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VIRUSES of wildlife:

Elephants and Rhinos



**Blijdorp Zoo
Rotterdam
October 11th 2011**





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DECLARATION OF INTEREST/ EMPLOYMENT:

Professor/Head Department of Virology Erasmus University/MC (NL)

Professor Virology Utrecht University (NL)

Chair Artemis Wildlife Health Institute (Europe)

CSO Viroclinics-Biosciences BV (NL)

Chair ESWI (Europe)

**Blijdorp Zoo
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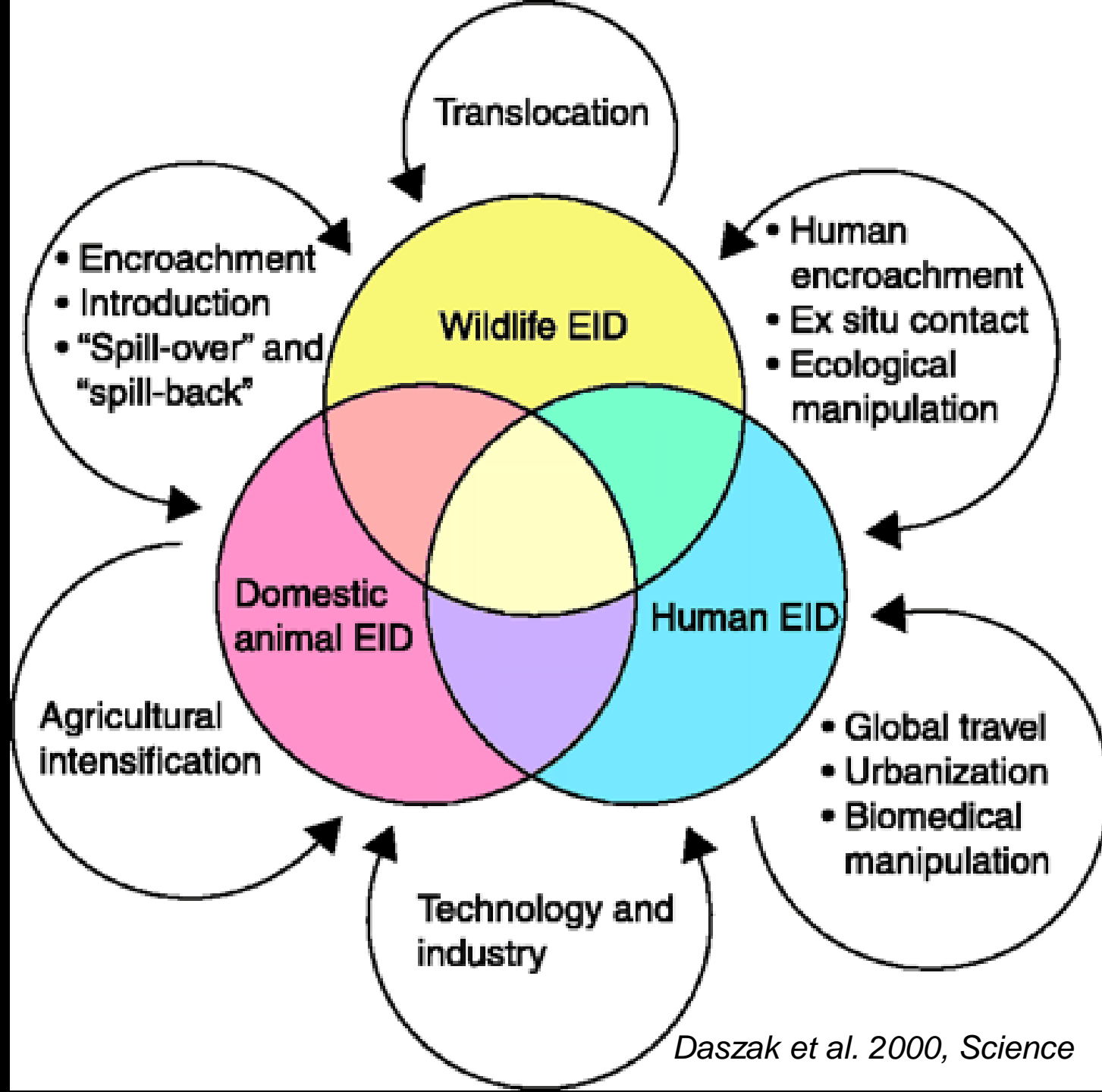




The paradox:

-Emerging virus infections in the past decade –

(source: WHO)



Daszak et al. 2000, Science



Pubmed articles on emerging viruses in wildlife, 2006 to 2010

| Virus group | No. of articles | Virus group | No. of articles |
|-------------------|-----------------|-----------------|-----------------|
| Adenovirus | 9 | Iridoviridae | 16 |
| Arenaviridae | 9 | Morbillivirus | 23 |
| Bat viruses | 35 | Papillomavirus | 13 |
| Bornavirus | 4 | Paramyxoviridae | 10 |
| Caliciviridae | 9 | Parvovirus | 5 |
| Circovirus | 8 | Picornaviridae | 9 |
| Coronavirus | 9 | Polyomavirus | 6 |
| Fish viruses | 67 | Poxvirus | 24 |
| Flaviviridae | 43 | Primate viruses | 54 |
| Hantavirus | 41 | Reoviridae | 16 |
| Hepatitis E virus | 6 | Retroviridae | 14 |
| Herpesvirus | 33 | Rhabdovirus | 55 |
| Influenza virus | 103 | Togaviridae | 23 |



Important new viral diseases in wildlife, 2006-2010

| Virus | Wildlife species | Novelty |
|----------------------------|-------------------------------|-------------------------|
| West Nile virus | Multiple birds and mammals | New range |
| Hepatitis E virus | Wild boar | New virus |
| HPAIV H5N1 virus | Multiple birds and mammals | New hosts |
| Canine distemper virus | Multiple carnivores | New hosts |
| Dolphin morbillivirus | Multiple cetaceans | New hosts, re-emergence |
| Phocine distemper virus | Multiple carnivores | New hosts, re-emergence |
| African swine fever virus | Wild boar | New range |
| Bluetongue virus | Multiple mammals | New hosts |
| Feline leukemia virus | Florida panther, Iberian lynx | New hosts |
| Border disease virus | Pyrenean chamois | New host |
| Coronaviruses | Multiple bats | New viruses |
| Nipah virus | Fruit bat | New range |
| Ebola virus | Multiple species | Increased incidence |
| Provent. Dilat. Dis. virus | Psittacines | Etiology determined |
| Usutu virus | Multiple birds | New range |
| Amphibian ranaviruses | Multiple amphibians | New viruses |

Elephant endotheliotropic herpes virus

- With few resources, researchers work to contain fatal **elephant virus**.
Am J Vet Res. 2011 Aug;72(8):1006.
- Nonfatal clinical presentation of **elephant** endotheliotropic herpes **virus** discovered in a group of captive Asian **elephants** (*Elephas maximus*).
Schaftenaar W, Reid C, Martina B, Fickel J, Osterhaus AD.
J Zoo Wildl Med. 2010 Dec;41(4):626-32.
- **Virus** suspect identified in **elephant** deaths.
Ferber D.
Science. 1999 Feb 19;283(5405):1093-4.

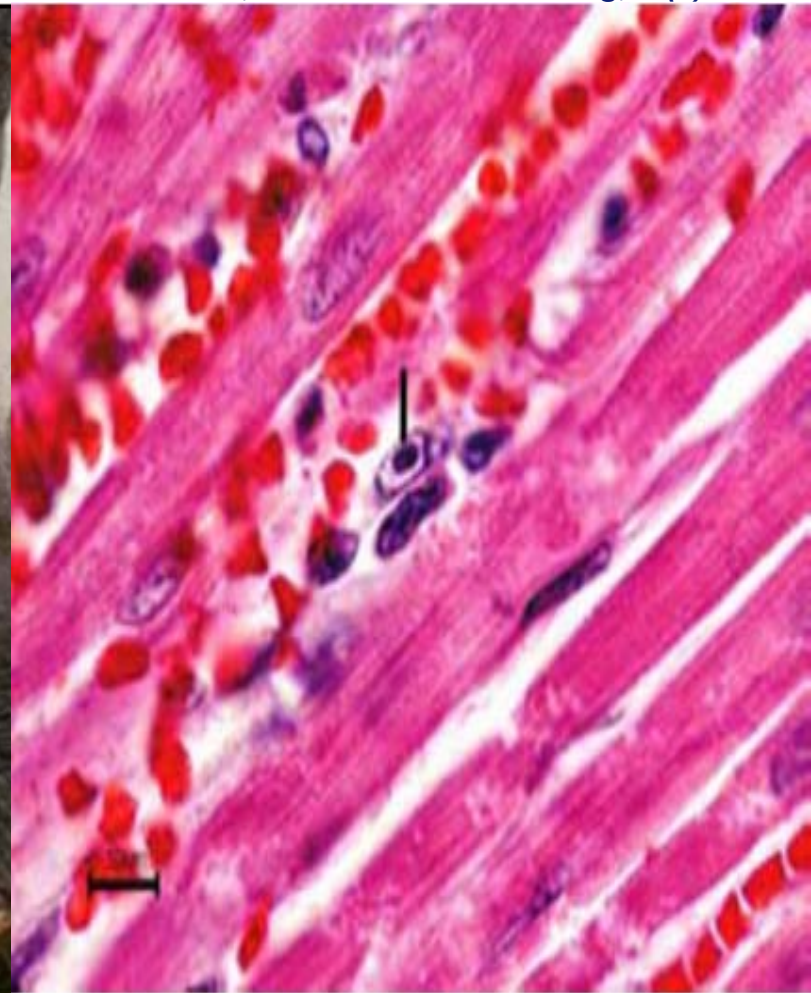
Herpesvirus claims another elephant as search for answers continues

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With few resources, researchers work to contain fatal elephant virus

R. Scott Nolen, Am J Vet Res. 2011 Aug;72(8):1006



Elephant endotheliotropic herpesviruses were first identified in 1995 when Kumari (above) died of infection at the National Zoological Park.

Courtesy of Jessie Cohen/Smithsonian National Zoological Park

Viral inclusion bodies in endothelial cells are a hallmark of herpesvirus infection.

Courtesy of Smithsonian National Zoological Park

Herpesvirus claims another elephant as search for answers continues

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With few resources, researchers work to contain fatal elephant virus



Sri Lankan scientists are collaborating with the National Elephant Herpesvirus Laboratory to shed light on the impact EEHVs are having on Asian elephants in the wild.

Courtesy of Amanda Perez/Smithsonian National Zoological Park

"There are plenty of ugly diseases that kill baby elephants, but this one is terrifying. This is the one we all worry about." —Dr. Ellen Wiedner, who treated one of only eight elephants to survive severe **elephant endotheliotropic herpesvirus** infection

Nonfatal Clinical Presentation of Elephant Endotheliotropic Herpes Virus Discovered in a Group of Captive Asian Elephants (*Elephas maximus*)

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Figure 1. Several elephant endotheliotropic herpes virus-1-related mucosal lesions on the palate of a 36-yr-old female Asian elephant. One lesion is located in a nonpigmented area surrounded by pigmented tissue. Note the orifice of the vomeronasal organ (arrow).



Figure 2. Elephant endotheliotropic herpes virus-related vesicles in the vestibulum vaginae of a 36-yr-old female Asian elephant carrying a dead full-grown calf that was 2 mo past due.



Serology: an indication for viral presence

- Serosurvey for selected infectious disease agents in free-ranging black and white rhinoceros in Africa.
Fischer-Tenhagen C, Hamblin C, Quandt S, Frölich K
- Canine distemper virus antibodies in the Asian elephant (*Elephas maximus*).
Oni O, Wajjwalku W, Boodde O, Chumsing W.
Vet Rec. 2006 Sep 23;159(13):420-1.
- Antibodies against some viruses of domestic animals in southern African wild animals.
Barnard BJ.
Onderstepoort J Vet Res. 1997 Jun;64(2):95-110.
- Enzyme-linked immunosorbent assays for the detection of antibody to Crimean-Congo haemorrhagic fever virus in the sera of livestock and wild vertebrates.
Burt FJ, Swanepoel R, Braack LE.
Epidemiol Infect. 1993 Dec;111(3):547-57.
- Serological evidence of herpesvirus infection in captive Asian elephants (*Elephas maximus*).
Metzler AE, Ossent P, Guscetti F, Rübel A, Lang EM.
J Wildl Dis. 1990 Jan;26(1):41-9

Serosurvey for selected infectious disease agents in free-ranging black and white rhinoceros in Africa.

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20/11/00

Fischer-Tenhagen C, Hamblin C, Quandt S, Frölich K

Two hundred and eighty one serum samples collected from free-ranging black (*Dicerosbicornis*) and white (*Ceratotherium simum*) rhinoceros, in the Republic of South Africa (RSA), Namibia, and Kenya from 1987–97, were examined for antibody to 16 different infectious agents.

Positive antibody titers were detected against Akabane (59.8%), bluetongue (55%), African horse sickness (27.9%), epizootic haemorrhagic disease of deer (19.4%), parainfluenza type 3 (25.3%), bovine herpes virus 1 (3.1%), equine herpes virus 1 (8.8%) and bovine viral diarrhea (1.2%) viruses, and four serovars of *Leptospira interrogans*, (ranging 1.2 to 8.8%).

Interspecies differences were detected for African horse sickness, epizootic haemorrhagic disease of deer and parainfluenza type 3 viruses.

There appeared to be some geographic variation in the prevalence of antibody for African horse sickness, bluetongue, epizootic haemorrhagic disease of deer, parainfluenza type 3, equine herpes virus 1 and *Leptospira interrogans* serovar *bratislava*.



Journal of Wildlife Diseases, 2000

Endogenous viruses in elephants



Endogenous non-retroviral RNA virus elements in mammalian genomes.

Horie M, Honda T, Suzuki Y, Kobayashi Y, Daito T, Oshida T, Ikuta K, Jern P, Gojobori T, Coffin JM, Tomonaga K.

Nature. 2010 Jan 7;463(7277):84-7.

Discovery and characterization of mammalian endogenous parvoviruses.

Kapoor A, Simmonds P, Lipkin WI.

J Virol. 2010 Dec;84(24):12628-35. Epub 2010 Oct 13.



Characterization of an endogenous retrovirus class in elephants and their relatives.

Greenwood AD, Englbrecht CC, MacPhee RD.

BMC Evol Biol. 2004 Oct 11;4:38.

Evolution of endogenous retrovirus-like elements of the woolly mammoth (*Mammuthus primigenius*) and its relatives.

Greenwood AD, Lee F, Capelli C, DeSalle R, Tikhonov A, Marx PA, MacPhee RD.

Mol Biol Evol. 2001 May;18(5):840-7.

Identification of new viral pathogens

| | |
|------|--|
| 1995 | CDV as the cause of mass mortality in Serengeti lions |
| 1996 | γ -herpesvirus in seals (phocid herpesvirus-2) |
| 1997 | monk seal morbilliviruses (MSMV-WA/G) |
| 1997 | influenza A (H5N1) virus in humans |
| 1998 | lentivirus from Talapoin monkeys (SIVtal) |
| 1999 | influenza B virus in seals |
| 2000 | human metapneumovirus (hMPV) |
| 2002 | re-emerging PDV in Europe |
| 2003 | SARS CoV cause of SARS in humans (Koch's postulates) |
| 2003 | influenza A (H7N7) virus in humans |
| 2004 | fourth human coronavirus (CoV NL) |
| 2005 | H16 influenza A viruses (new HA!) in black headed gulls |
| 2008 | dolphin herpesvirus |
| 2009 | deer astrovirus |
| 2010 | human astrovirus, human picobirnavirus |
| 2011 | ferret coronavirus, porcine picobirnavirus, stone marten anellovirus. influenza A (H1N1) virus in dogs... |

Identification of viral pathogens (1/2)

- 1977 non-mammalian papillomavirus: Finch papillomavirus
- 1984 phocid herpesvirus (phocid alpha herpesvirus-1, PhHV-1)
- 1985 phocid morbillivirus (phocid distemper virus, PDV)
- 1988 picornavirus in seals (affected by PDV infection)
- 1990 orthopoxvirus in seals (affected by PDV infection)**
- 1992 porpoise and dolphin morbilliviruses causing mortality
- 1992 rhabdovirus of dolphins





Poxvirus Taxonomy

Genus

avipoxvirus

capripoxvirus

leporipoxvirus

molluscipoxvirus

orthopoxvirus

parapoxvirus

suispoxvirus

yatapoxvirus

Species

camelpox virus

cowpox virus

ectromelia virus

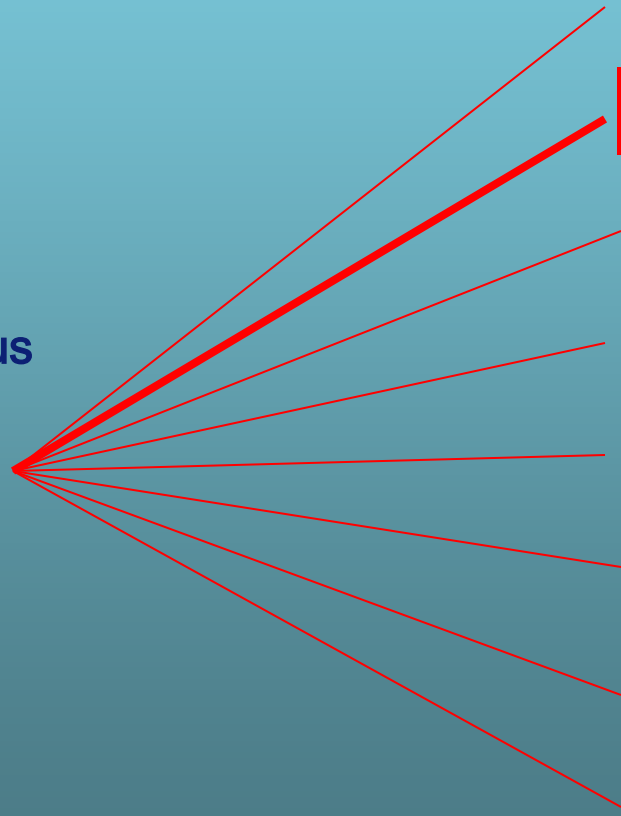
monkeypox virus

raccoonpox virus

vaccinia virus

variola virus

volepox virus



cowpox



Wolfs et al. E.I.D. 2002

Since eradication smallpox: more animal poxvirus infections in humans?

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monkeypox

Figure 1. African child with disseminated monkeypox. Note postauricular adenopathy (courtesy of Leo Lanole, Prince Albert Parkland Health Region, Saskatchewan, Canada).



cowpox

ProMED-mail 9 Jan 2003

cowpox



Stittelaar et al., Nature, 2006

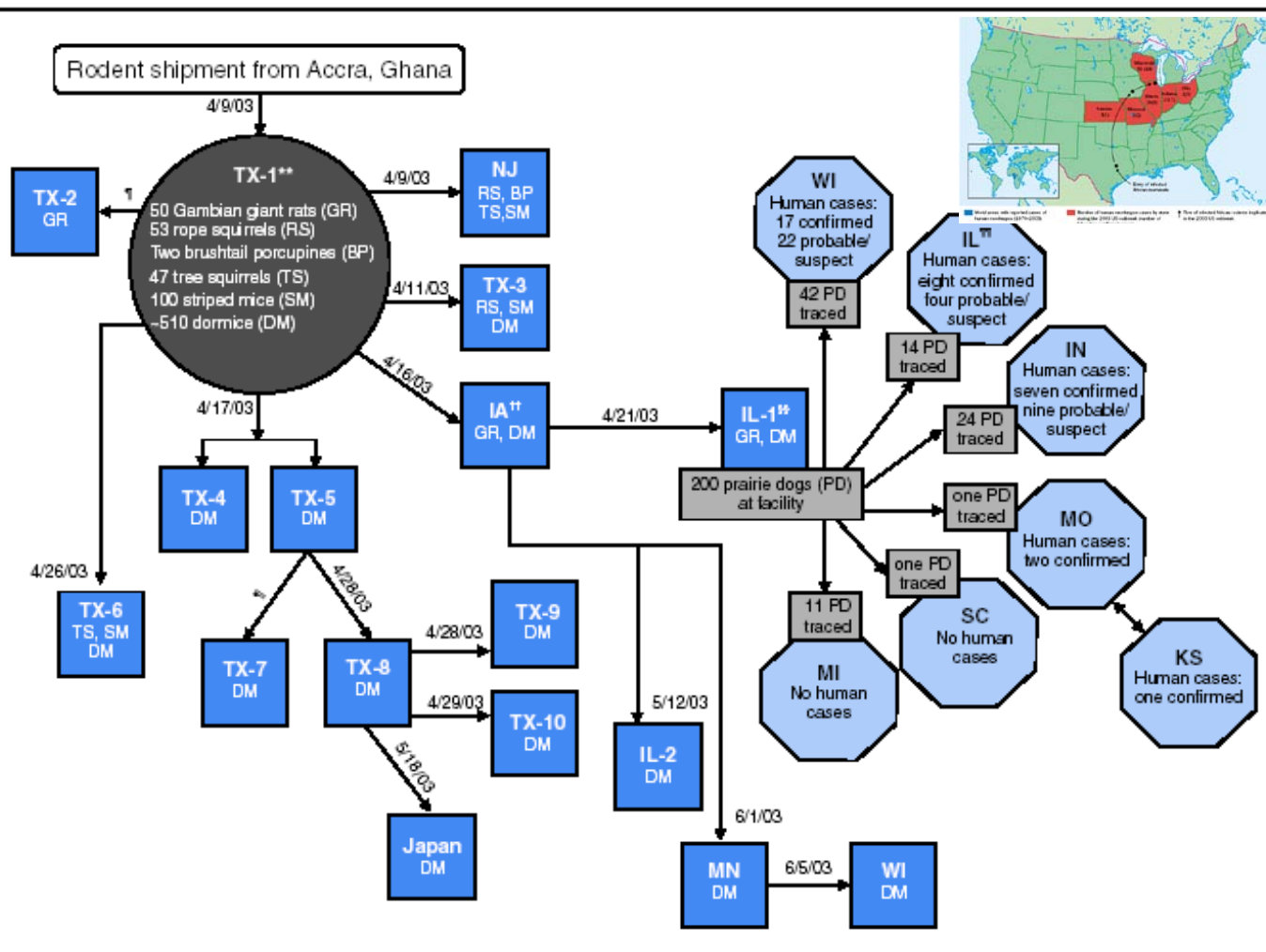
Pelkonen et al. E.I.D. 2003; 9:1458-1461

Multistate outbreak of monkeypox

Illinois, Indiana, and Wisconsin, 2003

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Cowpoxvirus infections in elephants

Human cowpox virus infection acquired from a circus elephant in Germany.

Hemmer CJ, Littmann M, Löbermann M, Meyer H, Petschaelis A, Reisinger EC.

Int J Infect Dis. 2010 Sep;14 Suppl 3:e338-40.

Rat-to-elephant-to-human transmission of cowpox virus.

Kurth A, Wibbelt G, Gerber HP, Petschaelis A, Pauli G, Nitsche A.

Emerg Infect Dis. 2008 Apr;14(4):670-1.

Cowpox virus infection causing stillbirth in an Asian elephant (*Elphas maximus*).

Wisser J, Pilaski J, Strauss G, Meyer H, Burck G, Truyen U, Rudolph M, Frölich K.

Vet Rec. 2001 Aug 25;149(8):244-6.

Characterization of orthopoxviruses isolated from man and animals in Germany.

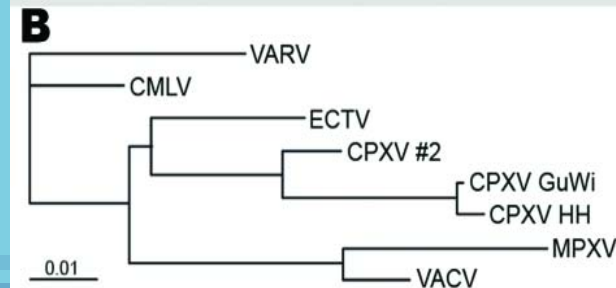
Meyer H, Schay C, Mahnel H, Pfeffer M.

Arch Virol. 1999;144(3):491-501.

A method for serological differentiation of closely related poxviruses.

Maltseva NN, Marennikova SS.

Acta Virol. 1976 Jun;20(3):250-52.



Human cowpox virus infection acquired from a circus elephant in Germany



Figure 1. Clinical evolution of the cowpox lesion over time (days after the onset of symptoms).

Characterization of orthopoxviruses isolated from man and animals in Germany

H. Meyer¹, C. Schay¹, H. Mahnel², and M. Pfeffer²

Fourteen orthopoxvirus strains isolated from humans, cats, a dog, a cow, and an elephant in Germany were characterized.

All were classified as cowpox virus based on haemorrhagic lesions induced on the Chorioallantoic membrane of chicken eggs and reactivity of a 160 kDa protein with anti-A-type inclusion protein hyperimmune serum in a Western blot. More detailed comparison of the isolates by restriction endonuclease mapping using *Hind*III and *Xho*I demonstrated a close relationship between all isolates and confirmed them as cowpox viruses.

One group consisting of five closely related isolates contained a unique 4.0 kb *Hind*III fragment.

In a Southern blot this fragment failed to hybridize with other cowpox virus isolates including the reference strain.



Fig.3. Map of Germany depicting where the 14 cowpox virus strains described in this study had been isolated

Poxvirus in a rhino

- Characterization of a fowlpox **virus** isolated from a **rhinoceros**].

Mayr A, Mahnel H.

Arch Gesamte Virusforsch. 1970;31(1):51-60.



EMC virus in elephants

Cardiovirus is a genus within the family Picornaviridae



- An outbreak of encephalomyocarditis-**virus** infection in free-ranging African **elephants** in the Kruger National Park.

Grobler DG, Raath JP, Braack LE, Keet DF, Gerdes GH, Barnard BJ, Kriek NP, Jardine J, Swanepoel R.

Onderstepoort J Vet Res. 1995 Jun;62(2):97-108

- Encephalomyocarditis **virus** infection of captive **elephants**.

Simpson CF, Lewis AL, Gaskin JM.

J Am Vet Med Assoc. 1977



EMC vaccination elephants

Hunter et al., Vaccine 1998



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The efficacy of an experimental oil-adinejuvanted encephalomyocarditis vaccine in elephants, mice and pigs.

- An oil-adjuvanted inactivated encephalomyocarditis (EMC) vaccine was developed.
- Mice showed protection against challenge and pigs developed high antibody levels.
- Vaccinated elephants developed high antibody titers which protected all vaccinates from a challenge roughly two months post-vaccination, whereas controls developed fatal or sub-clinical myocarditis.
- Ethics?

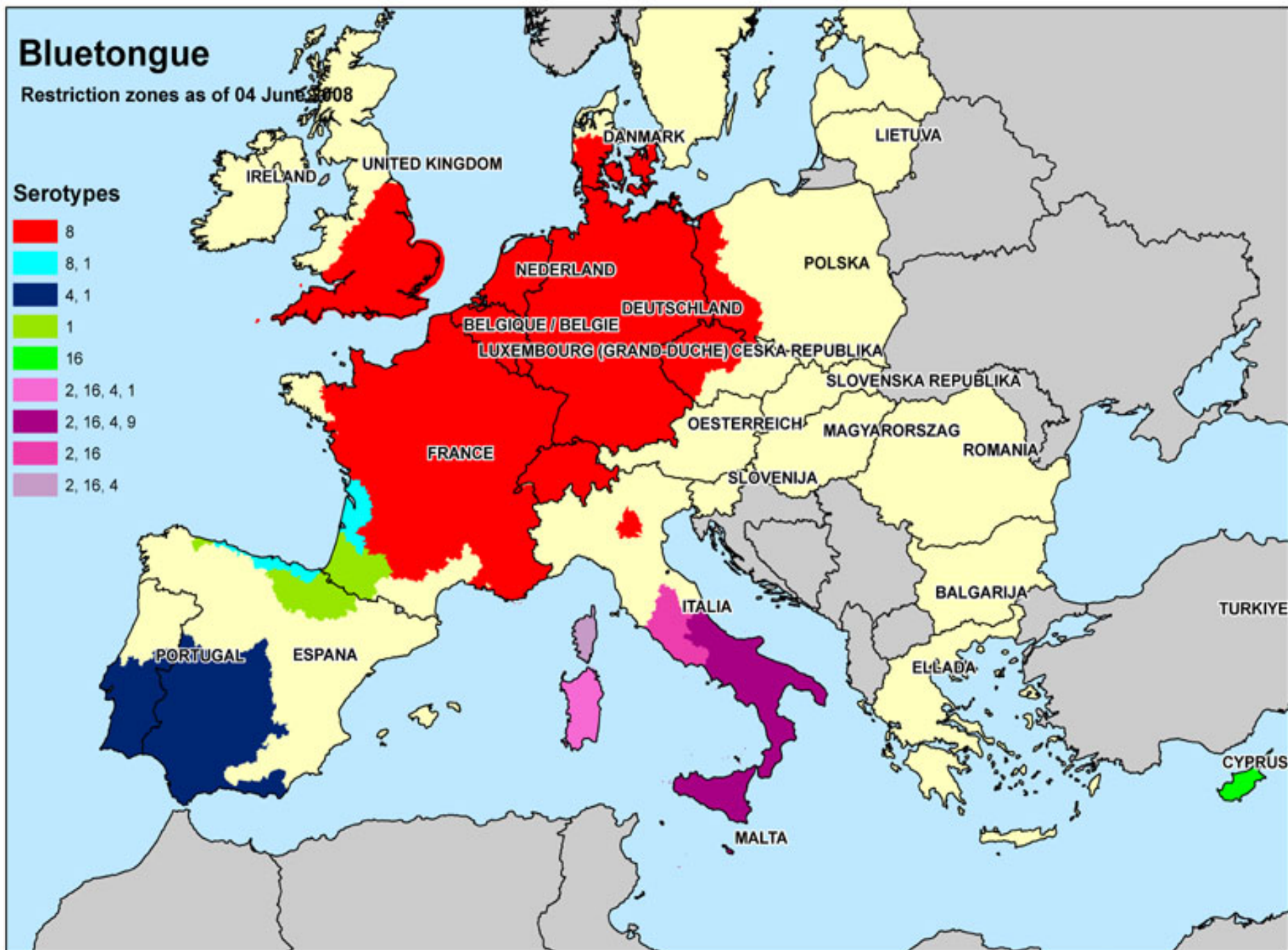
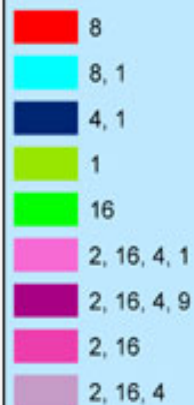


Figure 1 The heart function of the experimental elephants was monitored using an ECG after challenge with the EMC virus

Bluetongue

Restriction zones as of 04 June 2008

Serotypes



Bluetongue antibodies in wildlife Africa

Epidemiologic study of bluetongue in sheep, cattle and different species of wild animals in the Ivory Coast.

Formenty P, Domenech J, Lauginie F, Ouattara M, Diawara S, Raath JP, Grobler D, Leforban Y, Angba A.

Rev Sci Tech. 1994 Sep;13(3):737-51.

antibodies against BTV in:

kob (Kobus kob)

common waterbuck (Kobus ellipsiprymnus)

roan antelope (Hippotragus equinus),

buffalo (Syncerus caffer)

hartebeest (Alcelaphus buselaphus)

elephant (Loxodonta africana)



African horse sickness: elephants as a reservoir?

- Epidemiology of African horsesickness: antibodies in free-living elephants (*Loxodonta africans*) and their response to experimental infection.
Barnard BJ, Bengis RG, Keet DF, Dekker EH.
Onderstepoort J Vet Res. 1995 Dec;62(4):271-5.
- An attempt to define the host range for African horse sickness virus (Orbivirus, Reoviridae) in east Africa, by a serological survey in some Equidae, Camelidae, Loxodontidae and Carnivores
Binepal VS, Wariru BN, Davies FG, Soi R, Olubayo R.
Vet Microbiol. 1992 Apr;31(1):19-23
- Elephants and zebras as possible reservoir hosts for African horse sickness virus.
Davies FG, Otieno S.
Vet Rec. 1977 Apr 2;100(14):291-2.

FMDV in elephants

- Foot and mouth disease in elephant (*Elephas maximus*).
Rahman H, Dutta PK, Dewan JN.
Zentralbl Veterinarmed B. 1988 Jan;35(1):70-1.
- An outbreak of foot-and-mouth disease in Indian elephants (*Elephas maximus*).
Pyakural S, Singh U, Singh NB.
Vet Rec. 1976 Jul 10;99(2):28-9.



Morbilliviruses crossing species barriers

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PDV in European Harbour seals
Nature 1988 / Science 2002



CDV in Baikal seals
Nature 1988



CDV in Caspian seals
EID 2000



CDV in Serengeti lions
Vaccine 1994



DMV in Med. monk seals
Nature 1997



CDV in macaques
China, EID 2011

should we continue measles vaccination for ever?

CDV antibodies... what about disease?

- Canine distemper virus antibodies in the Asian elephant (*Elaphas maximus*).

Oni O, Wajjwalku W, Boodde O, Chumsing W.

Vet Rec. 2006 Sep 23;159(13):420-1.



Order *Mononegavirales*, family *Paramyxoviridae*

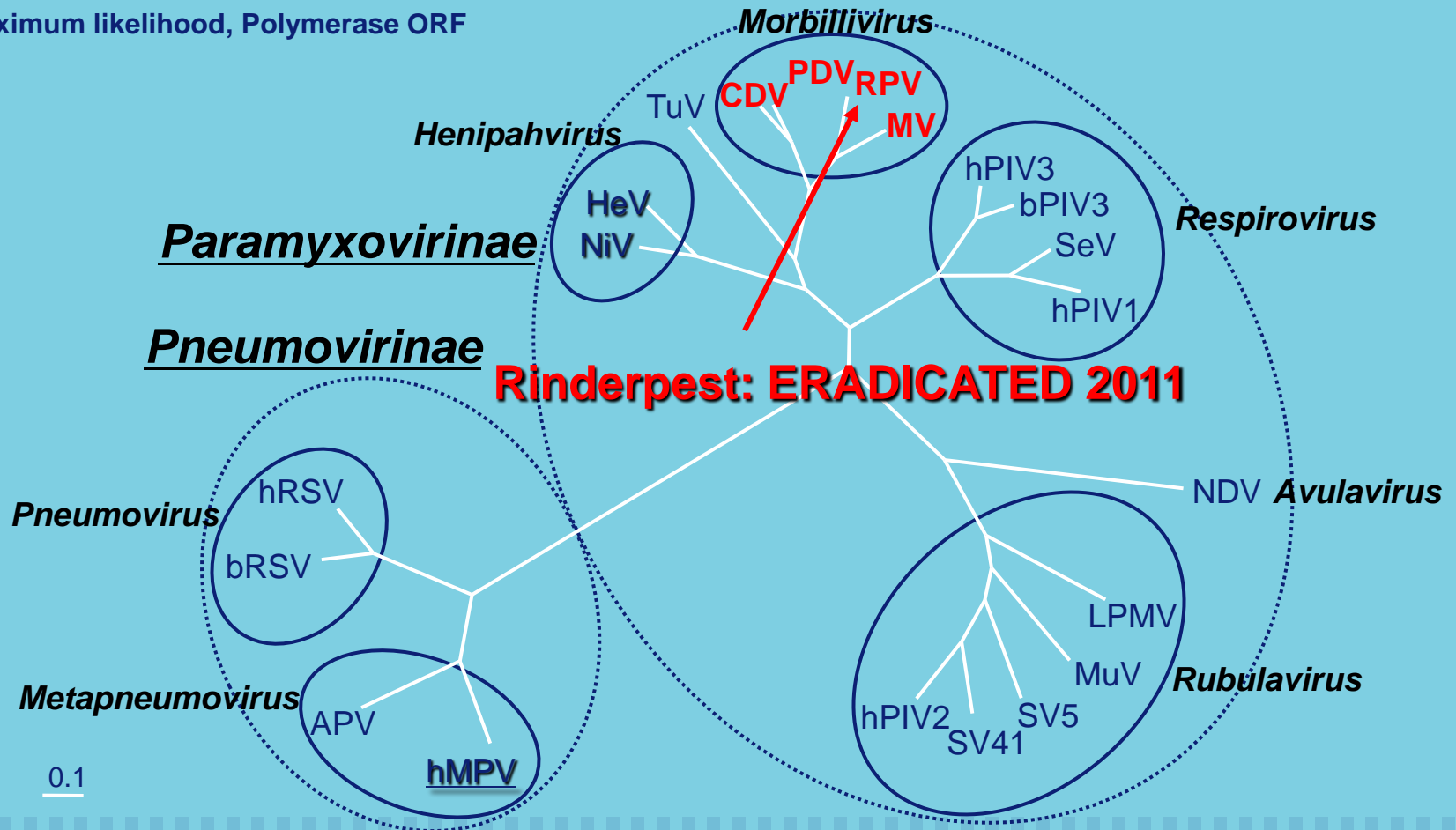
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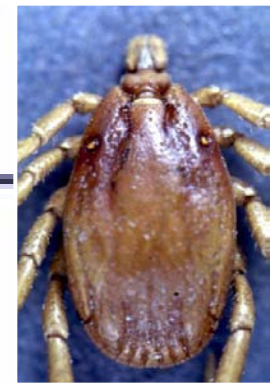


Morbilliviruses: a continuing story!!!

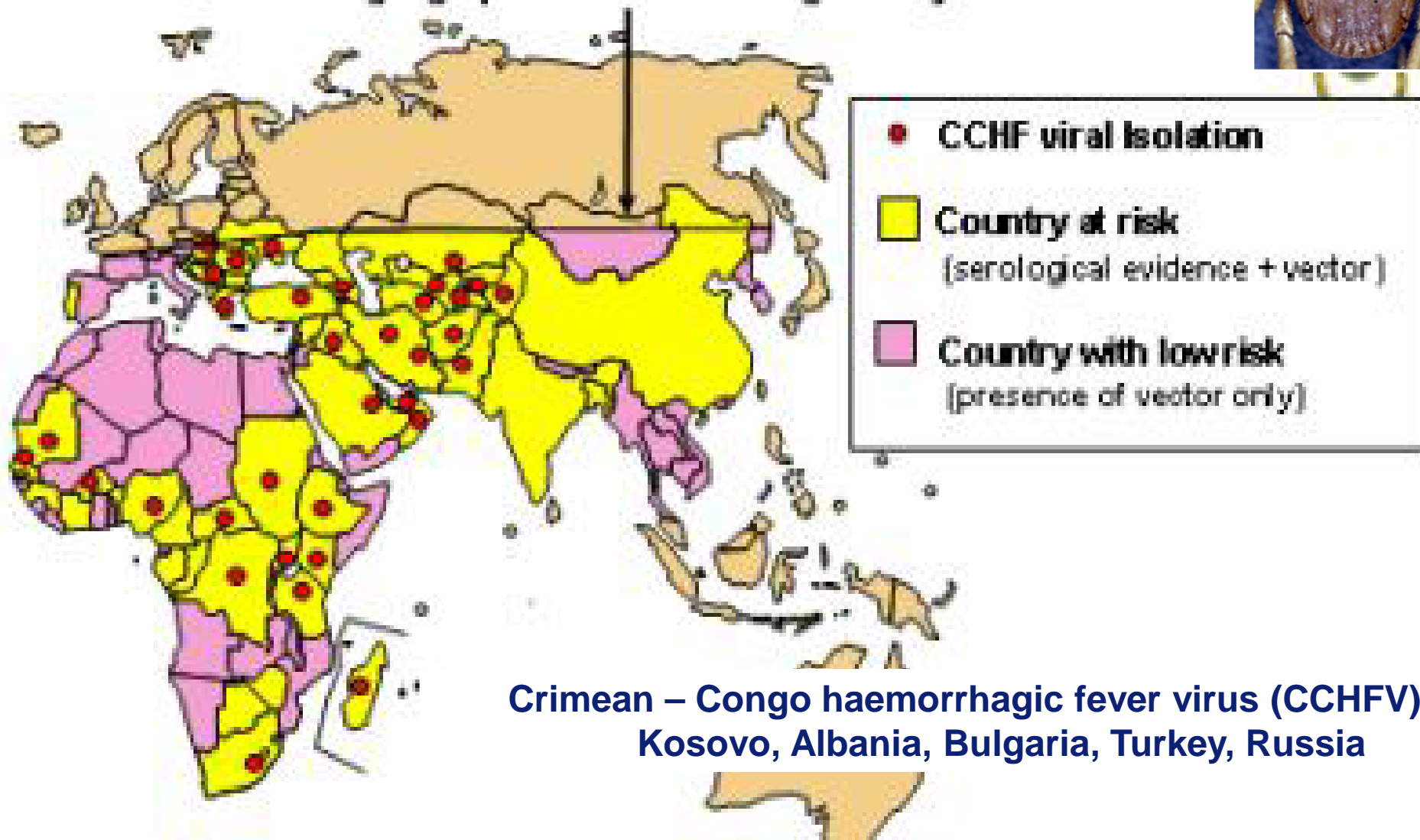
DNA Maximum likelihood, Polymerase ORF



CCHF: geographic distribution



North limit for the geographic distribution of genus *Hyalomma* ticks



Crimean – Congo haemorrhagic fever virus (CCHFV)
Kosovo, Albania, Bulgaria, Turkey, Russia

CCHV antibodies in African elephants

- Enzyme-linked immunosorbent assays for the detection of antibody to Crimean-Congo haemorrhagic fever **virus** in the sera of livestock and wild vertebrates.

Burt FJ, Swanepoel R, Braack LE.

Epidemiol Infect. 1993 Dec;111(3):547-57.

The CELISA was applied to the sera of 960 wild vertebrates from a nature reserve in SouthAfrica, and the prevalence of antibody was found to be greatest in large mammals such as rhinoceros, giraffe and buffalo, which are known to be the preferred hosts of the adult tick (*Hyalomma*) vectors of the virus.

TICK-BORNE ENCEPHALITIS -geographical distribution-

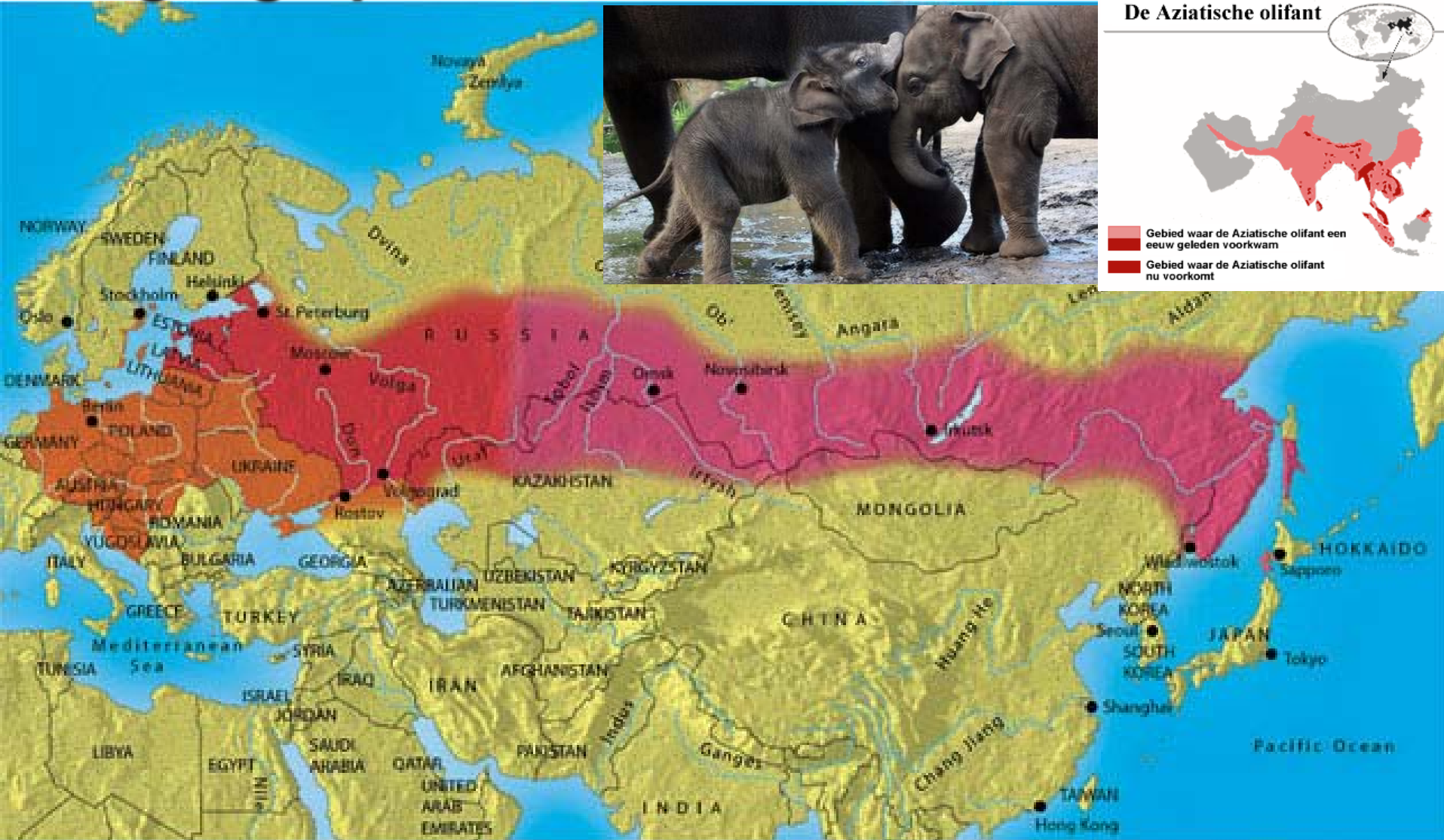
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De Aziatische olifant



- Gebied waar de Aziatische olifant een eeuw geleden voorkwam
- Gebied waar de Aziatische olifant nu voorkomt

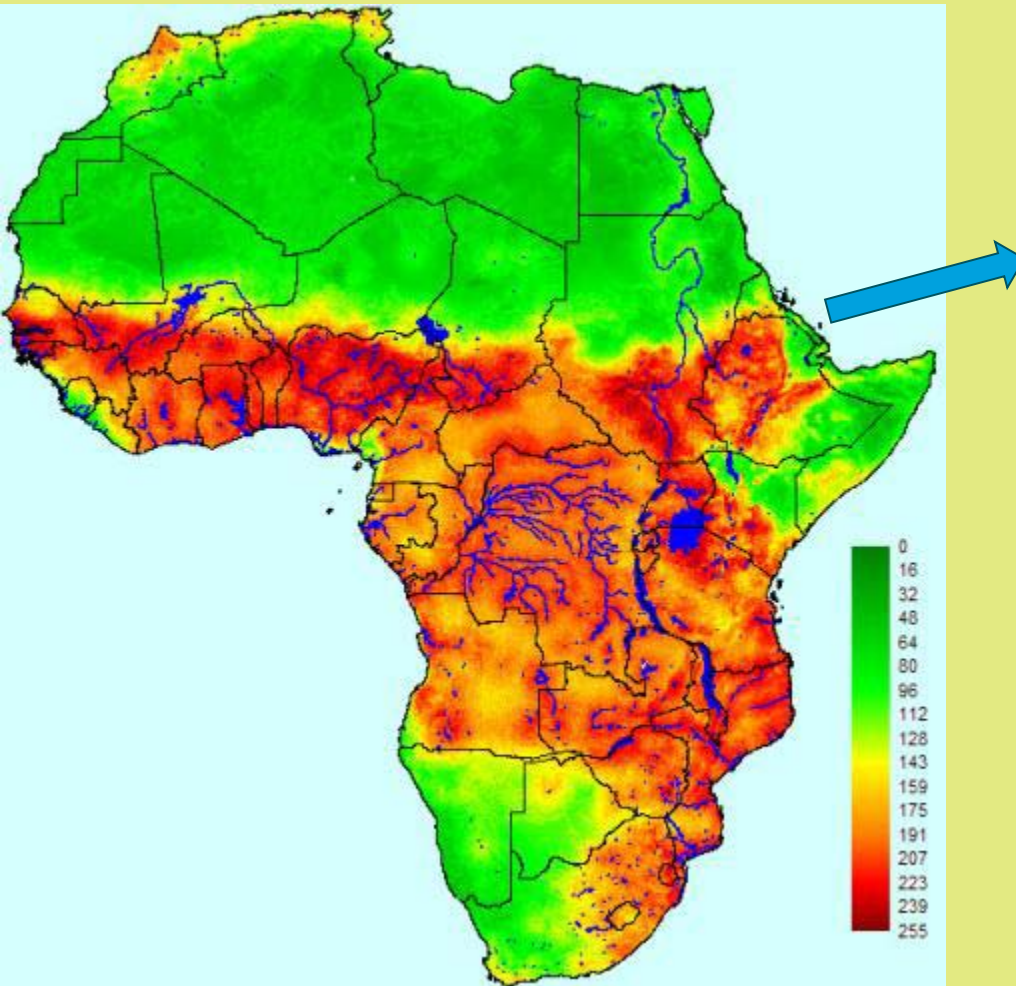


Western subtype

both subtype

Eastern subtype

Climate effects on mosquito-borne infections



RVF outbreaks: massive mortality ungulates (and HF in humans)



Food shortage, increased consumption of bush meat



Increased risk of zoonotic infections (eg monkeypox)

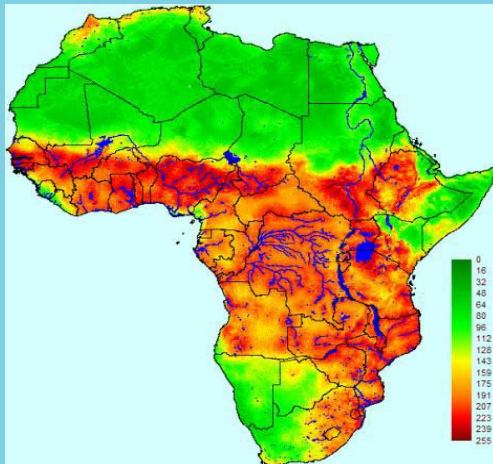
Rift Valley Fever (RVF) is a viral zoonosis (affects primarily domestic livestock, but can be passed to humans) causing fever. It is spread by the bite of infected mosquitoes, typically the Aedes or Culex genera.

Rift valley fever antibodies in elephants

Prevalence of antibodies against Rift Valley fever **virus** in Kenyan wildlife.

Evans A, Gakuya F, Paweska JT, Rostal M, Akoolo L, Van Vuren PJ, Manyibe T, Macharia JM, Ksiazek TG, Feikin DR, Breiman RF, Kariuki Njenga M.

Epidemiol Infect. 2008 Sep;136(9):1261-9. Epub 2007 Nov 8

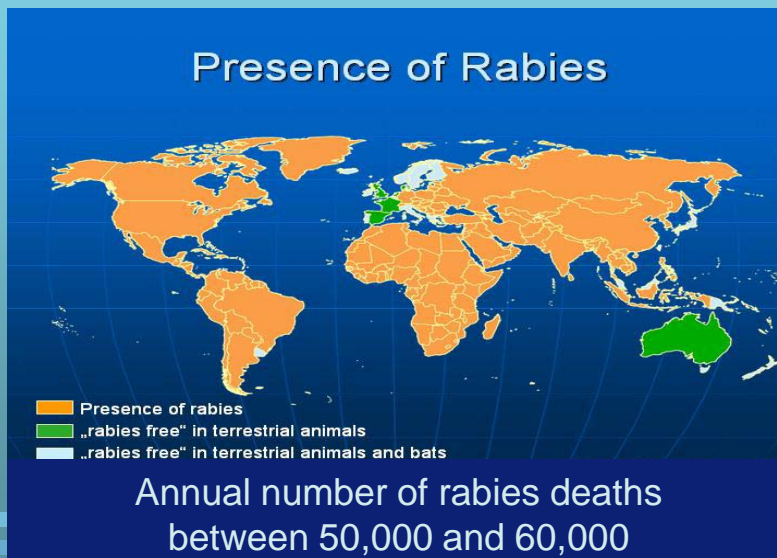


Duvenhage (rabies) virus infection in The Netherlands: “out of Africa”

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failure of treatment
of a patient in
The Netherlands!



- van Thiel et al., Euro surveill., 2008
- van Thiel et al., Plos Negl. Trop. Dis. 2009

Rabies in elephants

- Serum antibody titers following routine rabies vaccination in African elephants.

Miller MA, Olea-Popelka F.

J Am Vet Med Assoc. 2009 Oct 15;235(8):978-81.

- Results of vaccination of Asian elephants (*Elephas maximus*) with monovalent inactivated rabies vaccine.

Isaza R, Davis RD, Moore SM, Briggs DJ.

Am J Vet Res. 2006 Nov;67(11):1934-6.

- First reported case of elephant rabies in Sri Lanka.

Wimalaratne O, Kodikara DS.

Vet Rec. 1999 Jan 23;144(4):98.

CONCLUSIONS:

VIRUSES in elephants and rhinos

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Points to consider:

- problems in captivity (pox, herpes, EMC...)
- problems in the wild (herpes, EMC, rabies, FMDV...)
- reservoirs (BT, AHS, EMC, FMDV...)
- virus discovery (classical / advanced techniques)
- importance for translocation / conservation
- surveillance (syndromic, sampling / laboratory)
- prevention (wildlife- / captive management...)

