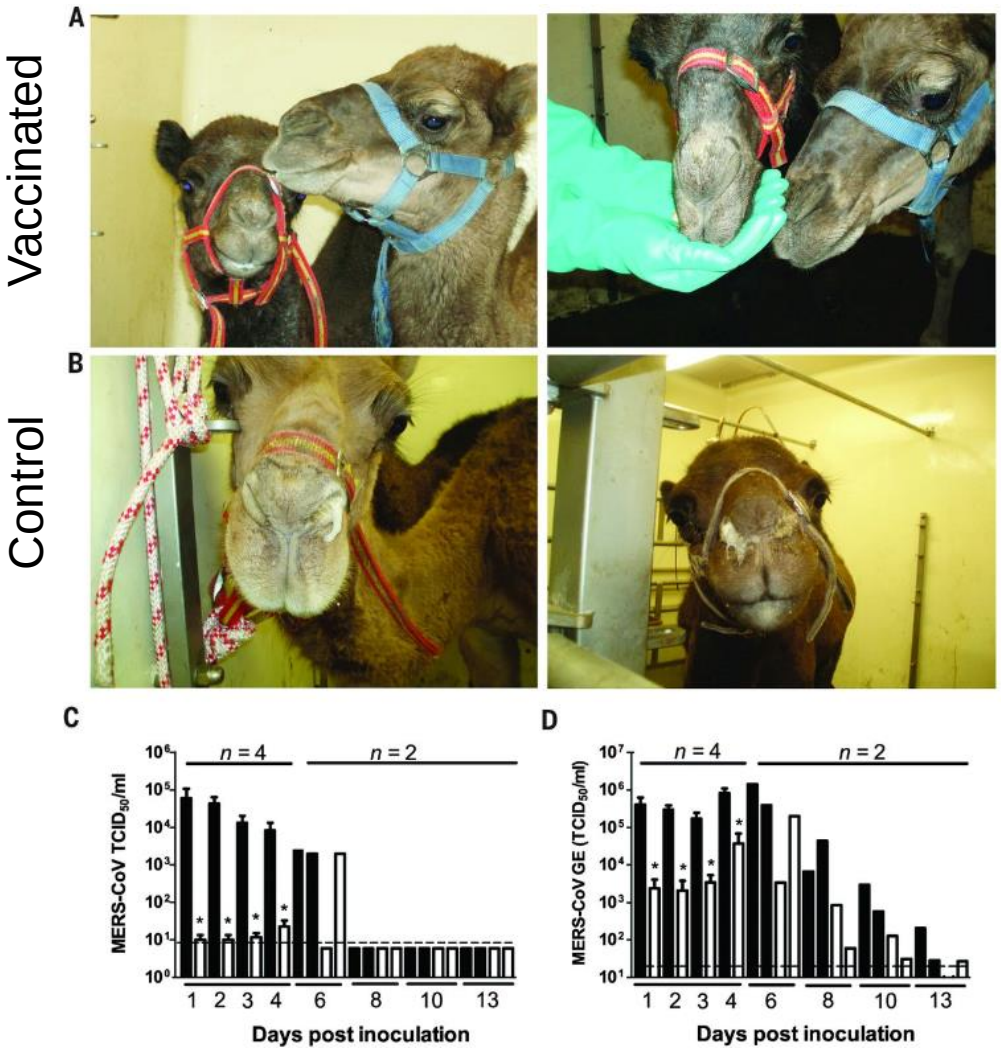
An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels

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,*]

Hurdles:

- Cannot induce sterilizing immunity
- Protective duration?





Detection of infection MERS-CoV in adult camel with high neutralizing antibody

- NT antibodies might not confer a complete protection against MERS-CoV?
- Possibility of re-infections?

A lot of unknown about MERS-CoV in camels.

Technical Appendix Table. Testing of dromedary camels by RT-PCR and serologic testing for MERS-CoV, Al-Hasa, Saudi Arabia*

Farm, sampling date	Camel no.	Calf/Adult†	Age	RT-PCR result: Nasal sample	RT-PCR result: Oral sample	RT-PCR result: Fecal sample	Copy/mL‡	Antibody titers§
Farm A								
2013 Nov 30	1	Adult	13 y	Pos¶	Neg	Neg	2.61×10^7	>5,120
	2	Adult	12 y	–	–	Neg		>5,120
	3	Adult	10 y	–	Neg	Neg		>5,120
	4	Adult	14 y	–	–	Neg		>5,120
2013 Dec 4	5	Adult	8 y	–	Neg	Neg		640
	6	Adult	9 y	–	Neg	Neg		2,560
	2	Adult	10 y	–	Neg	–		>5,120
	7Calf	Calf	1–2 y	–	Neg	Neg		1,280
	7Dam	Adult	9.5 y	–	Neg	–		2,560
	8	Adult	7 y	–	Neg	Neg		1,280
	9	Adult	6 y	–	Neg	Neg		1,280
	10	Adult	8 y	–	Neg	Neg		640
	11Calf	Calf	1–2 y	–	Neg	Neg		–
	11Dam	Adult	–	–	Neg	Neg		–
	12	Adult	12 y	–	Neg	Neg		320
2013 Dec 30	13	Calf	1 y	Pos¶#	–	–	1.30×10^8	<20
	14	Calf	1 y	Pos#	–	Neg	1.78×10^8	<20
	15	Calf	1 y	Pos	–	Neg	6.07×10^6	20
	16	Calf	1 y	Pos	–	Neg	3.78×10^7	>5,120
	17	Calf	40 d	Pos	–	Neg	4.86×10^4	80
	18	Calf	40 d	Neg	Neg	–		–
	19Calf	Calf	1 y	Pos	–	Neg	2.41×10^7	–
	19Dam	Adult	–	Neg	–	Pos¶# *	9.27×10^7	–
	20	Adult	8 y	Neg	–	Neg		>5,120
	21	Adult	7 y	Pos	–	Neg	3.31×10^3	320
	22	Calf	2 wk	Pos	–	Neg	3.38×10^3	1,280
2014 Feb 14	26	Calf	9 mo	Neg	–	Neg		>5,120
	13	Calf	1 y	Neg	–	Neg		640
	27	Calf	10 mo	Neg	–	Neg		40
	15	Calf	1 y	Neg	–	Neg		160
	17	Calf	3 mo	Neg	–	Neg		1,280
	11Dam	Adult	12 y	Neg	–	Neg		1,280
	19Calf	Calf	1 y	Neg	–	Neg		320
	28Calf	Calf	3 mo	Neg	–	Neg		20
	28Dam	Adult	10 y	Neg	–	Neg		1,280
Farm B, 2014 Feb 11	23Calf	Calf	2.5 mo	Neg	–	Neg		–
	23Dam	Adult	7 y	Neg	–	Neg		>5120
	24Calf	Calf	2 mo	Neg	–	Neg		–
	24Dam	Adult	6 y	Neg	–	Neg		1,280
	25Calf	Calf	2 mo	Neg	–	Neg		–
	25Dam	Adult	6 y	Neg	–	Neg		640

*RT-PCR, reverse transcription PCR; MERS-CoV, Middle East respiratory syndrome coronavirus; Pos, positive; Neg, negative; –, specimen not collected or age information not available.
†Calf defined as dromedary camel <2 y of age; adult defined as dromedary camel ≥2 y of age.
‡Data deduced from the upstream of E assay.
§Pseudotype neutralization antibody titers.
¶Full genome sequenced.
#Virus isolated.

Hemida et al., EID 2014

Summary

- Camels are a source of human infection but transmission (leading to disease or seroconversion) is inefficient and routes / modes of transmission to humans are poorly understood.
  - *Asymptomatic humans as a source of transmission?*
  - *Unusual route of exposure?*
  - *Host heterogeneity ?*
- MERS-CoV is genetically diverse but antigenically homogenous
- There are well validated methods for MERS detection
  - MERS-spike pseudoparticle neutralization test is a reliable and specific method for sero-epidemiology / confirmation that does not require BSL-3 containment.
- Promising experimental vaccines have been developed, but their potential use in the field are need to be evaluated

MERS: What we do **NOT** know

- Old disease or new:
  - Virus was circulating in dromedary camels for >30 years. Why no disease till 2012?
- Why not in Africa?
  - Virus is endemic in dromedary camels in Africa. Why no human cases reported?
- Why is zoonotic MERS apparently so rare / stochastic but efficient transmission within health care facilities?
  - Virus is common in dromedary camels, e.g. in calves and in abattoirs.
  - Why are the highly exposed groups not prominent in patients with MERS?
  - Parallels with avian flu H5N1
- Can it / what will lead to, increased transmission within the community? → SARS experience!!
- Route of transmission from camels to humans?
- Other animal hosts (reservoir, intermediate host)?

Technology transfer and collaborations

WHO site visit:
South Korea

WHO/FAO site visit:
Kingdom of Saudi Arabia

FAO site visit:
United Arab Emirates

WHO/FAO/Other trainings:
Kingdom of Saudi Arabia
United Arab Emirates
Overseas visitors

Collaborators from :
Australia
Egypt
KSA
France
Mongolia
South Korea
UAE
Others

Veterinary lab

Slaughterhouse

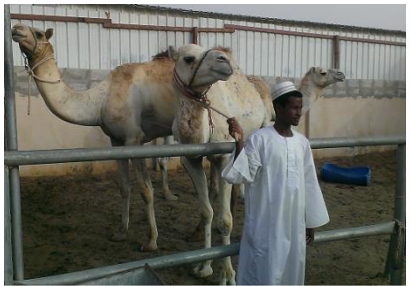
Whole sale market

Hospital

FAO training course in HK

Household visit

Camel farm



Racing court

Food and Agriculture Organization of the United Nations

World Health Organization

Emperie

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