



Epidemic risks in CEE with focus on highly pathogenic viruses: the case of Crimean-Congo Hemorrhagic fever virus

gap analysis

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ERIHNA: High-Consequence Infectious Diseases in Central Eastern Europe:
Gaps and Cooperation Opportunities

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Institute of Microbiology and Immunology

Faculty of Medicine, University of Ljubljana

- a leading teaching, research and diagnostic institution in Slovenia
- 18 highly specialized laboratories, covering clinical microbiology and immunology
- > 610 different microbiological tests
- > 550.000 tests/year
- BSL3+ facility
- BSL2 animal facility





Building laboratory diagnostic capacity



Development and implementation of advanced & automated diagnostic methods

Isolation of live pathogens

Partners in infectious diseases outbreak control - confirmatory testing



Surveillance on emerging pathogens and antimicrobial resistance

Field work: collection of environmental, human, animal samples

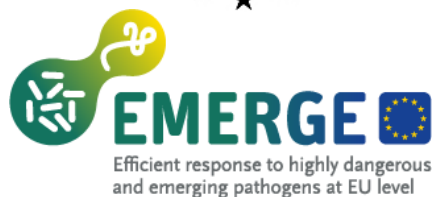
Supply of diagnostic material



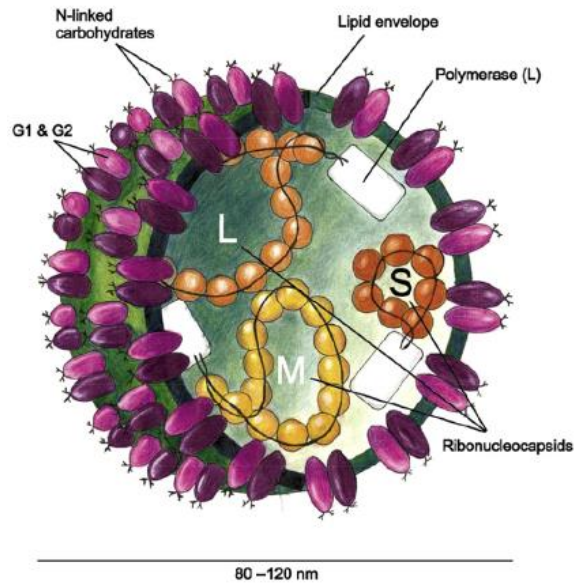
Hands-on training courses & access to facility for neighbouring countries

Partners in international research projects

Collaboration with Civil protection and disaster relief administration and armed forces

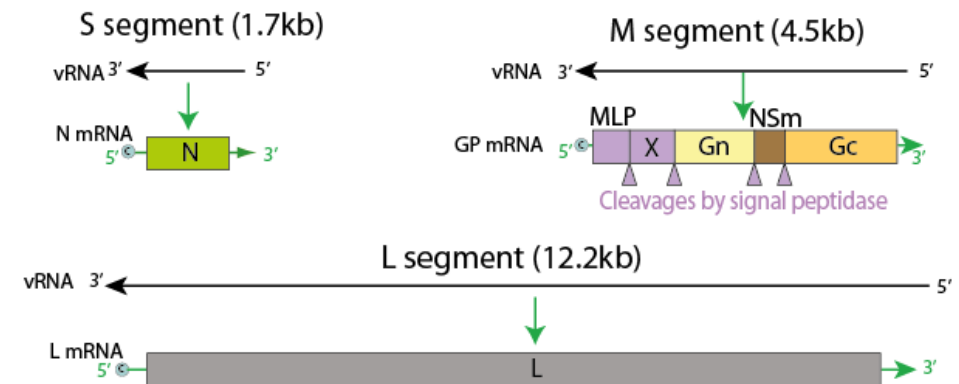


Crimean Congo hemorrhagic fever virus

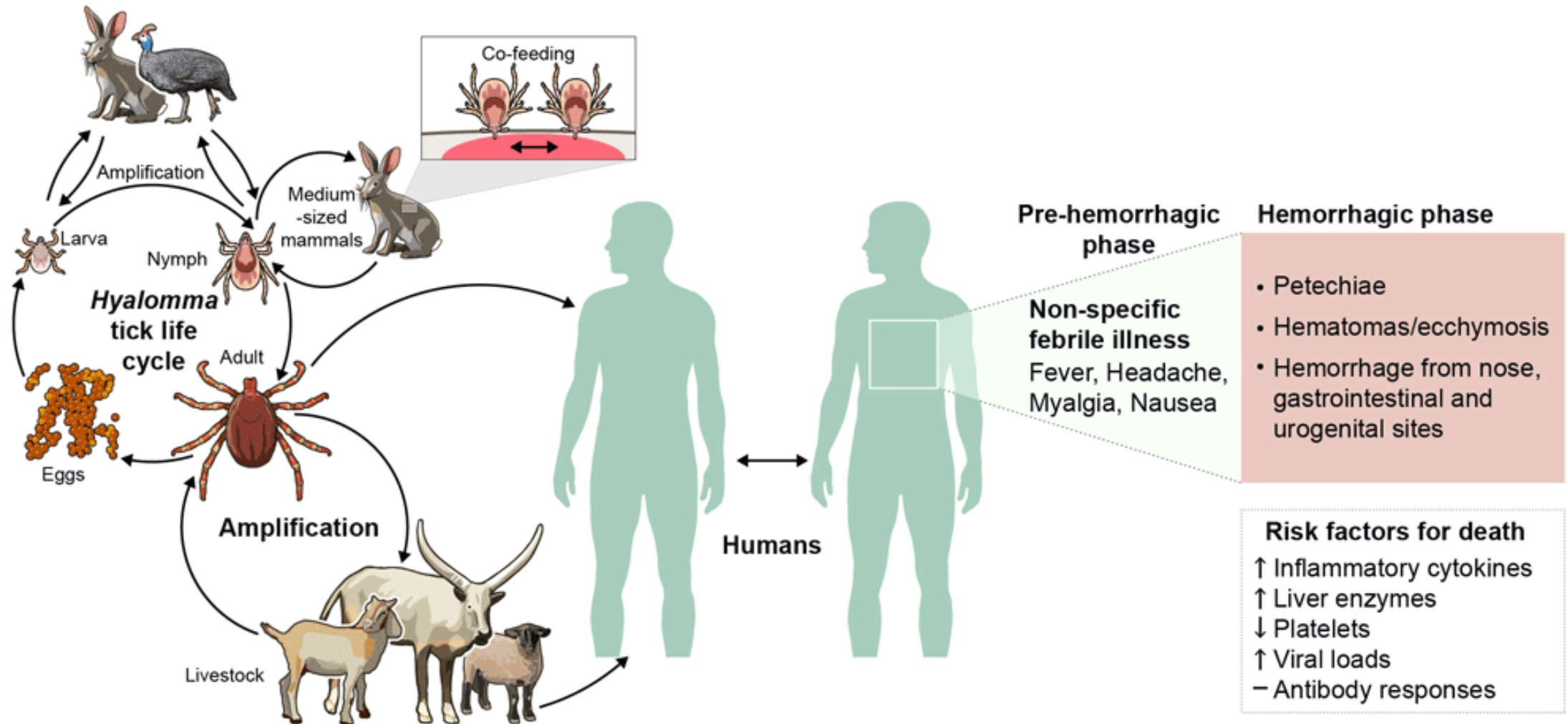


- genus *Nairovirus*, order *Bunyaviridae*
- Lipid bilayer
- S segment - nucleocapsid protein NP
- M segment - structural proteins (G1=Gn and G2=Gc, NSm)
- L segment - viral RNA polymerase

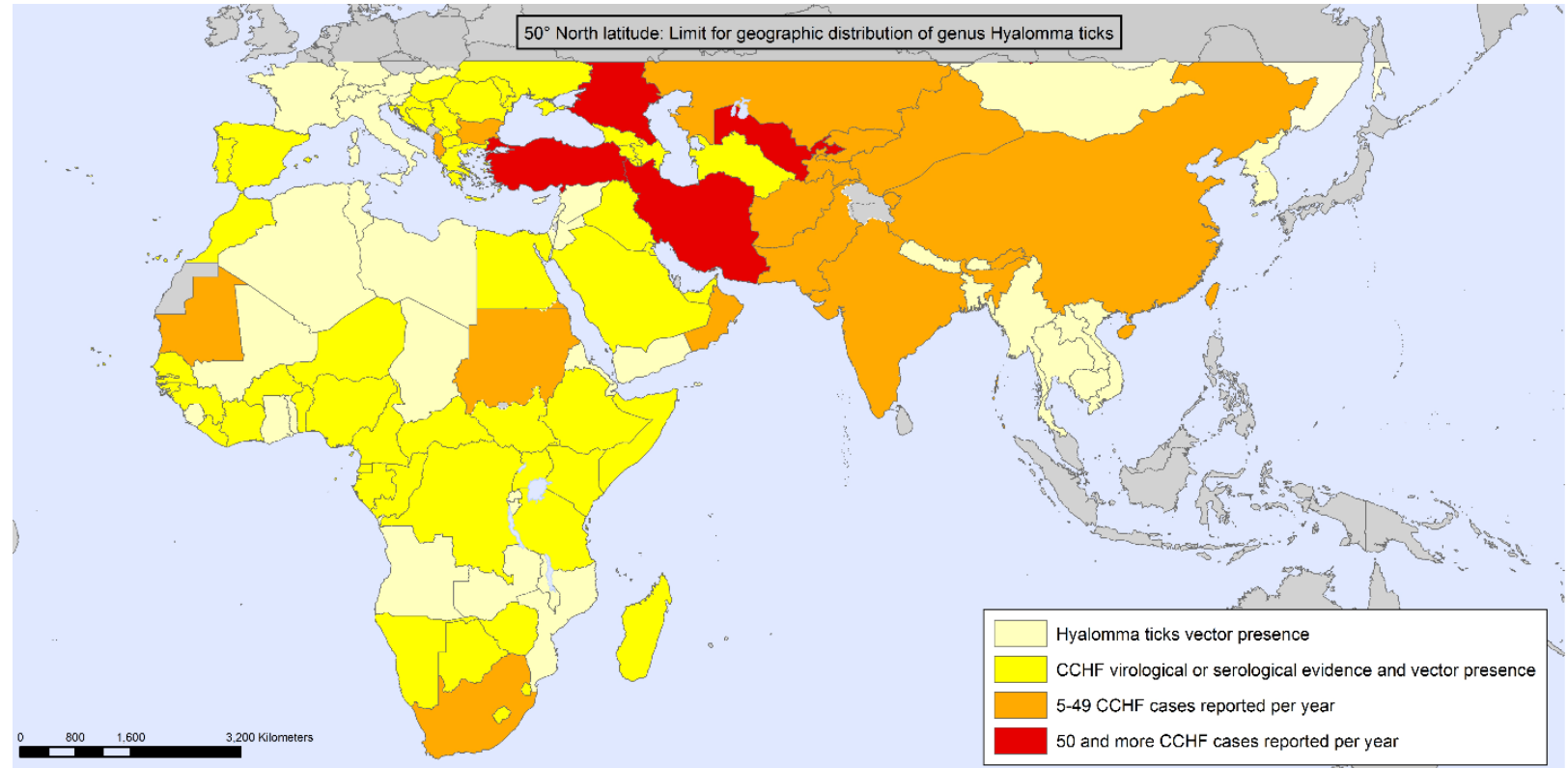
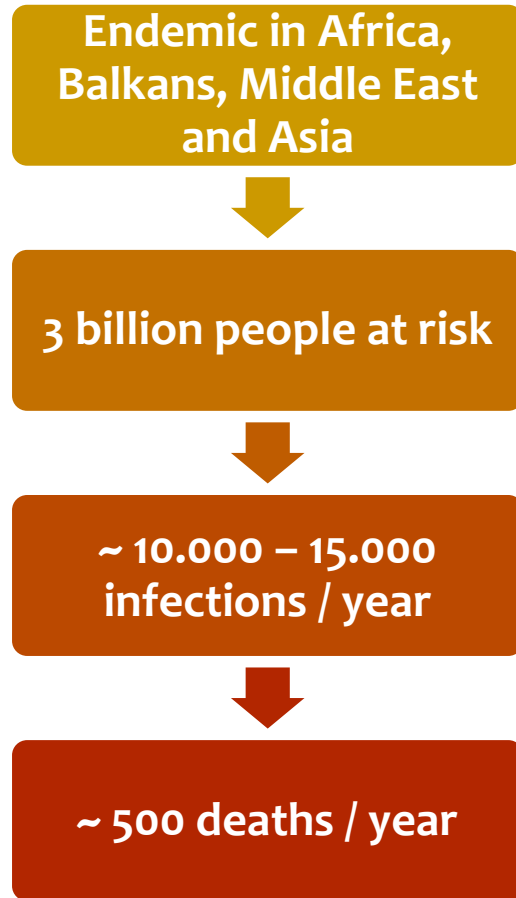
The virus is highly contagious and causes severe disease, it should only be handled in high-containment laboratories.



Natural reservoir and transmission to humans



Risk areas and burden of CCHF



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization
Map Production: Information, Evidence
and Research(IER)
World Health Organization

 **World Health Organization**
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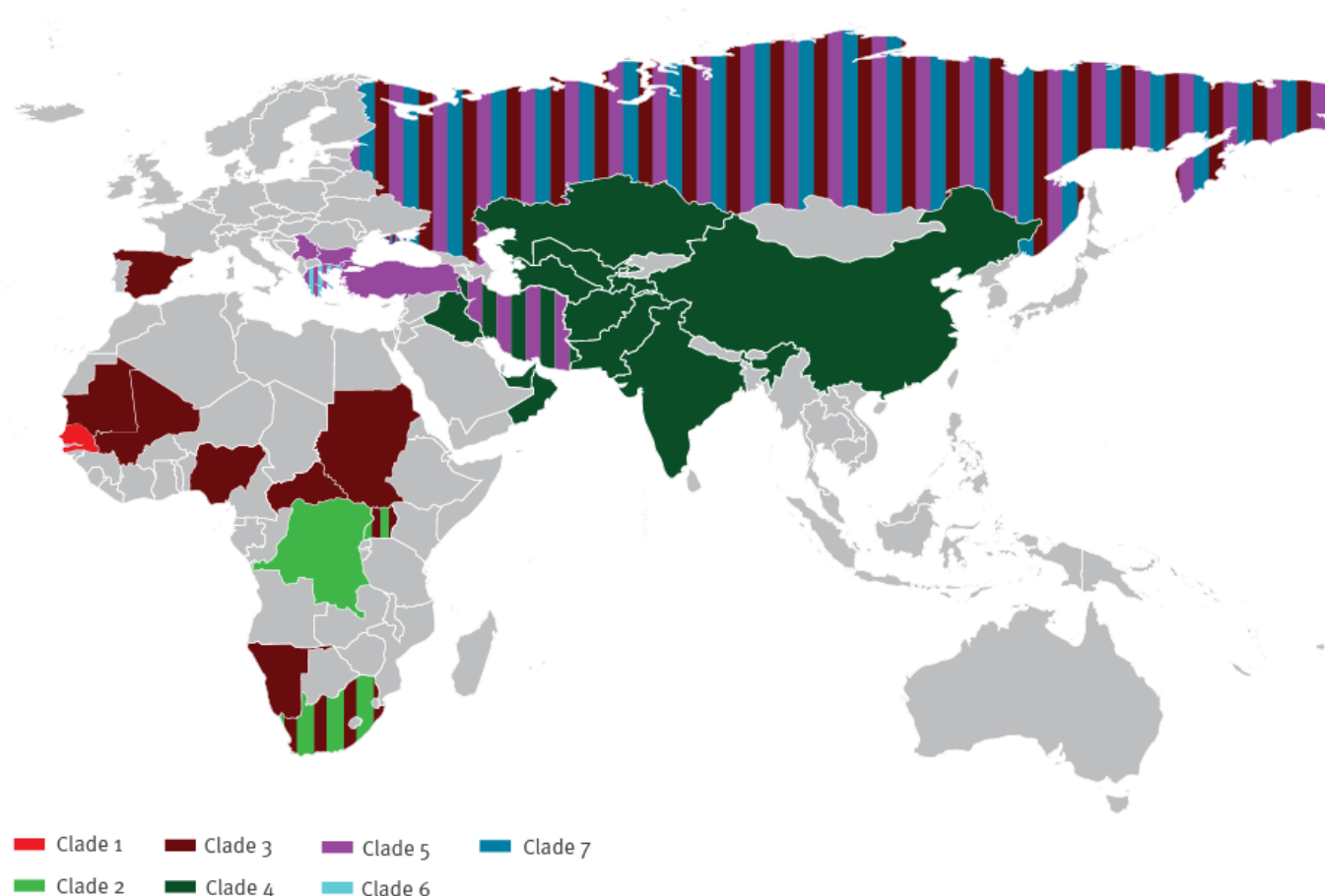


CCHF case trend and fatality rates

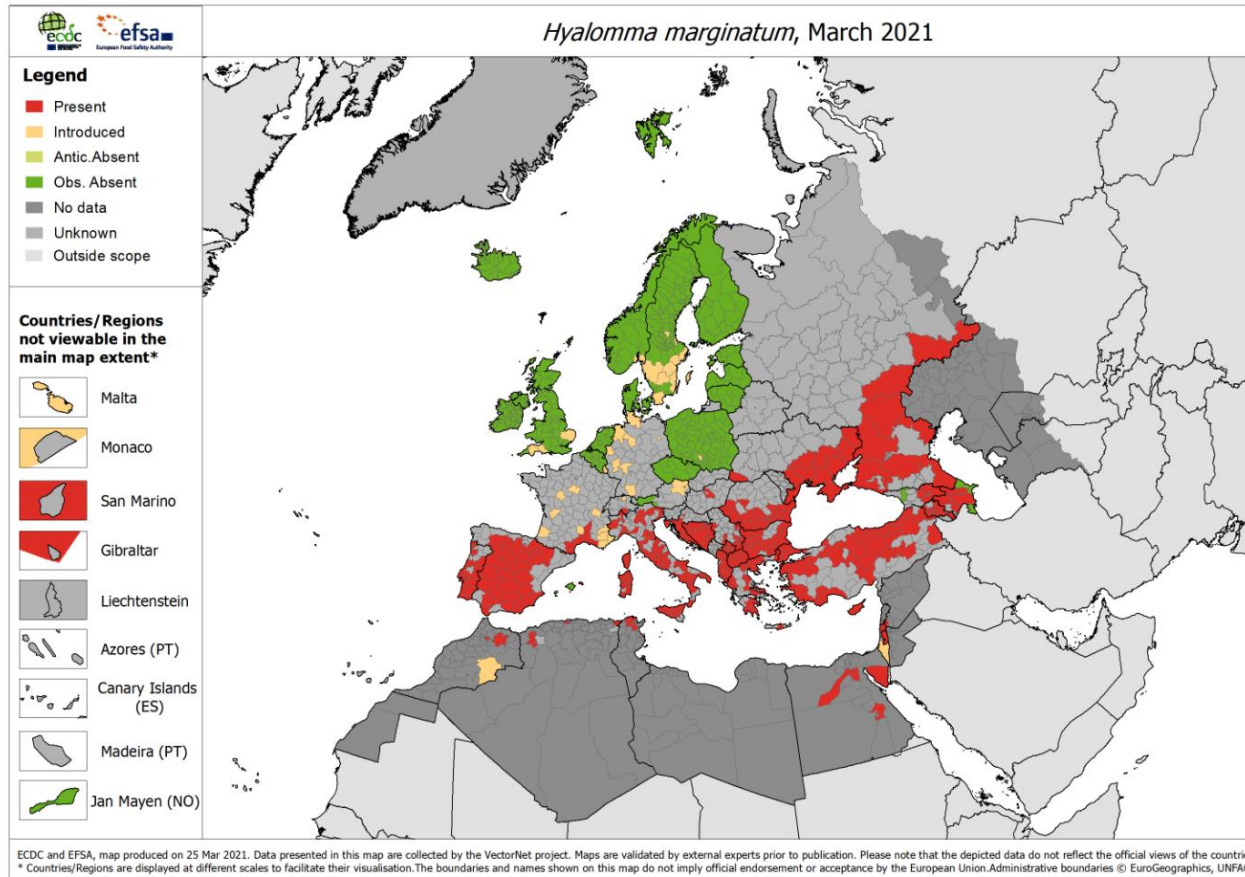
- Increasing trend of CCHF cases occurred through the past
- The means of annually and periodically CCHF fatality rates are about one-tenth of CCHF human cases.
- The mean of CCHF fatality rates: Africa (22.0 %), Asia (33.5 %), Europe (33.8 %).
- Among occupations involved in CCHF:
 - agricultural (28.9 %)
 - health-care (19.2 %)
 - slaughterhouse workers (16.7 %)
 - farmers (13.9 %)

World map of virus phylogenetic diversity

- **7 genetic lineages**
- **Clade I, II and III** - three lineages are found in Africa and Spain
- **Clade IV** - Central Asia (Kazakhstan, Tajikistan, Uzbekistan and China), and the fourth in Pakistan, Madagascar and some of the strains found in Iran.
- **Clade V** - first European lineage comprises the viruses found in East Europe covering a region from the Balkan peninsula, through Turkey and into Russia
- **Clade VI** - second European lineage, named AP92 and found mainly in Greece, forms an independent group (less pathogenic)
- **Clade VII** - third European lineage comprises the viruses found in East Europe



CCHF in Europe



Cases of human infections have been reported from:

- Albania, Armenia, Bulgaria, Kazakhstan, Kosovo, Russia, Serbia, Tajikistan, Turkey, Turkmenistan, Ukraine, and Uzbekistan
- 2008 – first fatal case in Greece
- 2016 - first locally acquired case in Spain



CCHF burden in Europe

Albania

- First case in 1986;
- 5 -10 cases annually
- since 2000 cases are concentrated in Northern area
- Households outbreaks inside
- 1,3 % seroprevalence in general population

Bulgaria

- Virus first isolated in 1960s
- 1953-1974: 1,105 reported cases
- 1997-2004: 124 cases
- Since 2013 from 1 – 8 cases every year
- Endemic area: central, eastern & southwestern Bulgaria
- Vaccination for high risk groups
- Seroprevalence in ticks: 2,0 – 4,8 %

Greece

- 1975 - CCHF virus (AP92) was isolated from *Rhipicephalus bursa* collected from goats
- seroprevalence of 1,1 % (range 0-6,3 %), but without any recognized disease
- 2008 first fatal case – woman exposed to tick bite during farming activities in rural areas near Komotini
- RT-PCR positive – CCHFV belongs to the Europe 1 lineage.

Turkey

- First recognized in 2002
- Cases reported every year
- Nearly 1000 cases reported annually
- prevalent in Turkey's northern regions
- peak usually in the second half of spring and the first half of summer
- CFR around 4,6 %
- Family outbreaks, nosocomial infections

Spain

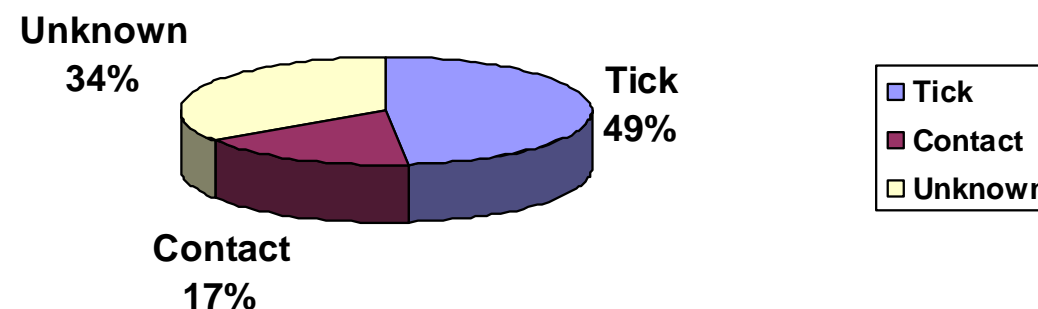
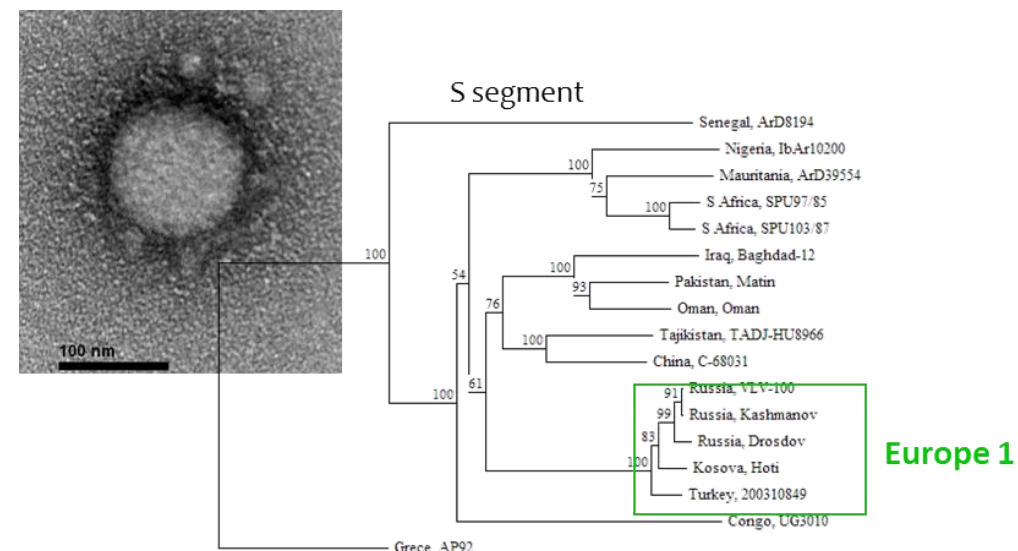
- 2016, Spain reported its first autochthonous cases in the province of Ávila, Castile-León.
- Nosocomial infections
- Retrospective study – first case 2013
- 2013 -2021: 10 cases / 3 deaths (3 %)

CCHF in Kosovo

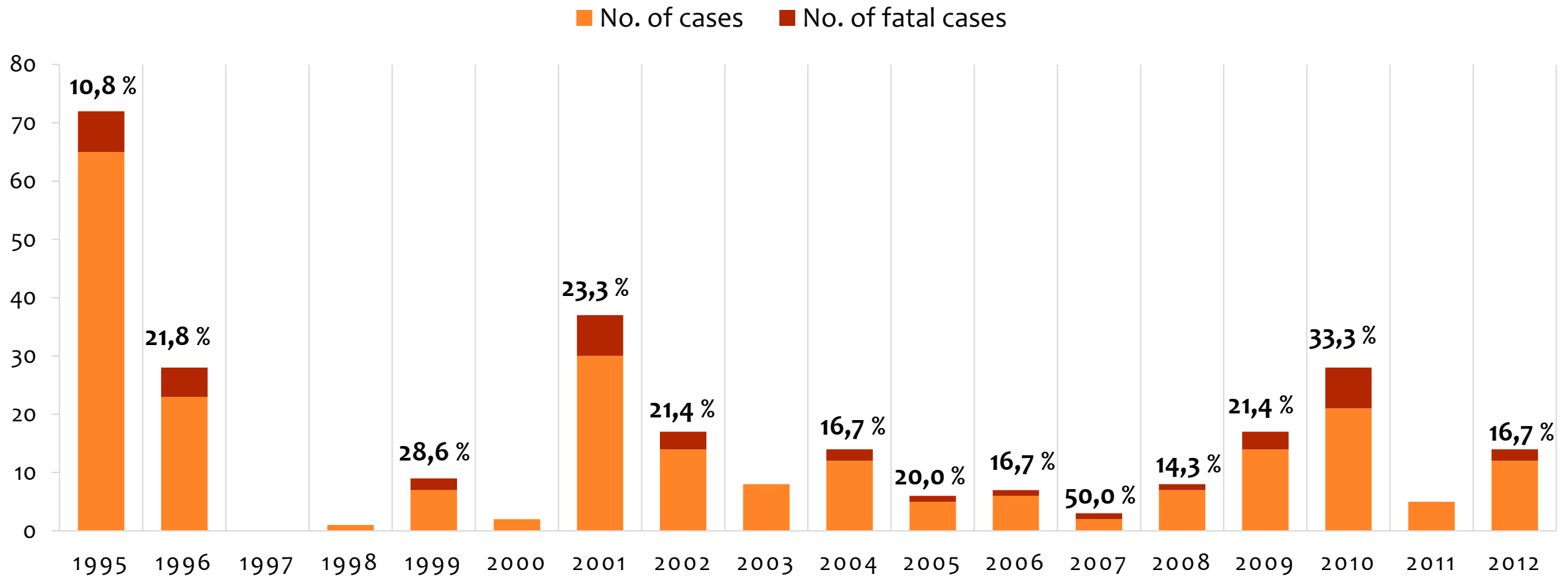
- First cases in 1954 in Nishor village (8 deaths)
- 1970 - outbreak among shepherds in village Čiflik:
 - 13 cases /2 fatal
 - 269 *H. marginatum* ticks collected – 3 strains of CCHF virus were isolated (A. Gligić)
 - 3 % seroprevalence in residents of Čiflik
 - 691 livestock tested for CCHF antibodies: 14 % in cattle and 32.6 % in sheep
- 1991-1992: 76 suspected cases (5 confirmed - 3 fatal)
- 1995-2000 disease was present in sporadic or epidemic form

2001 – outbreak in Kosovo

- Breakdown of healthcare and public health systems after war
- 114 suspected cases : 31 cases / 7 deaths (23,3 %)
- 7/24 PCR positive cases were seronegative at the time of first sample being taken
- virus was sequenced & isolated in cell culture (Kosova-Hoti)
- Sequence analysis confirmed intrahospital and intrafamilial transmission



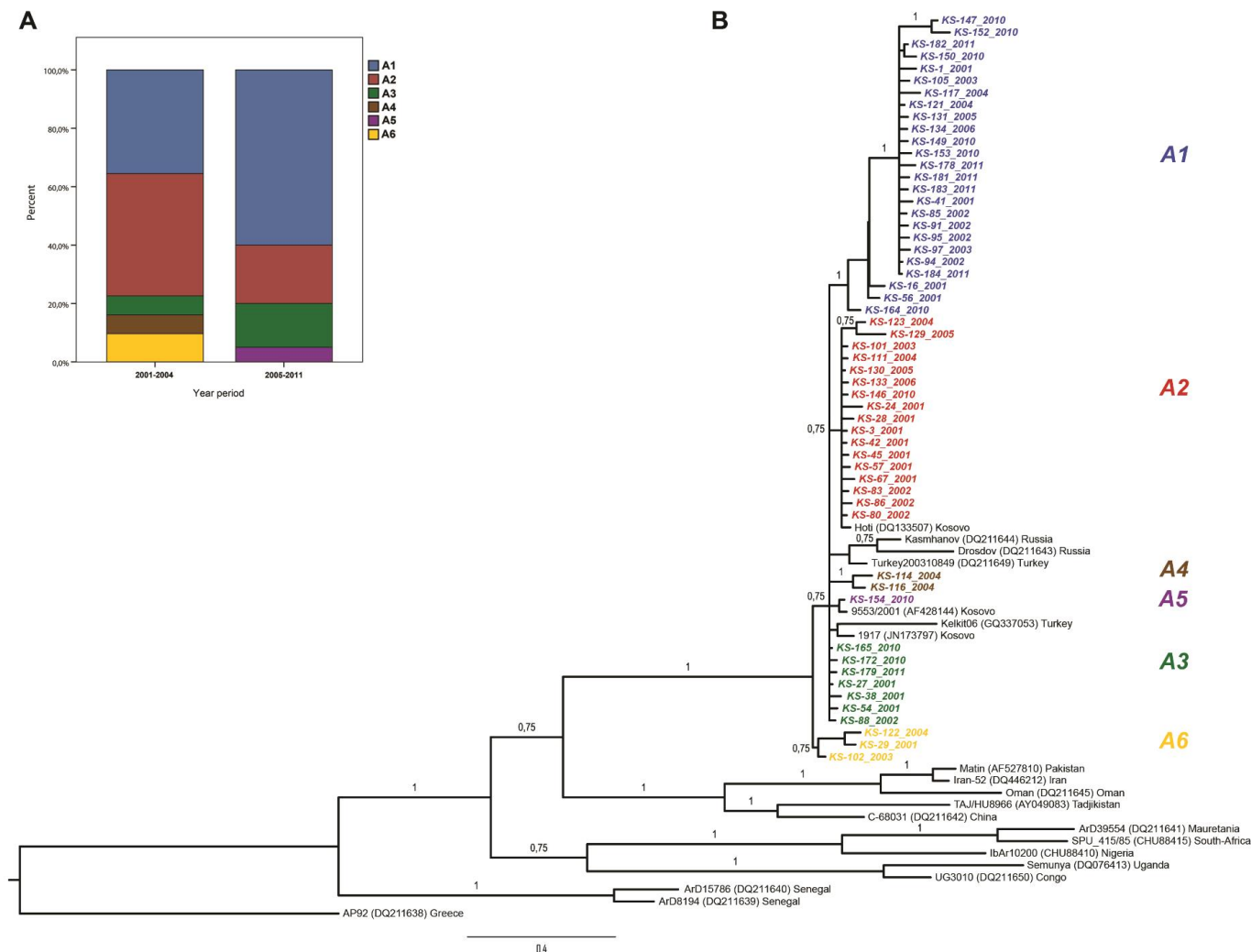
High case fatality rate





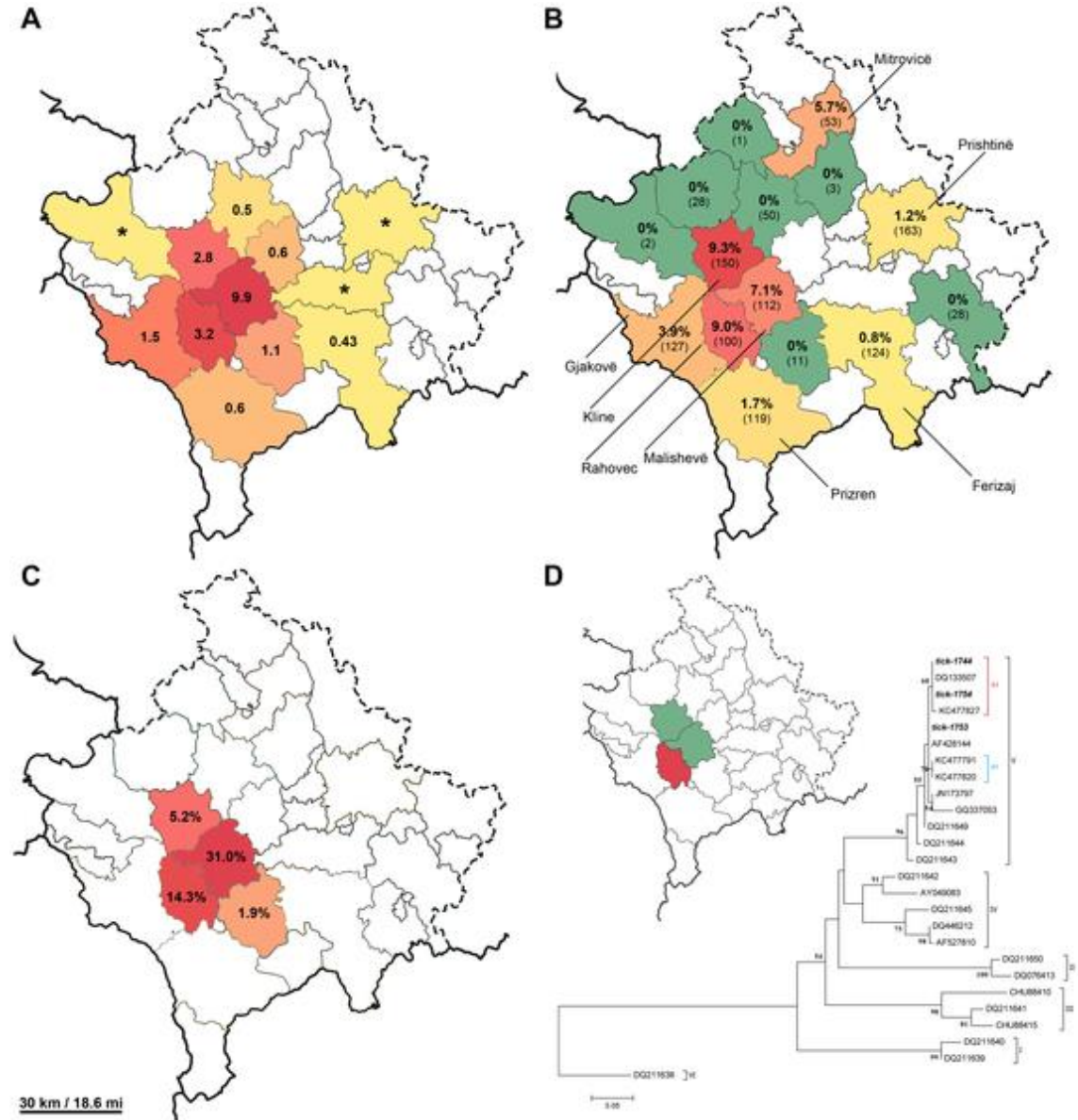
Genetic variability of CCHFV in Kosovo

- we sequenced partial S segment from patients from 1991 to 2013
- high genetic variability in relation to the studied area
- variants are mostly uniformly distributed throughout Kosovo
- suggest the presence of a new distinct lineage within the European CCHF phylogenetic clade



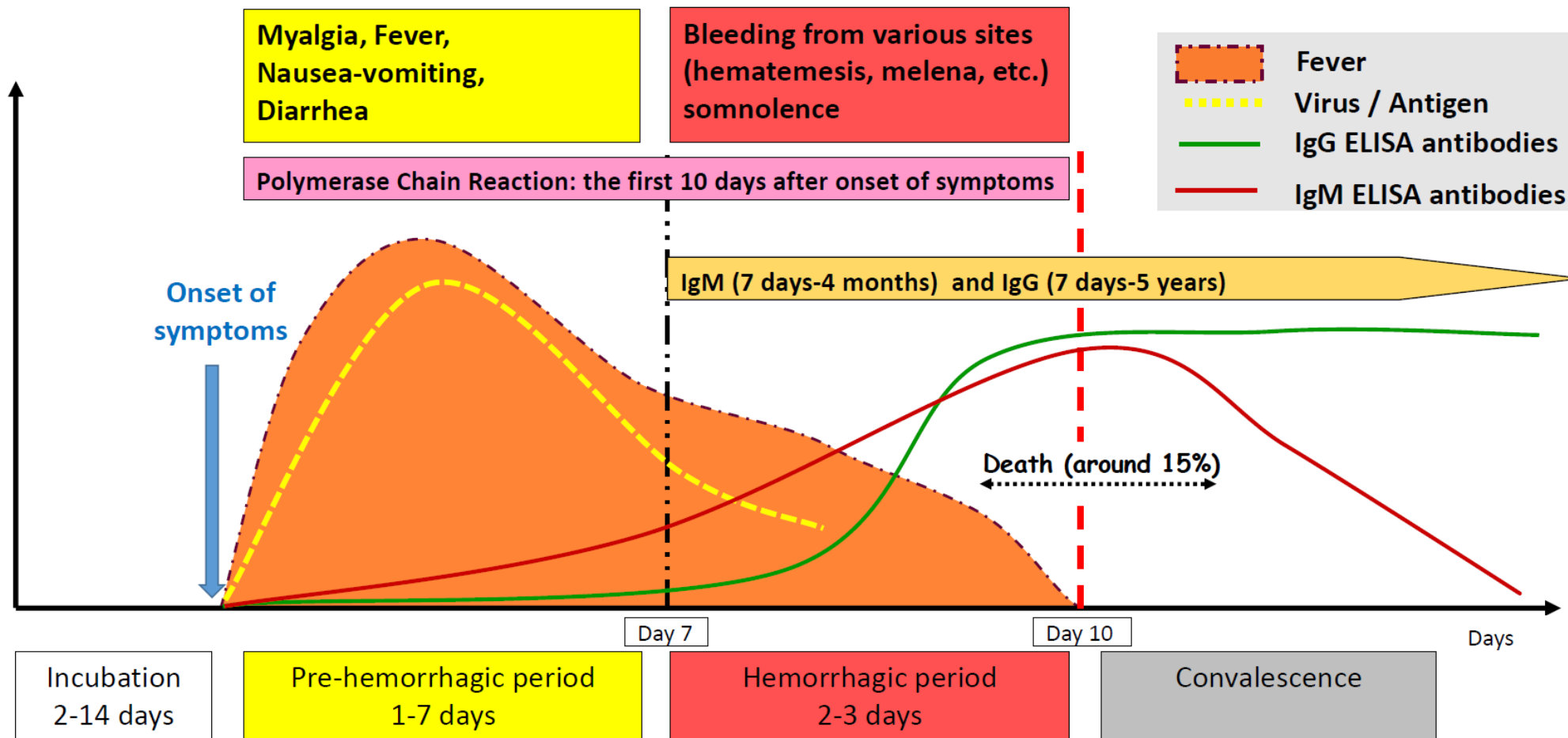
Prevalence of CCHF in Kosovo

- A. Cumulative incidence of CCHF in each municipality (from 1995 to 2013)
- B. Seroprevalence of CCHF in healthy human population
- C. Seroprevalence in cow
- D. Seroprevalence in ticks





Clinical features of the disease



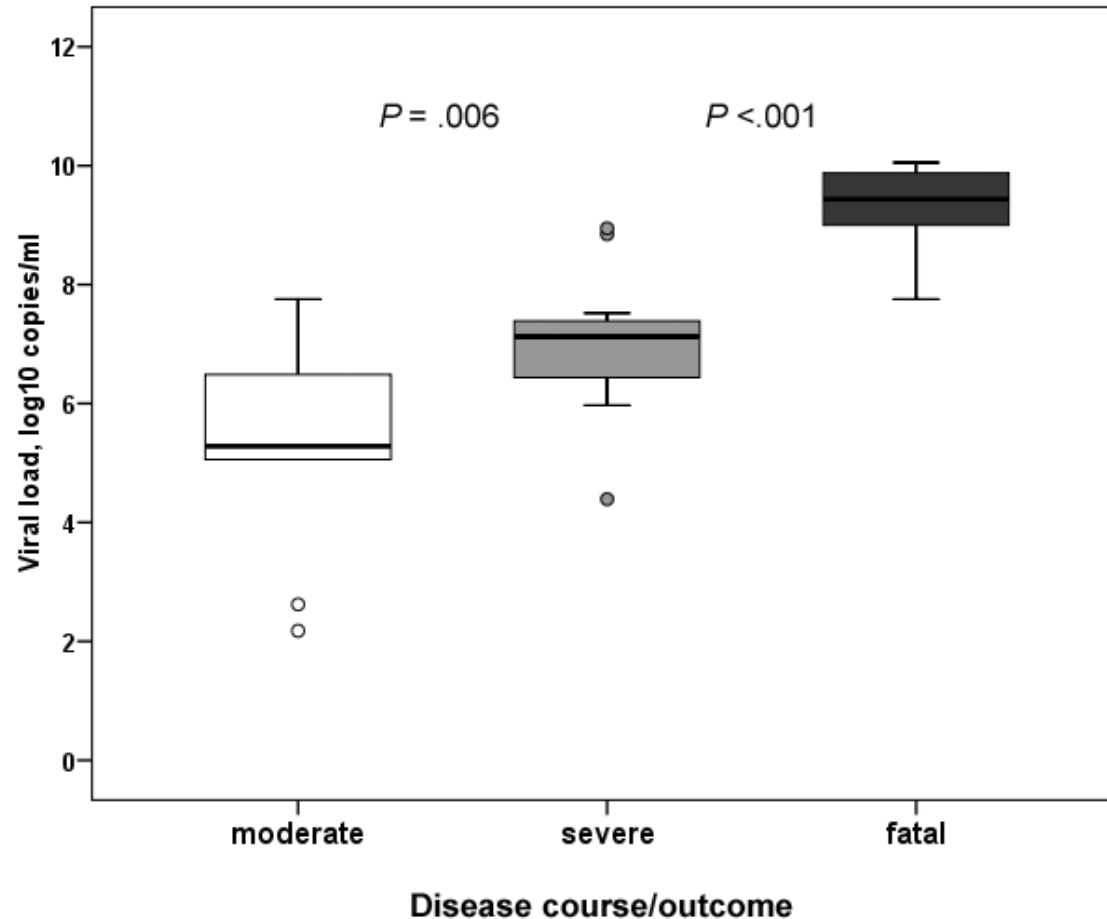
Laboratory diagnostics

Handling and processing specimen requires suitably equipped laboratories under maximum biological containment conditions and trained staff.

Definitive diagnosis requires testing:

- detection of viral genome by RT-PCR up to 10–15 days post onset of illness.
- detection of specific IgM antibodies from day 5
- IgG seroconversion or 4x titer increase – delayed diagnosis
- virus isolation by cell culture

Viral load & clinical course/disease outcome

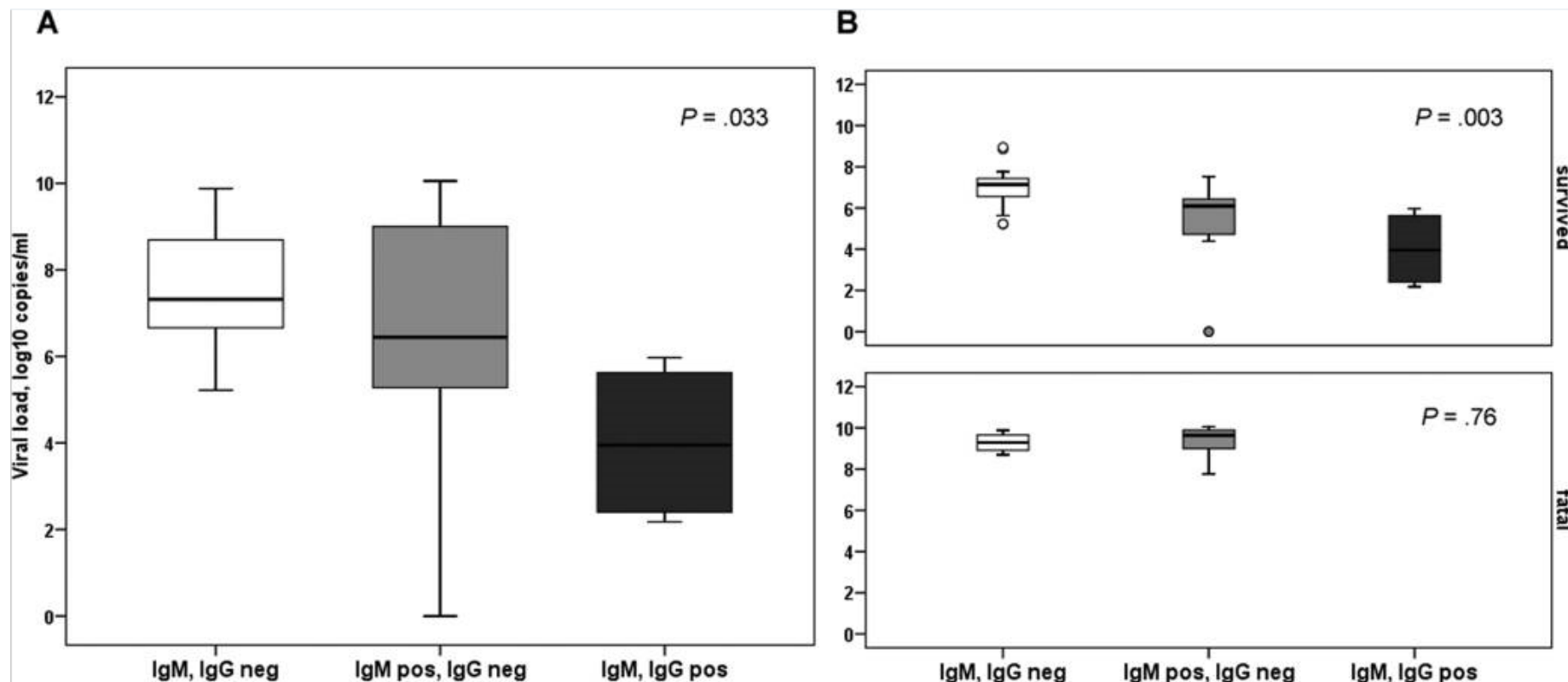


- Viral load of $>10^8$ copies/mL is a strong factor for fatality
- Viral load is also useful for estimating need for infection control measures



Viral load & antibody status

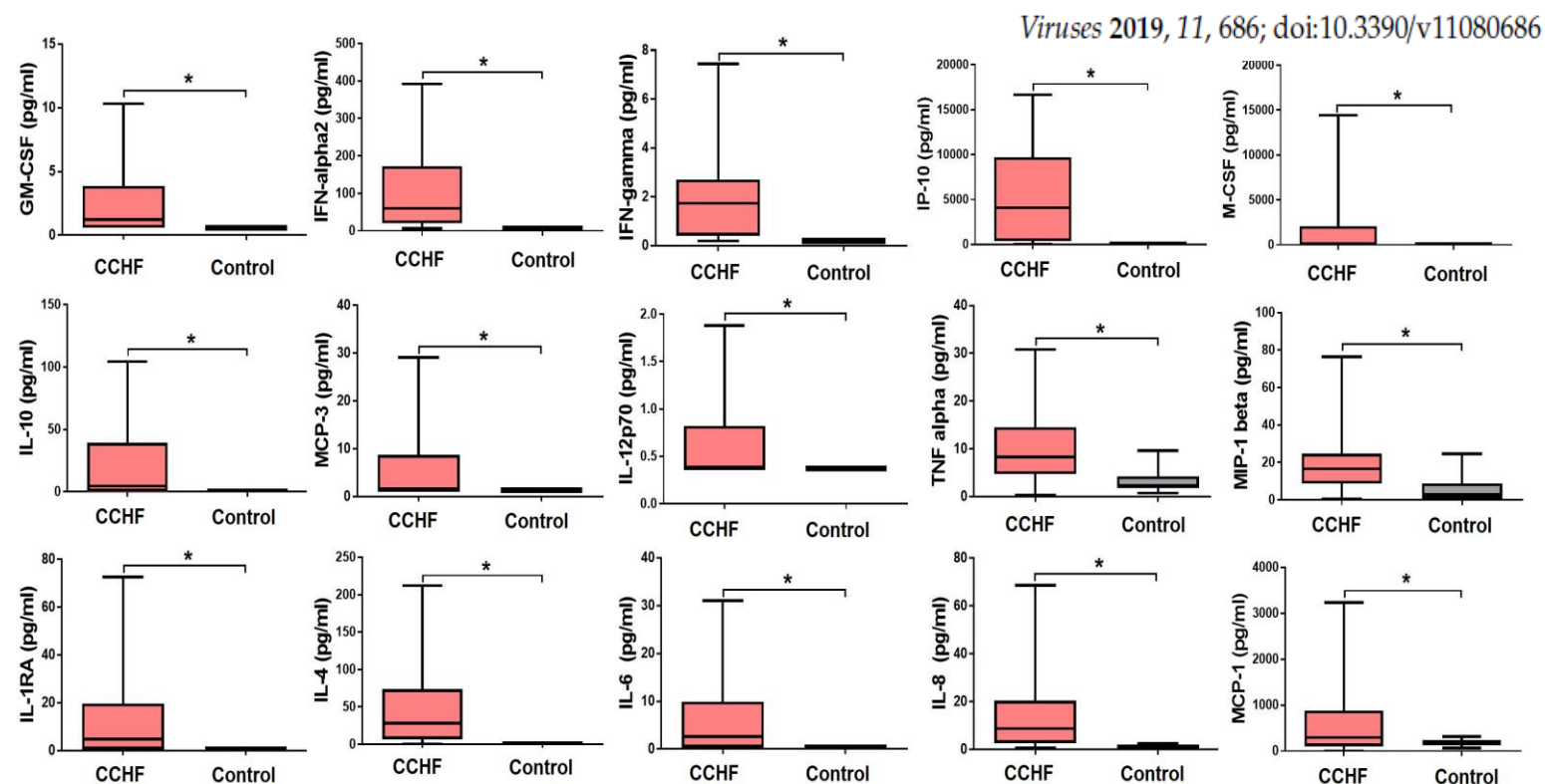
A positive linear dependence of viral load on the day of illness in fatal cases; and negative linear dependence in the survivors.



Pathogenesis in humans

Endothelium is the major target of CCHFV and can be activated directly by the virus and/or indirectly by virus-induced soluble mediators

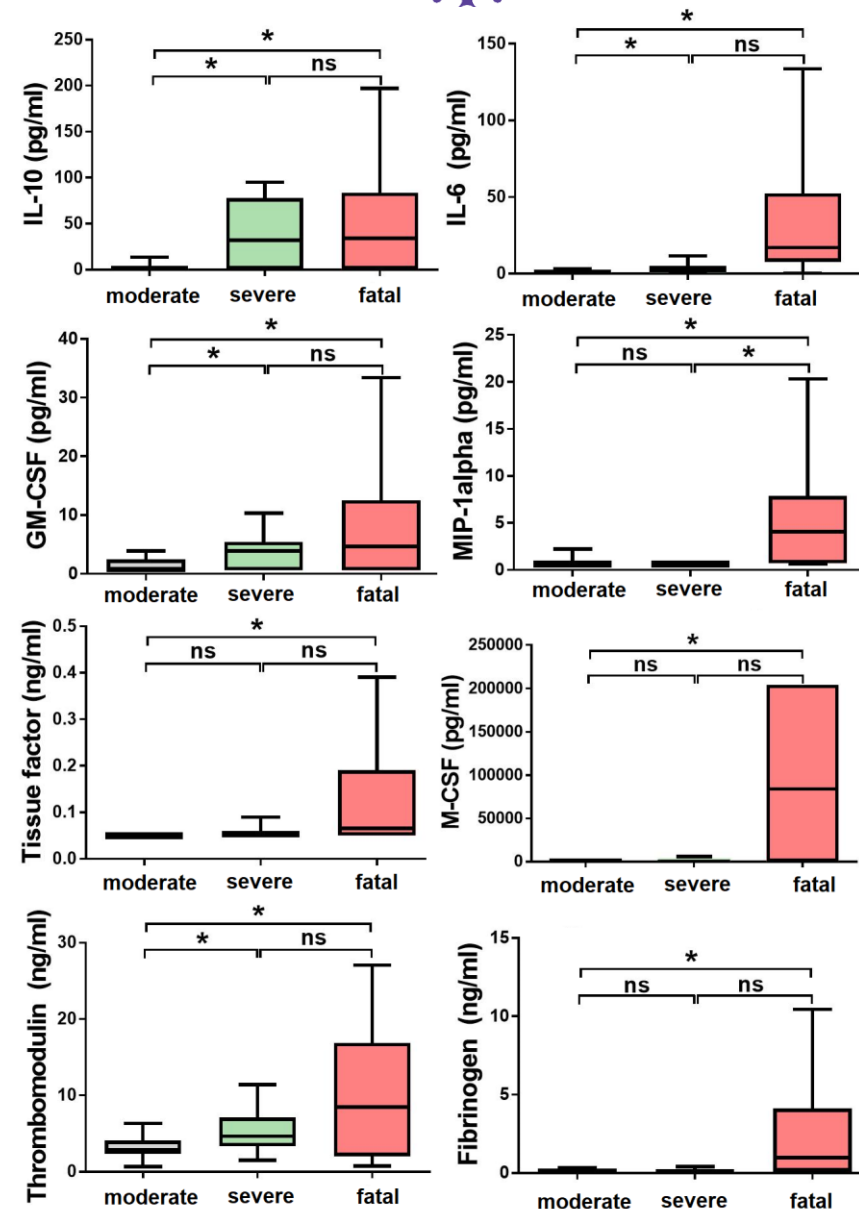
- CCHF patients have **STRONG INNATE** and TH1 immune response
- Viral load was strongly associated with IP-10





Biomarkers of endothelial dysfunction

- CCHF patients had significantly elevated levels of **d-dimer, fibrinogen, vWF, PECAM-1 and Thrombomodulin** compared to control group.
- Viral load was associated with endothelial markers too: Ang-2, PAI -1, ADAMTS3, TF, TM, PECAM-1 and d-dimer.
- **FATAL vs. SURVIVORS**
 - fatal: ↑ IL-6, IL-10, MIP-1, GM-CSF, M-CSF, fibrinogen, TF and TM
 - survivors: ↑ sCD40L and PF4 - markers of platelet activation



Pathogenesis research -animal models

- **Type I interferon-deficient mice**

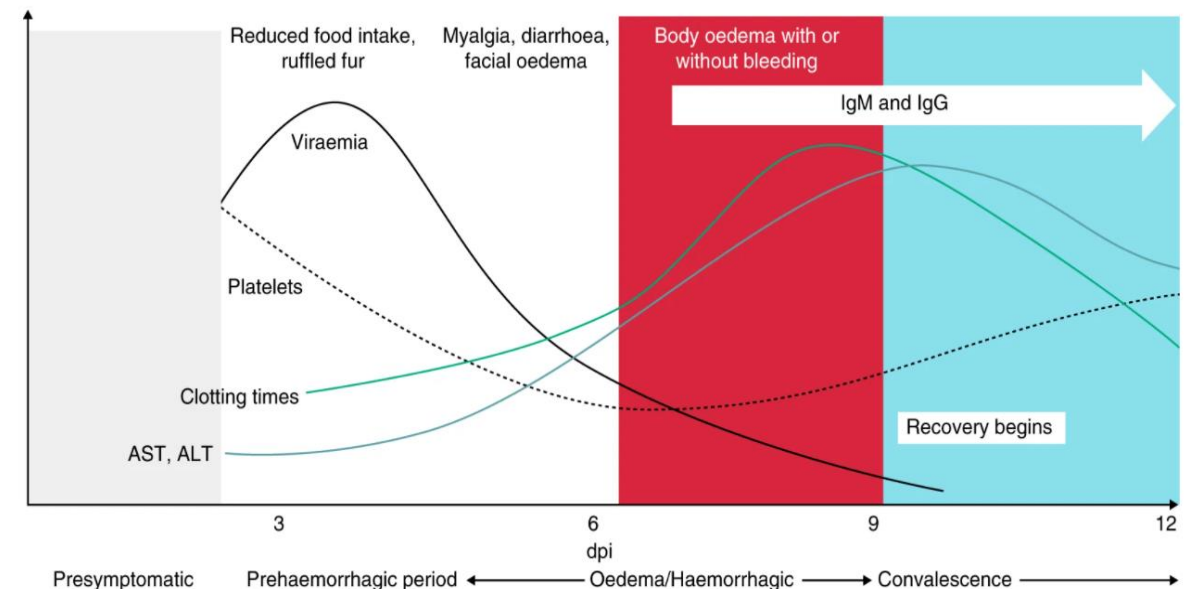
- Genetic knock out or antibody blockade
- Develop viremia, inflammatory immune response, liver failure
- Multiple strains can be used
- Valuable for therapeutic interventions

- **Humanized mice**

- Engrafted with humanCD34+
- Neurological type of disease
- Strain-specific virulence

- **Cynomolgus macaques**

- CCHFV-Hoti
- A full spectrum of disease outcomes
- can be used to study host and viral determinants





Prevention & Treatment & Vaccine



- **Prevention**

- avoiding or minimising exposure to ticks by using tick repellent and wearing protective clothing
- Avoiding contact with the blood or tissue of infected animals and humans (protective).

- **Treatment**

- Early aggressive intensive care support
- Support of coagulation system with blood component therapy.
- Supportive drug therapy + antiviral drugs

- **Vaccine**

- Bulgarian vaccine: suckling mouse brain (inactivated)
- Originated in the USSR in 1970 (use for military and medical personnel, farmers, and persons living or working in endemic regions in Bulgaria)



Future collaboration = Stronger together

GAP	CHALLENGE
Lack of sufficient research and endemic potential of the disease is posing serious threats to public health	Collect more data on vectors and reservoir hosts; Studies to understand and reveal the natural cycle of CCHFV Understanding environmental conditions that can influence the spatio-temporal distribution and dynamics of CCHF
Lack of standardization of case definitions, laboratory diagnosis procedures, reporting and documentation of the disease	Development of standardized and validated diagnostic methods Further research on investigations of CCHF pathogenesis
Treatment and prevention related knowledge is limited	Plan a basic research on possible additional antiviral drug therapy and/or vaccine Plan a randomized clinical trial to ascertain the benefits of ribavirin treatment
Lack of infrastructure capacity in endemic countries	In the wake of COVID-19 pandemic the healthcare system has come under immense pressure and routine health services have been disrupted. Disease surveillance and reporting activities for different diseases, even HPA like CCHF have been disrupted.



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thank you!