Public Health England

Development of a vaccine candidate against Crimean-Congo Haemorrhagic Fever (CCHF) virus

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National Infections Service: PHE
Porton Down

Public Health England

Infectious Disease Programmes

TB
Vaccines
Antibiotics
Diagnostics

Toxins
Botulinum
Clostridium
Immunotherapy
Diagnostics

Mening & Pertussis
Correlates
Vaccines

Biosafety
Detection
Decontam
HCAI/vCJD
Training

Animal Models
Efficacy studies
Aerosol
Pathol/ Imaging
Immunology

Research

Diagnostics Technology
Diagnostics
Bio/Molecular

GxP
Immune Assay
Clinical Trials/NVEC
Assay Validation
Product release assays

Immune Modulation
Inflammation
Adjuvants

Emerging Diseases
Virology/Influenza
Bacteriology

Medical Counter Measures
NIAID
Anthrax

Detection, treatments and vaccines

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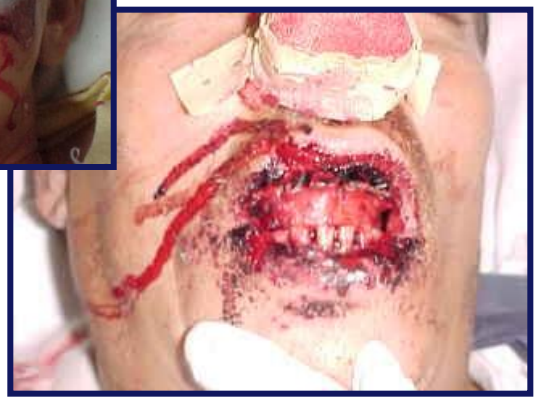
Background

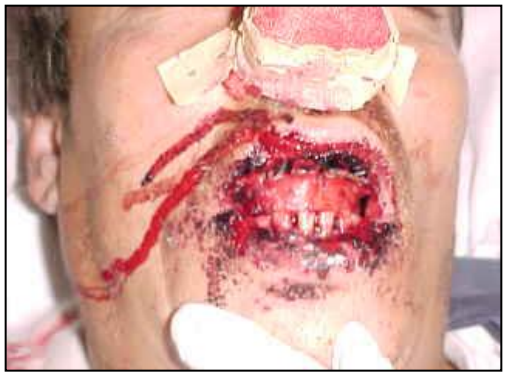
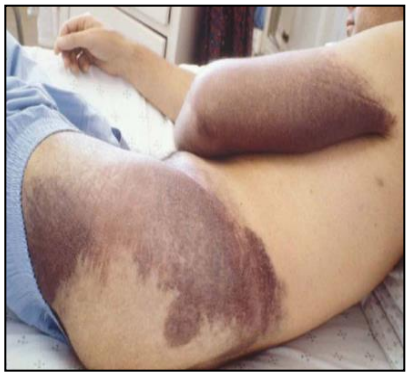


Crimean-Congo Haemorrhagic Fever (CCHF) virus:

- Severe human infection.
- Fatality rate 30% (9-50%).
- No FDA or European approved vaccine or treatment.
- ACDP - Hazard Group 4 pathogen.
- Reservoired in ticks & wild life mammals, amplified in cattle sheep, goat, camel [No disease in animals]
- Transmission by tick bite or direct / indirect contact with infected blood/body fluids.

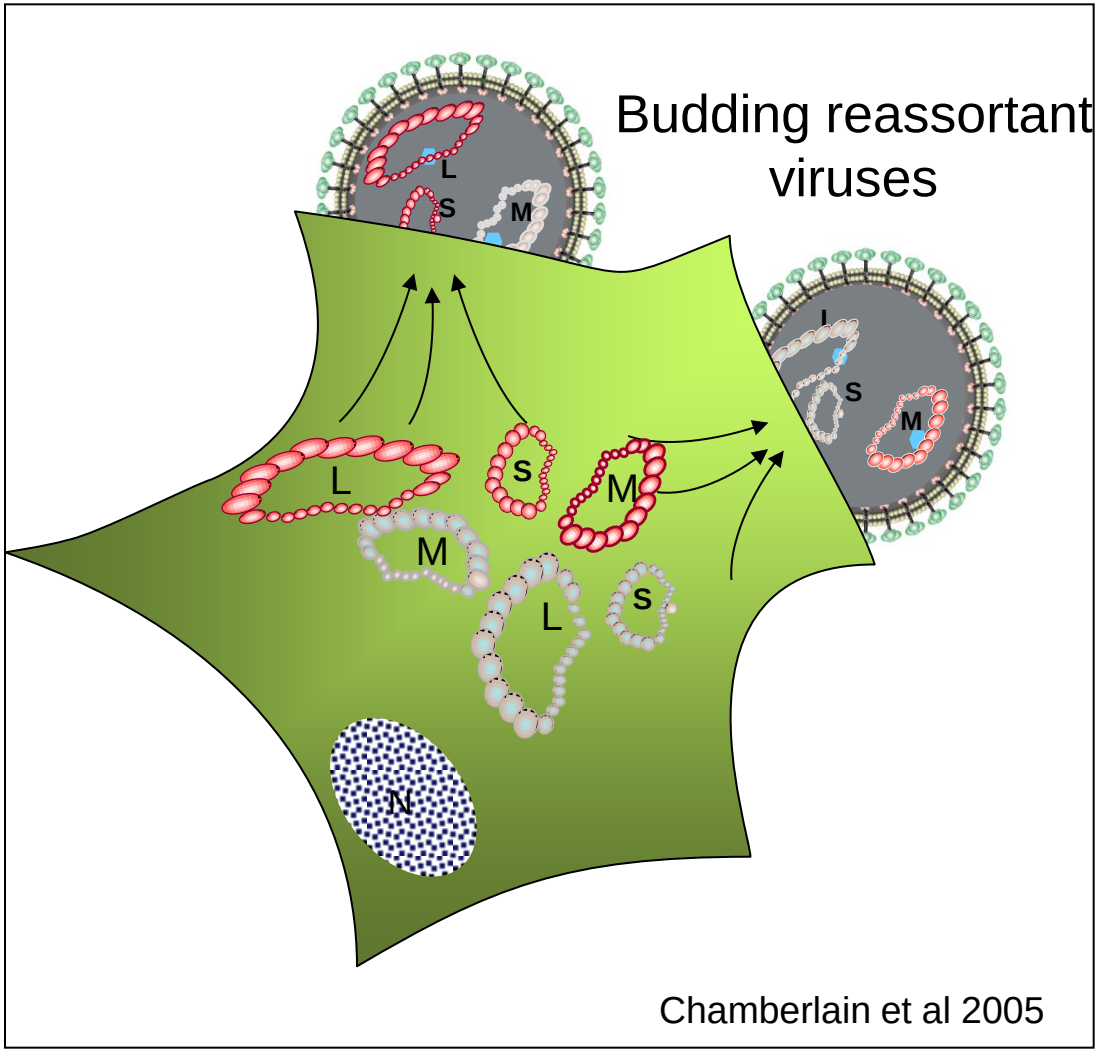
CCHF - Clinical Disease



- Incubation period 2-9 days
- Haemorrhagic state develops 3 - 5 days
- Petechial rash / ecchymoses in the skin
- Bleeding from the mucous membranes
  Epitaxsis, Haematuria, Haemoptysis
- Loss of blood pressure - shock
- Death 7-9 days
  [massive bleeding / cardiac arrest]


Re-assortment in CCHF viruses could lead to new viruses and new disease…



Exchange of M segments influence host range

Envelope glycoproteins influence cellular tropism

➡ *altered pathogenicity*

But can we:

… Detect

….Protect against

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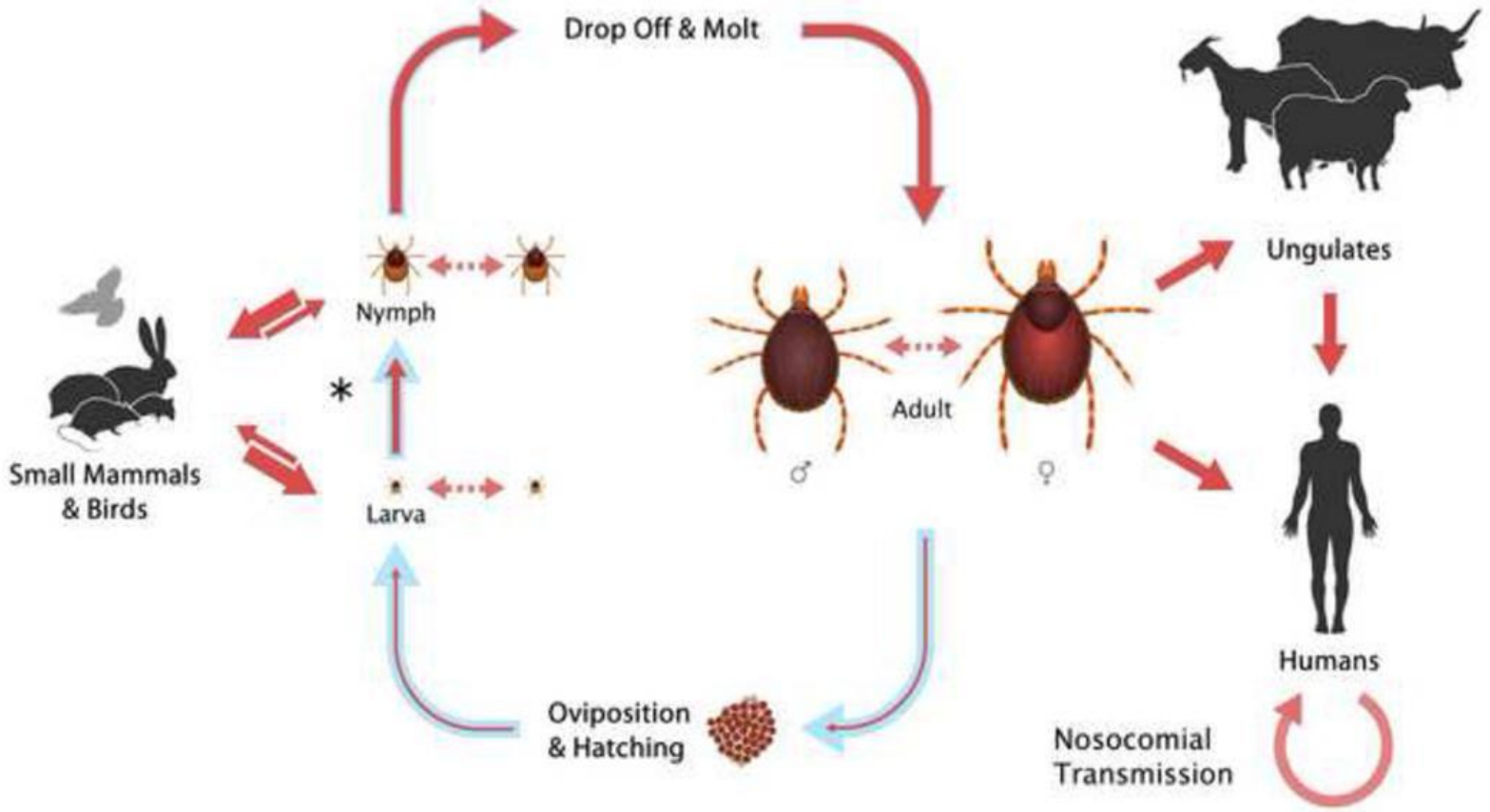
CCHFV Transmission cycle



Bente et al 2012

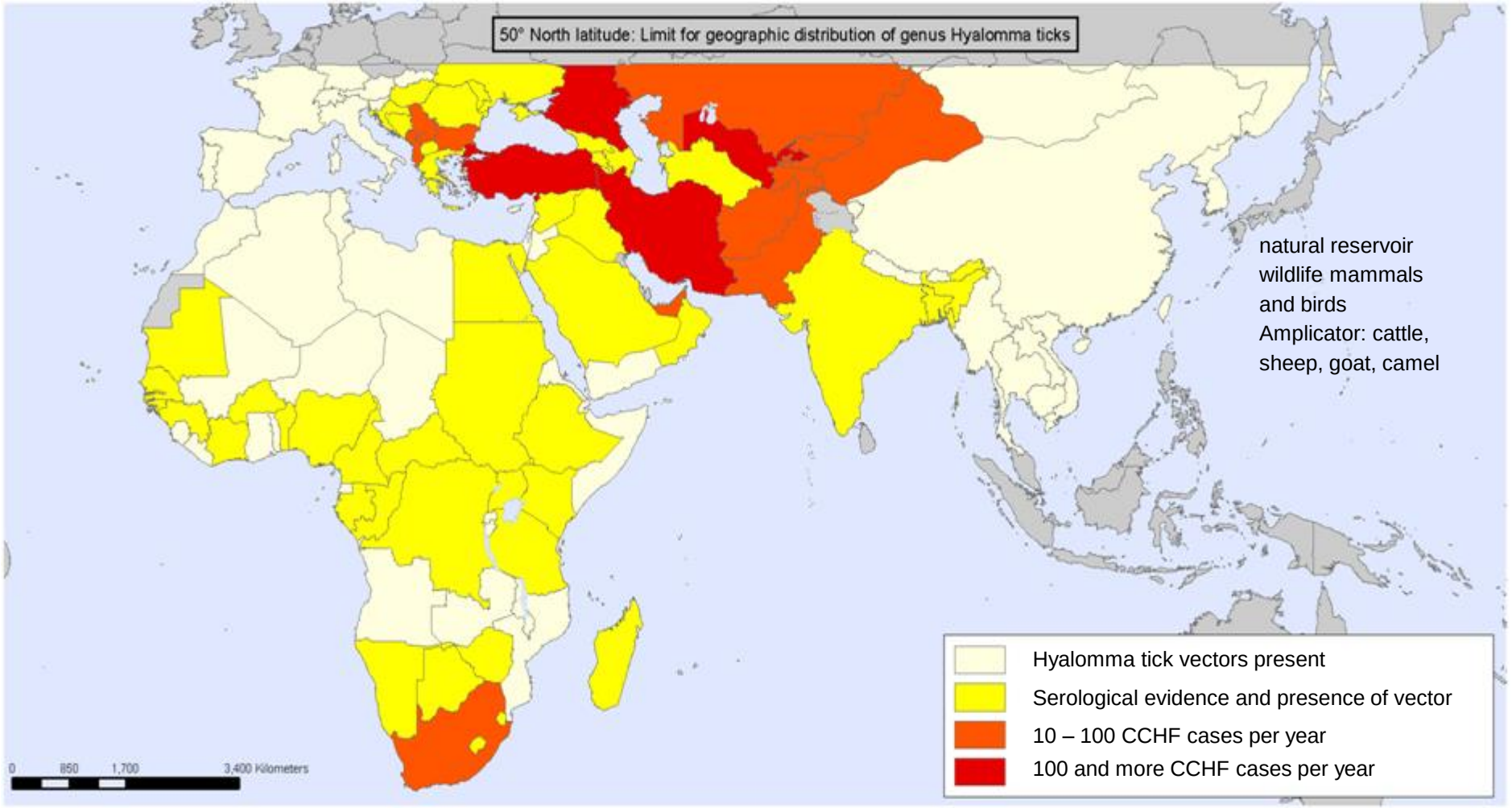
Transmission to Health Care Workers

Year	Country	Primary cases	HCW Contacts	2ary/3ary HCW cases	Exposure
1976	Pakistan	1	ND*	10	Hospital care
1979	Dubai	1	ND	6	Hospital care
1979	Iraq	1	ND	2	Hospital care
1984	South Africa	2	35	8	Hospital care
1994	Pakistan	1	12	3	Surgery
1994	Pakistan	3	40	0	NA
1994	Pakistan	1	ND	3	Surgery
1995	Oman	2	ND	0	NA
1999	Iran	3	ND	0	NA
2000	Kenya	1	ND	0	NA
2000	Pakistan	1	ND	2	Hospital care
2001	Yugoslavia	1	ND	1	Intubation
2001	Albania	1	ND	1	Electrocardiogram
2002	Pakistan	3	154	2	Muco-cutaneous
2003	Turkey	1	5	0	NA
2002-2003	Turkey	50	62	0	NA
2003	Mauritania	1	ND	6	Hospital care
2004	Senegal-France	1	181	0	Hospital care
2005	Turkey	2	5	0	NA
Total		77	494	44	

World Health Organization

***ND: not documented;**
NA: not applicable

Geographic distribution of CCHF



CCHF: sporadic ~ 2000 cases/year

World Health Organization

Case numbers likely to be an under estimate

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Importance of CCHF

1. Spread of vector across Europe.

Euro Surveill. 2010;15(10):pii=19504.

Crimean-Congo hemorrhagic fever in Europe: current situation calls for preparedness

H C Maltezou (helen-maltezou@ath.forthnet.gr)[1], L Andonova[2], R Andraghetti[3], M Bouloy[4], O Ergonul[5], F Jongejan[6], N Kalvatchev[7], S Nichol[8], M Niedrig[9], A Platonov[10], G Thomson[11], K Leitmeyer[12], H Zeller[12]

Travel Medicine and Infectious Disease (2010) 8, 139–143

Crimean–Congo hemorrhagic fever: Risk for emergence of new endemic foci in Europe?

Helena C. Maltezou [a,*], Anna Papa [b]

International Journal of Infectious Diseases (2009) 13, 659–662

Crimean-Congo hemorrhagic fever in southeastern Europe

R.M. Vorou *

2. Increased incidence in tourism areas.

International Journal of Infectious Diseases (2009) 13, 713–716

Crimean-Congo hemorrhagic fever in Greece: a public health perspective

Helen C. Maltezou [a,*], Anna Papa [b], Sotirios Tsiodras [a,c], Vasiliki Dalla [d], Efstratios Maltezos [e], Antonios Antoniadis [b]

Clin Microbiol Infect 2010; 16: 843–847

Emergence of Crimean–Congo haemorrhagic fever in Greece

A. Papa[1], V. Dalla[2], E. Papadimitriou[1], G. N. Kartalis[2] and A. Antoniadis[1]

International Journal of Infectious Diseases (2009) 13, e431–e436

An outbreak of Crimean-Congo hemorrhagic fever in western Anatolia, Turkey

Bülent Ertugrul [a,*], Yavuz Uyar [b], Kamil Yavas [c], Cetin Turan [a], Serkan Oncu [a], Ozlem Saylak [a], Ahmet Carhan [b], Barcin Ozturk [a], Nermin Erol [c], Serhan Sakarya [a]

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Importance of CCHF

3. Threat is national and international

Antiviral Research 98 (2013) 248–260

The impact of Crimean-Congo hemorrhagic fever virus on public health

Marc Mertens [a], Katja Schmidt [a], Aykut Ozkul [b], Martin H. Groschup [a,*]

Euro Surveill. 2010;15(10):pii=19504.

Crimean-Congo hemorrhagic fever in Europe: current situation calls for preparedness

H C Maltezou (helen-maltezou@ath.forthnet.gr)[1], L Andonova[2], R Andraghetti[3], M Bouloy[4], O Ergonul[5], F Jongejan[6], N Kalvatchev[7], S Nichol[8], M Niedrig[9], A Platonov[10], G Thomson[11], K Leitmeyer[12], H Zeller[12]

RAPID COMMUNICATIONS

Sequencing and phylogenetic characterisation of a fatal Crimean – Congo haemorrhagic fever case imported into the United Kingdom, October 2012

B Atkinson[1], J Latham[1], J Chamberlain[1], C Logue[1], L O'Donoghue[1], J Osborne[1], G Carson[1], T Brooks[2], M Carroll[1], M Jacobs[2], S Hopkins[2], R Hewson (Roger.Hewson@hpa.org.uk)[1]

1. Microbiology Services Division, Health Protection Agency, Porton Down, Salisbury, United Kingdom
2. High Security Infectious Disease Unit, Royal Free Hospital, London, United Kingdom

BBC NEWS

5 October 2012 Last updated at 10:13

Crimean-Congo Viral Haemorrhagic Fever case in Glasgow

Non-fatal case of Crimean-Congo haemorrhagic fever imported into the United Kingdom (ex Bulgaria), June 2014

S Lumley (Sarah.Lumley@phe.gov.uk)[1], B Atkinson[1], S D Dowall[1], J K Pitman[2], S Staplehurst[3], J Busuttil[3], A J Simpson[3], E J Aarons[3], C Petridou[4], M Nijjar[4], S Glover[4], T J Brooks[3,5], R Hewson[1,5]

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

CCHF listed in top 10 vector-borne diseases that have the greatest potential to affect European citizens

World Health Organization

CCHF Vaccine ⟷ Priority area

WHO Workshop Oman Dec 2015

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Importance of CCHF

4. Potential bioweapon

FAS
FEDERATION of AMERICAN SCIENTISTS
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Biological and Chemical Weapons >> Biosecurity and Biodefense Resource

Biological Threat Agents

- Anthrax
- Botulinum Toxin
- Brucellosis
- Cholera
- *Clostridium Perfringens* Toxin
- Crimean-Congo Hemorrhagic Fever
- Ebola Hemorrhagic Fever
- Melioidosis
- Plague
- Q Fever
- Ricin
- Rift Valley Fever
- Saxitoxin
- Smallpox
- Staphylococcal Enterotoxin B
- Trichothecene Mycotoxin
- Tularemia
- Venezuelan Equine Encephalitis

Strengthening National Public Health Preparedness and Response to Chemical, Biological and Radiological Agent Threats. Edited by C.E. Cummings and E. Stikova.
IOS Press, 2007.

Crimean-Congo Hemorrhagic Fever – A Biological Weapon?

Etem AKBAS*
Mersin University Faculty of Medicine,
Department of Medical Biology and Genetics, Turkey

CDC Centers for Disease Control and Prevention
CDC 24/7: Saving lives, protecting people, reducing health costs

Bioterrorism Agents/Diseases

5. Threat to armed forces.

The Washington Times

EXCLUSIVE:
CCHF virus poses new threat to troops

Dr. Radia said the hemorrhagic fever is similar to Ebola in that the viral strains is internal degeneration and external bleeding. From the Black Sea to Upper Tunisia, you'll see a dozen or more cases a year. Afghanistan falls right in the middle.

The disease was first reported in the Crimea in 1944, then in the Congo in 1956, according to the World Health Organization. An outbreak was reported eight years ago in Quetta, the capital of Pakistan's Baluchistan province, which borders Afghanistan.

Dr. Radia said U.S. soldiers in Afghanistan not only have to worry about the Taliban but also a host of ailments not commonly found in the West.

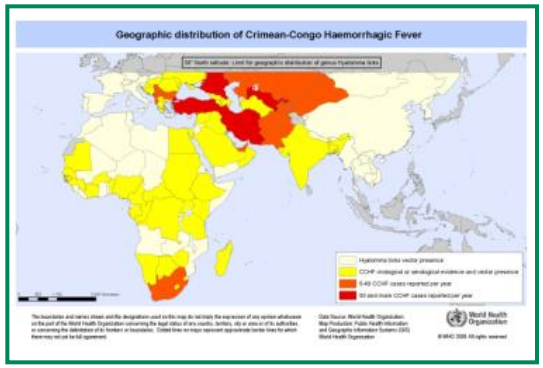
"Diarrheal diseases, typhoid fever, skin ailments and tuberculosis are some of what we have to be on the lookout for," he said. "It's important for soldiers to get medical help early on if they are not feeling well or have been bitten. It's too risky not to have a doctor take a look at it."

Dr. Radia also advised soldiers to wash their hands

Clin Microbiol Infect 2010; 16: 209–224

The past and present threat of vector-borne diseases in deployed troops

F. Pages[1,2], M. Faulde[3,4], E. Orlandi-Pradines[1,2] and P. Parola[2,5]



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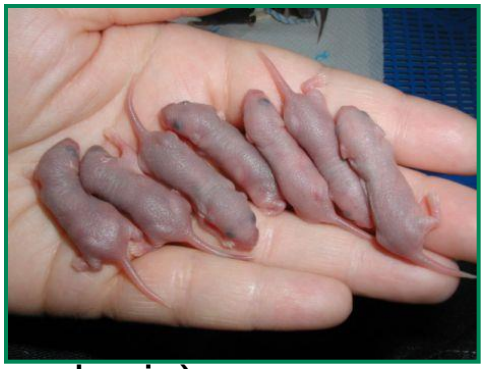
Vaccines & Therapies for CCHF

No vaccines or antiviral drugs are approved for CCHF by FDA or EMA.



Bulgarian vaccine candidate has major disadvantages:

- Requires live CCHF virus
- Crude preparation (non-standardised homogenisation of mouse brain)
- No efficacy studies, no interest to generate data package since 70s
- Is not acceptable to FDA/MHRA/EMA approval

Alternative approach badly needed for a modern CCHF vaccine that can meet regulatory approval and is proven to be effective.

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Development of the vaccine candidate

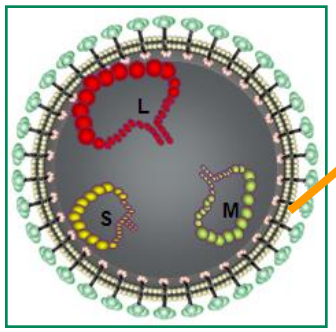
Our approach: We have used Modified Vaccinia Ankara (MVA) as a viral vector to induce immune responses against an inserted CCHF antigens.

Favourable properties of MVA:

- Human safety history: >100,000 doses in 1970s with no adverse effects.
- Human cells non-permissive.
- Induction of humoral and cellular immunity.
- Industrial GMP established.
- Thermostable.
- Production of recombinant proteins.
- Clear commercial opportunities
  - Vaxgene, OBM, Bavarian Nordic, Jansen/Emergent ➔ all in clinical trials with MVA-based vaccines.
  - Approximately extra 100,000 people vaccinated with no adverse signs.
- Inexpensive, low cost approach

Public Health England

Development of the vaccine candidate



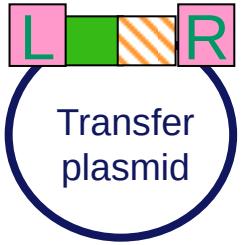
Antigen sequence

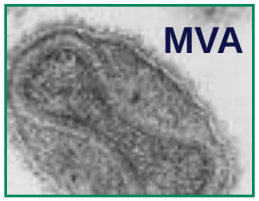
Wyatt & Moss

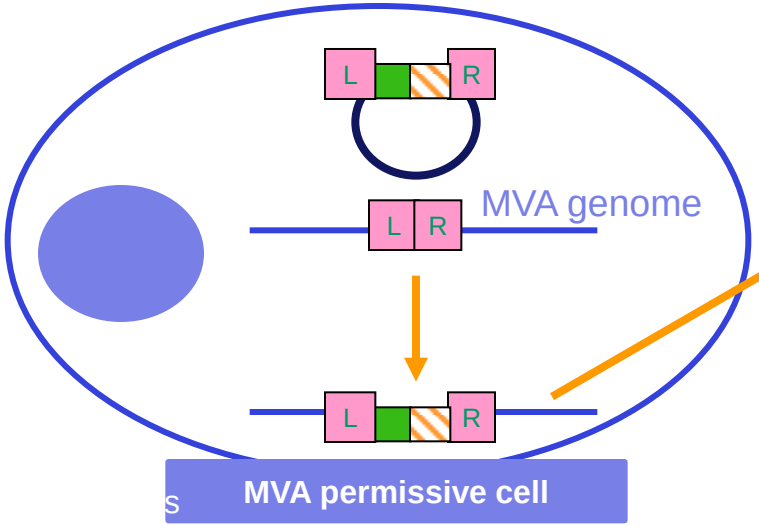
GFP for selection of recombinant viruses

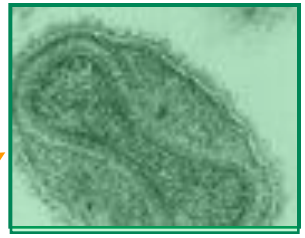
N-terminal tPA for secretion & Nab induction

C-terminal V5 for *in vitro* antibody recognition









GFP+ plaque purification

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Choice of CCHF vaccine antigen

Nucleoprotein [NP] (S-segment of CCHFv)

- Highly conserved between CCHFv strains.
- Most immunogenic protein in CCHFv.
- Successfully used for other viruses.

Pathogen	Vaccine construct	Protection effects
Ebola virus	Venezuelan equine encephalitis virus replicons	Protection in C57BL/6 mice
	Cytomegalovirus	Protection in mice
Hantavirus	Recombinant vaccinia virus	Partial protection in Mongolian gerbils
Influenza virus	DNA prime and recombinant adenovirus boost	Protection in mice
	Recombinant adenovirus	Protection in mice
Lassa virus	Recombinant vaccinia virus	Protection in guinea pigs
Measles	Recombinant vaccinia virus	Protection from encephalitis in rats
Pichinde virus	Recombinant vaccinia virus	Delayed mortality in Syrian hamsters
Rabies virus	Raccoon poxvirus	Protection in mice against lethal challenge
Rift Valley fever virus	DNA vaccine	Partial protection of mice against lethal challenge

Glycoprotein [GP] (M-segment of CCHFv)

- External envelope spike glycoprotein – readily accessible by antibodies.
- GPs commonly and successfully used for other virus pathogens.

➔ Two vaccine constructs made: MVA-NP and MVA-GP.

Confirmation of antigen expression

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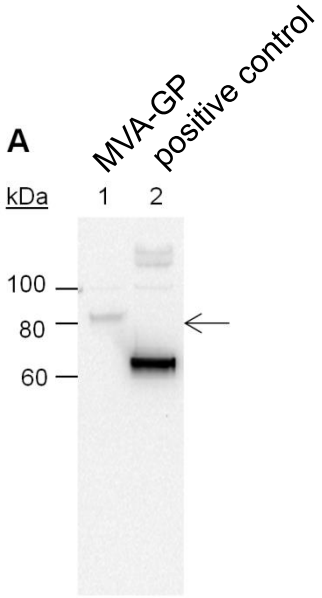
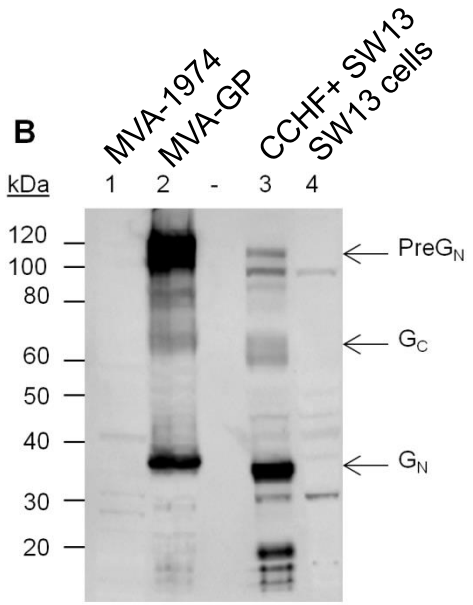


Anti-V5 antibody
(expected size of GP-V5 fusion protein = 76.6kDa, positive control protein = 62kDa)



Anti-CCHF rabbit polyclonal sera
(similar post-translational cleavages in MVA-GP to native protein)

(NB: Findings were similar with MVA-NP construct showing positive protein expression)

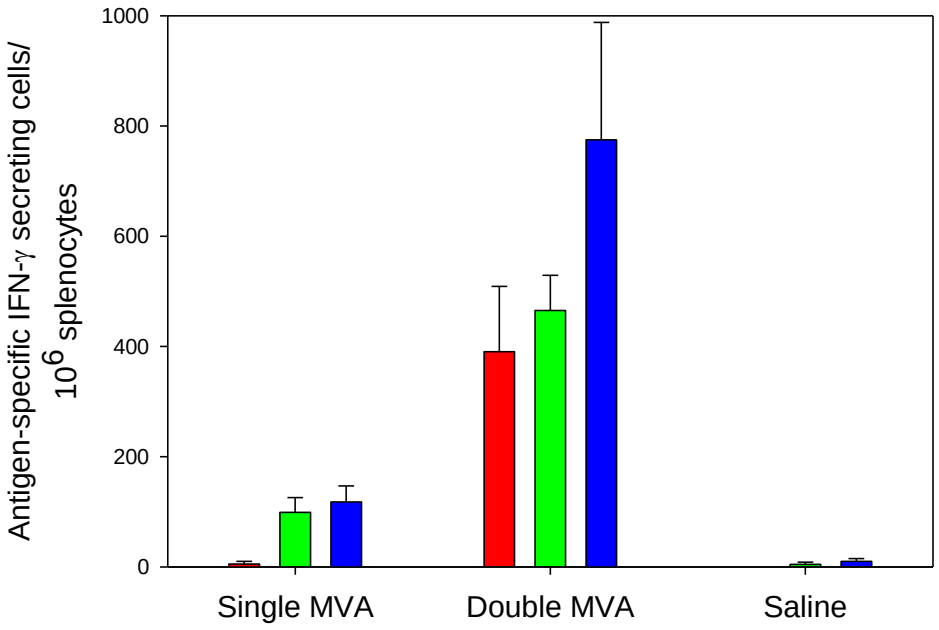
Single vs. booster dosing

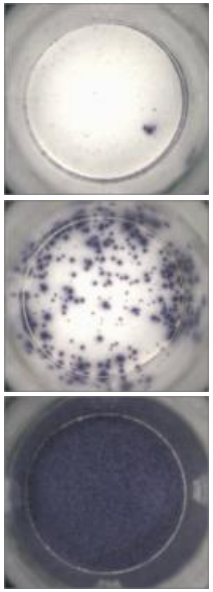
Balb/C mice, 10^7 pfu delivered i.m.

Single MVA-NP dose
Double MVA-NP dose
Saline control

Animals culled (n=3/group) at days 3, 8 and 12 post-vaccination for immunogenicity studies.

Antigen-specific T-cell responses made to CCHF NP peptides.
(20mers overlapping by 8aa, two pools containing 31 peptides)





Media

NP peptide pool

PMA + ionomycin

➔ Prime-boost approach gave greater frequencies of Ag-specific T-cells

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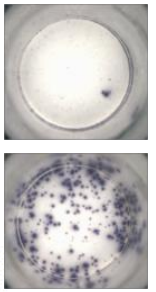
Responses in A129 vs. wild-type mice

IFN-γ ELISPOT assay

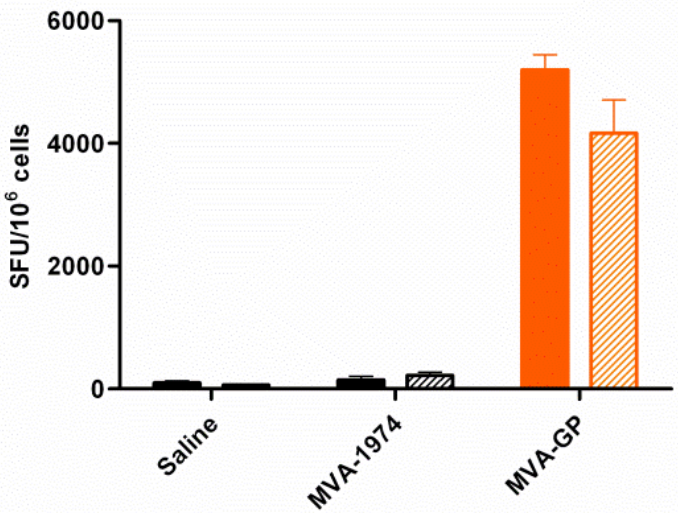
Solid bars = 129Sv/Ev mice; hatched bars = A129 mice [IFN-α/βR$^{-/-}$]



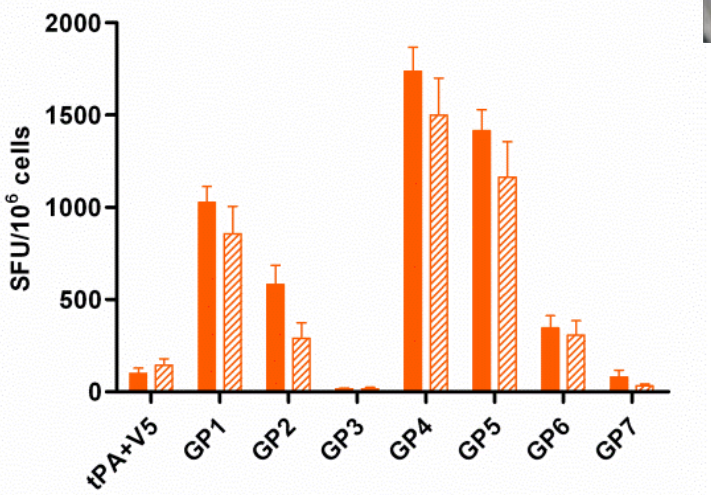
Media

GP peptides

Summed antigen responses



Individual peptide pools



Results for MVA-GP shown.

- ➔ Similar responses in 129Sv/Ev and A129 mice were detected.
- ➔ Immunogenicity was not evenly distributed across the antigen.
- ➔ Responses were specific to the glycoprotein, and similar between mouse strains.

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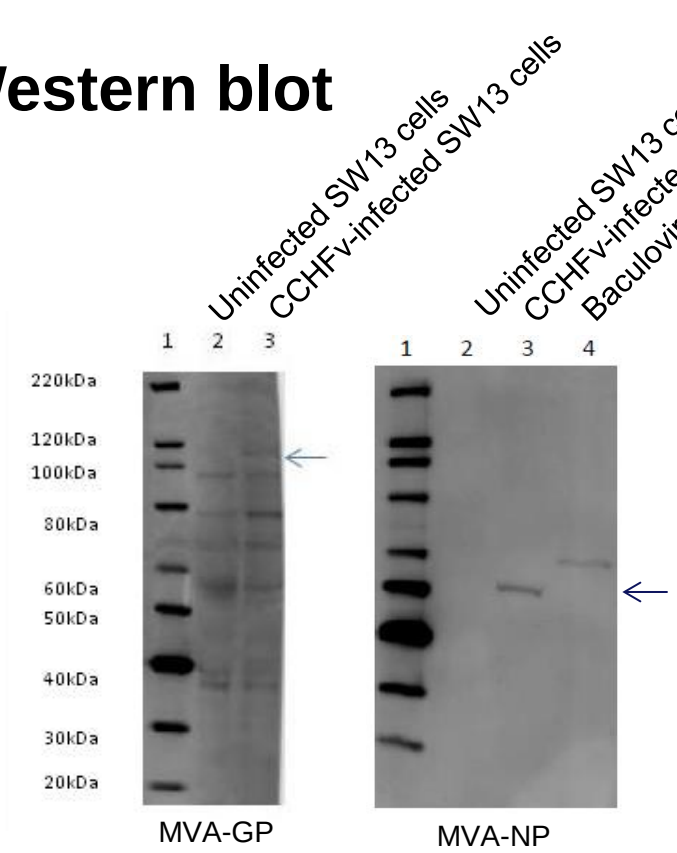
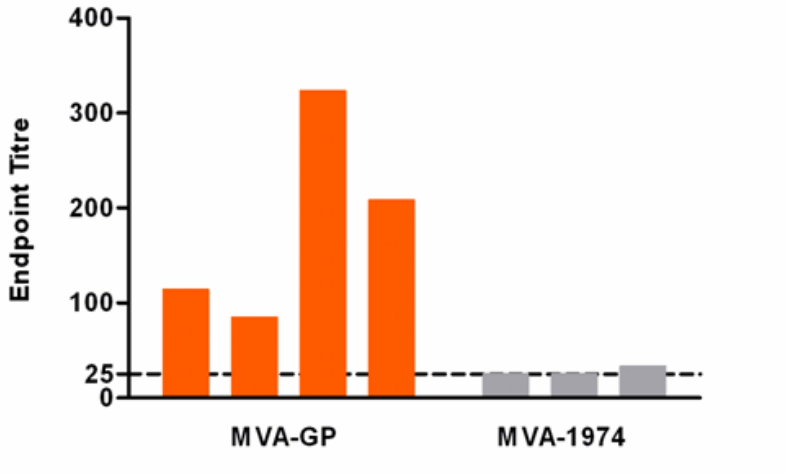
Antibody responses

Western blot



ELISA studies



Both MVA-GP and MVA-NP vaccines induced antigen-specific antibodies.

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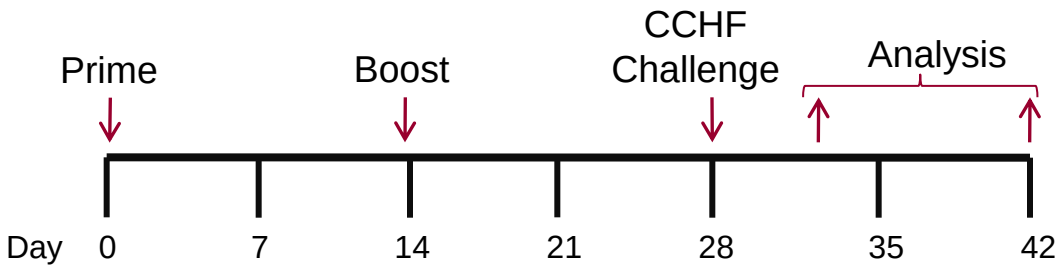
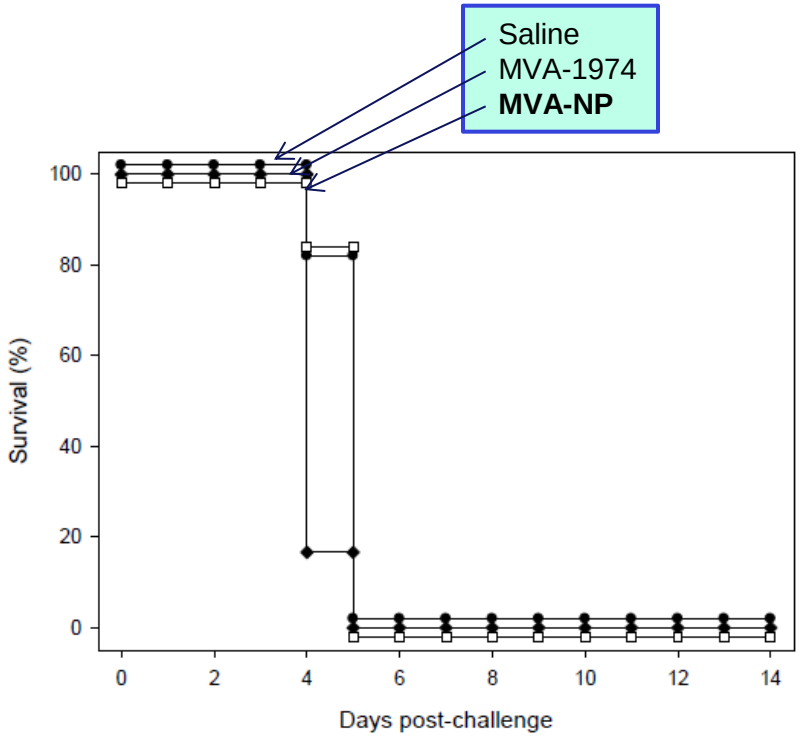
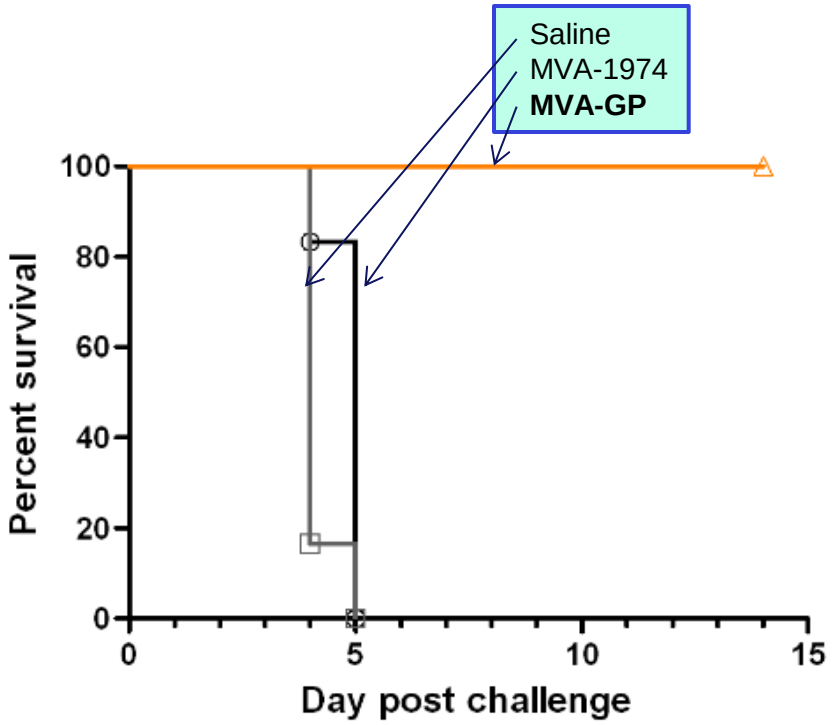


Efficacy studies





No protective effects seen with MVA-NP, but 100% protection from lethal challenge with MVA-GP

➔ First demonstration of CCHF vaccine efficacy

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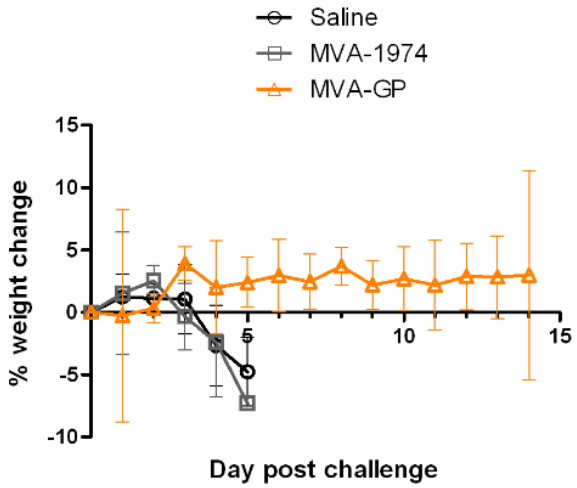
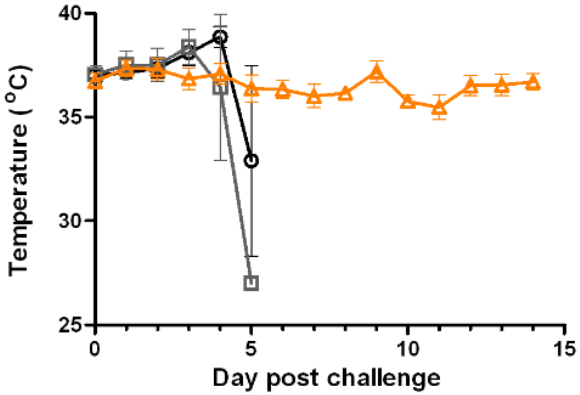
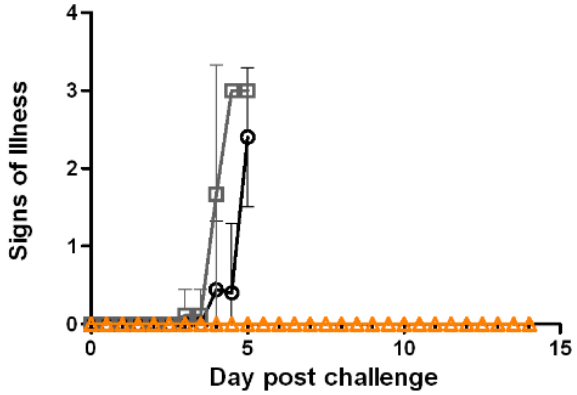
Clinical measurements

MVA-GP immunised animals showed no clinical evidence of CCHFv infection post-challenge:

- No loss in weight.
- No significant temperature deviations.
- Clinical signs scored healthy on all occasions.







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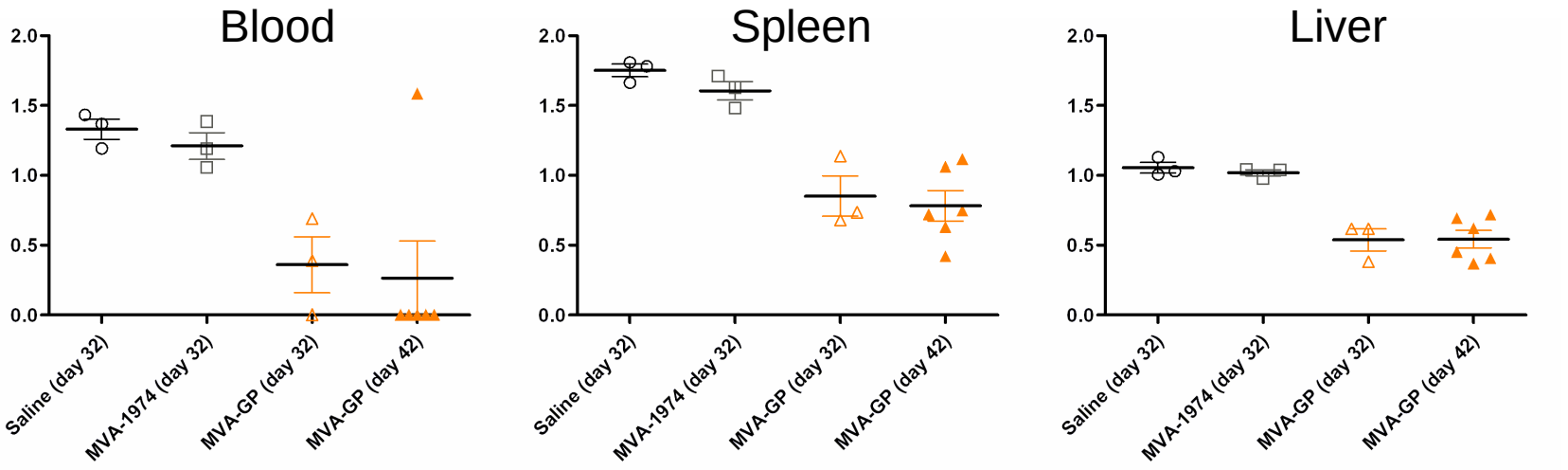
Viral loads

RT-PCR for CCHFv gene expression (normalised to mouse HPRT gene expression).



Day 32 = 4 days post-challenge
Day 42 = 14 days post-challenge (end of study)

Viral load was significantly lower in MVA-GP vaccinated mice than in control groups.

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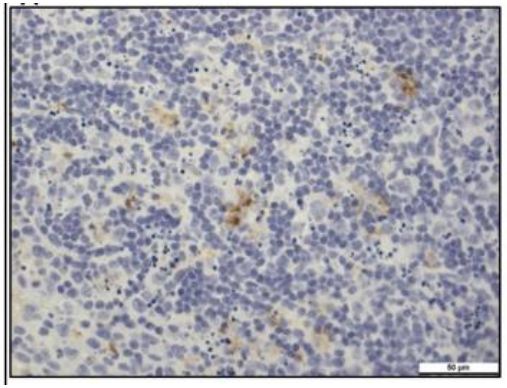
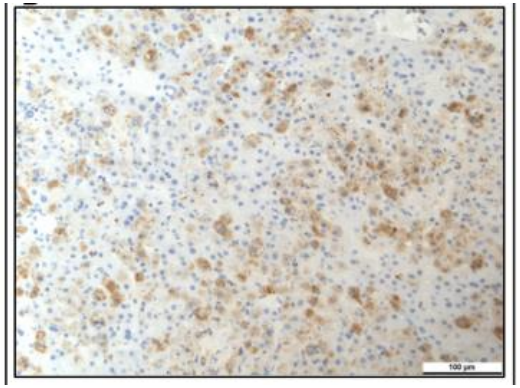
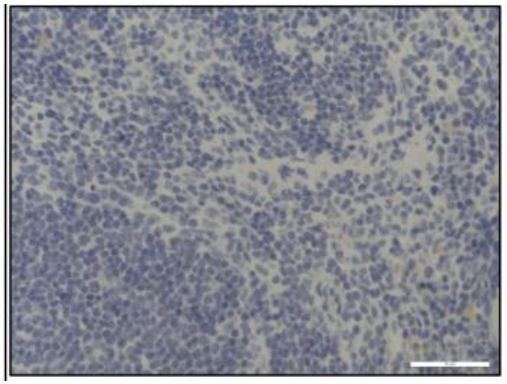
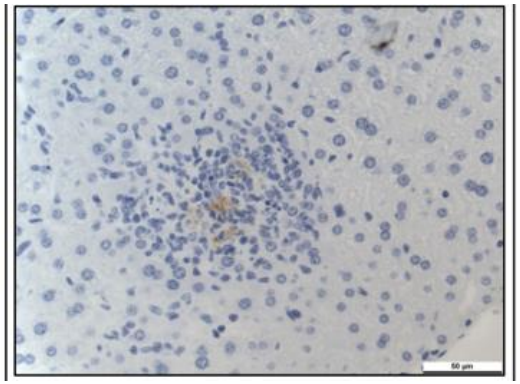
Histology

Immunostaining

Immunised A129 mice,
4 days post-challenge

	Spleen	Liver
MVA-1974		
	A few, scattered cells with cytoplasmic staining within the parenchyma.	Frequent, diffuse, positively stained hepatocytes.
MVA-GP		
	Normal parenchyma.	A few, positively stained cells within an inflammatory cell focus.

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Mechanism of Protection

Previous reports and anecdotal evidence point to importance of antibody response in protection

Ergonul, O., *Crimean-Congo haemorrhagic fever*. Lancet Infect Dis, 2006. 6(4): p. 203-14.

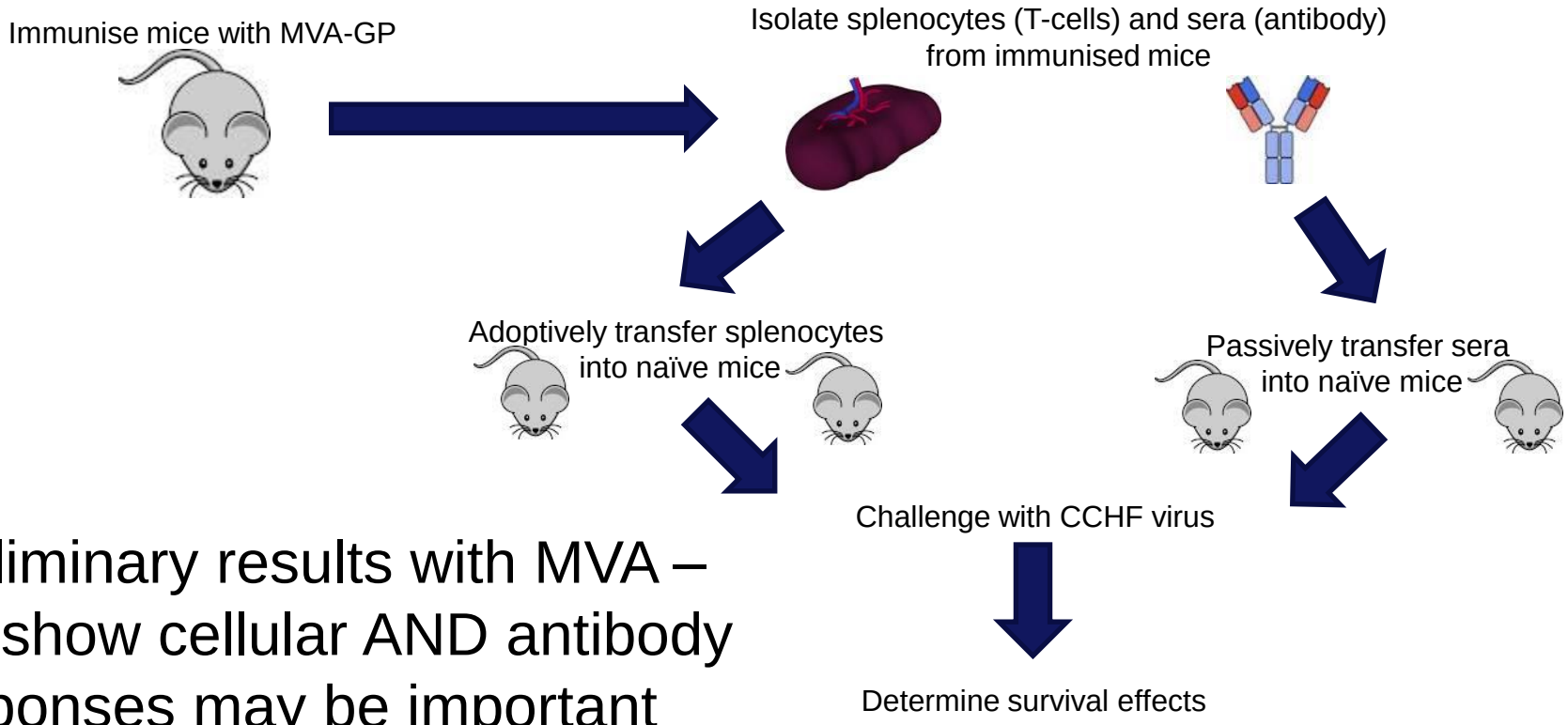
Kubar, A., et al., *Prompt administration of Crimean-Congo hemorrhagic fever (CCHF) virus hyperimmunoglobulin in patients diagnosed with CCHF and viral load monitorization by reverse transcriptase-PCR.* Jpn J Infect Dis, 2011. 64(5): p. 439-43.

Tishkova, F. et al., *CCHF survivors show strong neutralising antibodies are protected from further infection.* Mikrobiologiya i Virusologiya

Public Health England

Mechanism of Protection

Passive/Adoptive transfer



Preliminary results with MVA – GP show cellular AND antibody responses may be important

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Conclusions

- Vaccine is based on CCHF glycoproteins expressed in a viral vector.
- CCHF-specific antibodies and T-cells.
- 100% protection from disease in a pre-clinical model.
- MoA appears to rely on both T cell and antibody

Next steps include:

- NHP pre-clinical data package
- Assess cross neutralisation of CCHFv strains
- Assess prime boost stretegies

Buttigieg et al., (2014) PLOS one.9 (3) 91516-28

Alternative Vaccine Approaches

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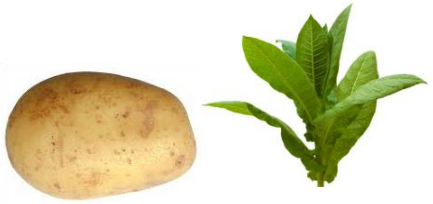
DNA-based vaccines expressing the CCHFv M segment

Spik K, et al., (2006) Vaccine 24: 4657–66.

Recombinant tobacco leaves expressing G_N and G_C

Ghiasi et al., (2011). Clin Vaccine Immunol 18: 2031–7.

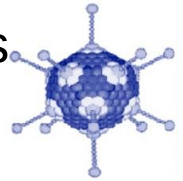


Inactivated virus from cell culture

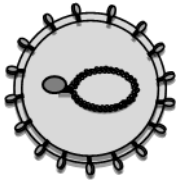
Canakoglu et al., (2015). PLOS NTD.

Recombinant Adenovirus

Feldmannu et al.,



CCHF Virus Like Particles:



Anti Tick vaccines: Cement & midgut antigen ($Bm86$) partially protective

Labuda et al 2006 PLOS one 2 (4) e24

Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial

Ana Maria Henao-Restrepo, Ira M Longini, Matthias Egger, Natalie E Dean, W John Edmunds, Anton Camacho, Miles W Carroll, Moussa Doumbia, Bertrand Draguez, Sophie Duraffour, Godwin Enwere, Rebecca Grais, Stephan Gunther, Stefanie Hossmann, Mandy Kader Kondé, Souleymane Kone, Eeva Kuisma, Myron M Levine, Sema Mandal, Gunnstein Norheim, Ximena Riveros, Aboubacar Soumah, Sven Trelle, Andrea S Vicari, Conall H Watson, Sakoba Kéïta, Marie Paule Kieny, John-Arne Røttingen**

Summary

Background A recombinant, replication-competent vesicular stomatitis virus-based vaccine expressing a surface glycoprotein of Zaire Ebolavirus (rVSV-ZEBOV) is a promising Ebola vaccine candidate. We report the results of an interim analysis of a trial of rVSV-ZEBOV in Guinea, west Africa.

100% Efficacy for preventing tertiary cases in ring vaccinations:
16 cases in 21 day vaccine delay compared to 0 for no delay

VSV as a vector for a CCHF vaccine?

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Vaccine Target

- Healthcare workers in endemic countries
- At risk occupations; abattoirs, farmers
- At risk local population in endemic countries
- International response healthcare workers
- Military personnel
- Farm animals

Public Health England

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