Crimean-Congo hemorrhagic fever virus in Asia Years of experience in epidemiology, diagnosis, treatment and outbreak investigation

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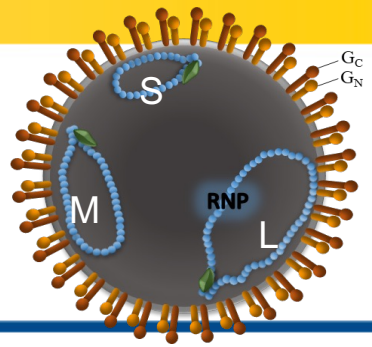
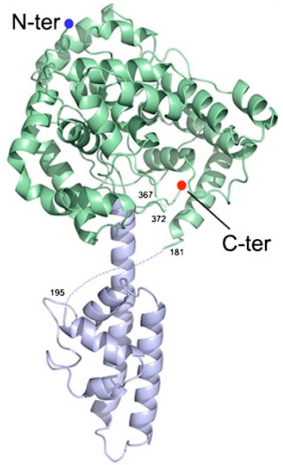
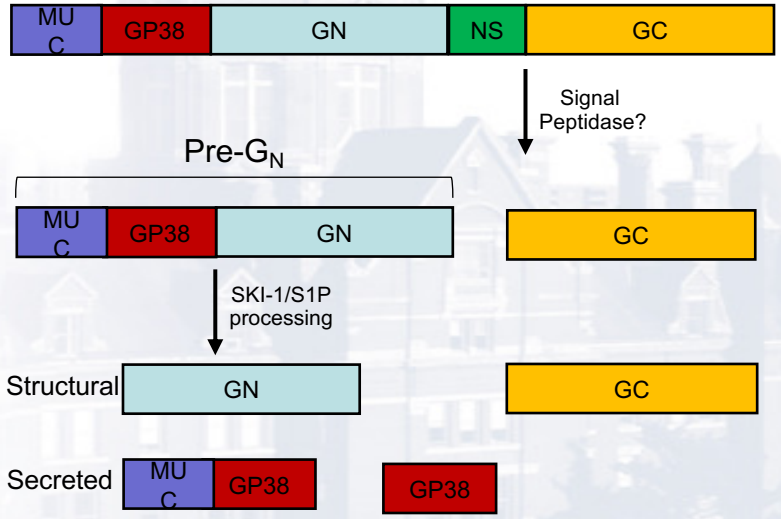
Organism and Virus Structure



- Crimean-Congo hemorrhagic fever virus (CCHFV)
- Negative-sense RNA virus
- The *Orthonairovirus* genus
- Family of *Nairoviridae*
- Order of *Bunyavirales*
- Extensive genetic diversity in viruses from different geographic regions





Guo et al, 2012. PNAS. Doi:10.1073/pnas.1200808109

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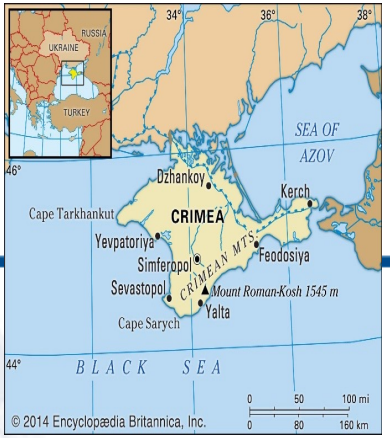
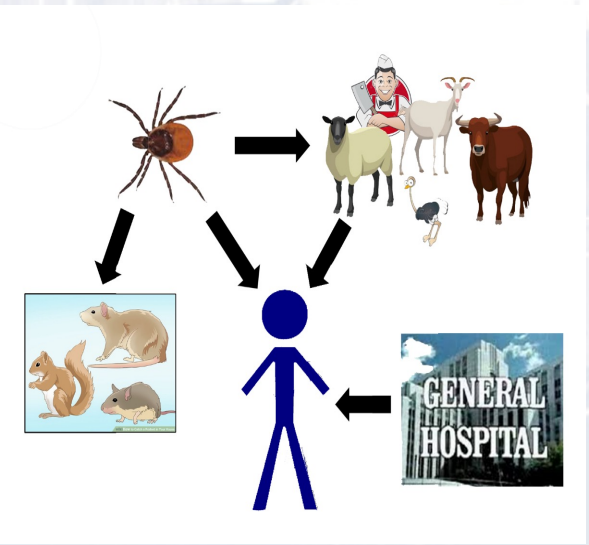
History



- CCHFV was introduced by Chumakov et al. following the 1944–1945 outbreak among Soviet military personnel in Crimea.
- In 1969: Also detected in Congo
- Outbreaks continue to occur
- Potential bioterrorist agent, CDC/NIAID Category C pathogen



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CCHF

- A tick-borne zoonotic disease
- Characterized by fever and hemorrhage
- Transmission: bite of Ixodes ticks of the genus *Hyalomma* or by direct contact with blood or tissues infected humans or viremic livestock.
- CCHF is a severe disease in humans, with a high mortality rate (2.8–70%)

Papa A, Mirazimi A, Köksal I, et al. J Clin Virol 2015; 64:137.

Table 2
History of Crimean–Congo hemorrhagic fever in Persia/Iran.

Date	Description	Comment	References
1203 CE	Detailed description of hemorrhagic fever and its putative causative agent (vulture louse)	Description identical to Galen's, thus may not be specific to CCHF	Jurjani, 1976
1887–1888	Description of a fatal hemorrhagic disease among the nomadic Yomut Turkomen in northern Iran	Likely CCHF, but key details, such as fever and season, are missing	Brown (1893)
19th Century	Reports of a sometimes fatal disease though to be caused by *Argas persicus* in the Mianeh region in NW Iran	Unlikely to be CCHF, though some clinical features suggestive	Nuttall (1908)
1940's–1960's	Seasonal and sometimes fatal hemorrhagic fever known locally as *Gara Mikh* typhoid fever in East Azerbaijan, Iran	Clinical and epidemiologic features consistent with CCHF	Aminolashrafi and Nooranian (1966)
1966–1969	Report of 41 cases of hemorrhagic fever from East Azerbaijan, Iran	Possible CCHF outbreak	Aminolashrafi (1970)
1970–1971	Sheep serum sent tested positive for CCHFV antibodies	First documentation of CCHFV in livestock	Chumakov and Smirnova (1972)
1971–1973	Report of 60 cases of hemorrhagic fever from East Azerbaijan, Iran	First suspected cases of CCHF in humans	Asefi (1973)
1970–1971	Sera of humans in northern Iran tested positive for anti-CCHFV antibodies	First documentation of CCHFV infection	Saidi (1974)
1974–1975	Hemorrhagic fever epidemic in northern Iran	Suspected CCHF, but not proven	Ardoin and Karimi (1982)
1999	Nosocomial transmission of CCHF	First confirmed cases of CCHF in Iran	Mardani (2001)

TABLE 2

Clinical manifestations, demographic variables, risk factors and outcome of nosocomial and index cases of Crimean-Congo hemorrhagic fever, Iran*

Case	Age, years	Sex	Bleeding manifestations	Fever	Job	Contact type/details of exposure	Outcome	Incubation period, days†
Index 1	55	M	GI bleeding, epistaxis	Yes	Shepherd	Animal contact	Dead	NA
Secondary 1	32	M	Petechia	Yes	Physician	Physical contact without gloves, blood splashing into face, performing gastric lavage	Alive	14
Tertiary 1	26	F	Hematemesis, vaginal bleeding, epistaxis, hematuria	Yes	Physician	Physical contact without gloves, blood sampling, providing intravenous access, touching skin, contact with sweat and saliva, sexual contact	Dead	12
Index 2	65	M	GI and pulmonary hemorrhage	Yes	Farmer	Animal contact	Dead	NA
Secondary 2	32	M	Petechia, purpura	Yes	Physician	Physical contact without gloves, intubation, resuscitation, blood splashing into face	Alive	22

* GI = gastrointestinal; NA = not applicable.

† Incubation period is the period between infection (first exposure to index case) and the appearance of symptoms of the disease.

A, et al. N Engl J
Jul 13;877(2):154-1

- Male, seventh day of illness, clinical condition deteriorated rapidly
- Fulminant hepatic failure, severe respiratory insufficiency, encephalopathy
- Evaluated for liver transplantation, died on day 9
- Did not suspect CCHFV until the *second* patient who was a Medical Care worker presented with a clinical picture similar to that of the index patient.

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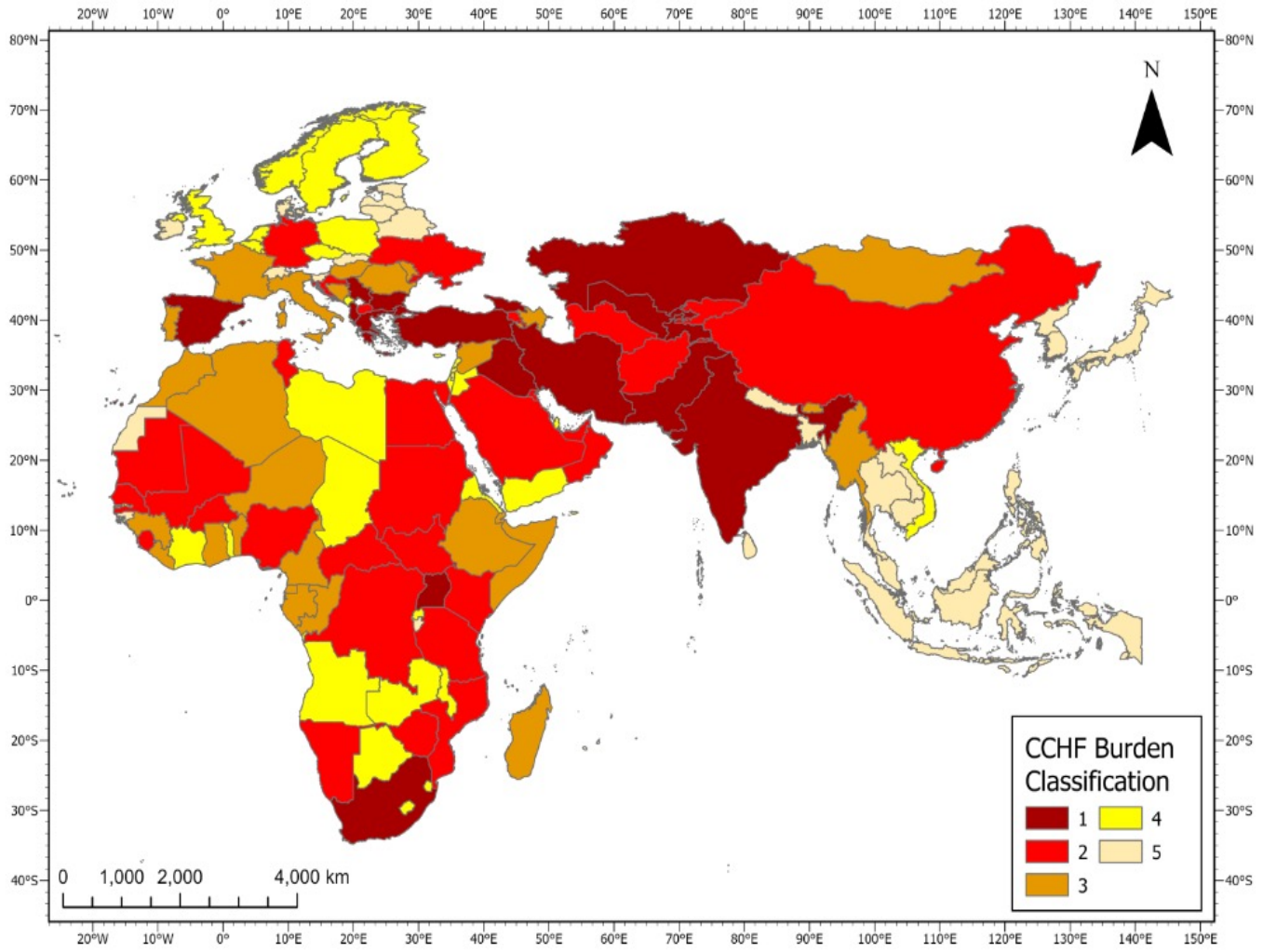
Global CCHF Epidemiology



Based on the results of our search, we developed a classification scheme that integrated vector, animal, and human data to define CCHFV circulation:

Level 1: CCHF cases reported annually through established surveillance level.

Level 2: CCHF cases reported intermittently in absence of robust surveillance.

Level 3: No CCHF cases reported; no robust surveillance established, but available data point toward the possibility of undetected/unreported CCHF cases.

Level 4: No CCHF cases reported; no robust surveillance or epidemiologic/epizoologic studies, but Hyalomma ticks are present.

Level 5: no available data

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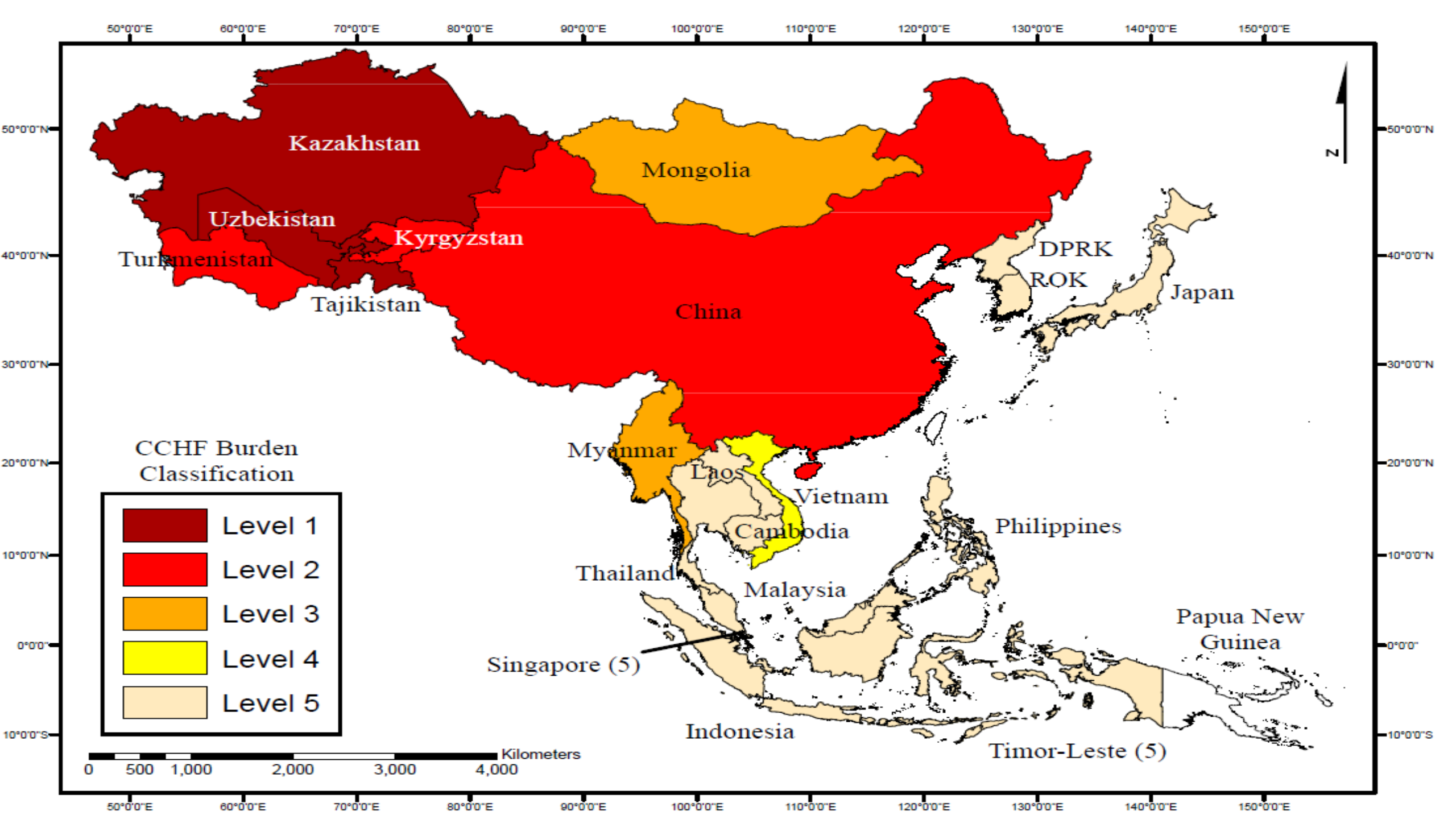
Kazakhstan
Mongolia
Uzbekistan
Kyrgyzstan
Turkmenistan
Tajikistan
China
DPRK
ROK
Japan
Myanmar
Laos
Vietnam
Cambodia
Philippines
Thailand
Malaysia
Papua New Guinea
Singapore (5)
Indonesia
Timor-Leste (5)
CCHF Burden Classification
Level 1
Level 2
Level 3
Level 4
Level 5
Kilometers
0 500 1,000 2,000 3,000 4,000
N
50°0'0"N
40°0'0"N
30°0'0"N
20°0'0"N
10°0'0"N
0°0'0"
10°0'0"S
50°0'0"E
60°0'0"E
70°0'0"E
80°0'0"E
90°0'0"E
100°0'0"E
110°0'0"E
120°0'0"E
130°0'0"E
140°0'0"E
150°0'0"E

Table 1. Total confirmed CCHF cases in Central Asia (1944–2021)

Country	Total confirmed cases	Total deaths	Year (s)
Kazakhstan	801	101	1948–2021
Kyrgyzstan	19	NA	1948, 1951, 1953, 2018–2021
Tajikistan	527	81*	1944–2020
Turkmenistan	14	10	1944, 1946
Uzbekistan	665	66	1944–1983, 1998-2007, 2001–2004, 2013-2015, 2017–2018
Total	1966	258	1944–2021

Table 3. Total confirmed CCHF cases in Eastern and South-eastern Asia by country (1944–2021)

Country	Total confirmed cases	Total deaths	Year (s)
Brunei Darussalam	NA	NA	NA
Cambodia	NA	NA	NA
China**	287	59	1964–2003
Democratic People's Republic of Korea	NA	NA	NA
Indonesia	0*	0*	NA
Japan	0*	0*	NA
Lao People's Democratic Republic	NA	NA	NA
Malaysia	NA	NA	NA
Mongolia	NA	NA	NA
Myanmar	NA	NA	NA
Philippines	NA	NA	NA
Republic of Korea	NA	NA	NA
Singapore	0*	0*	NA
Thailand	0*	0*	NA
Timor-Leste	NA	NA	NA
Viet Nam	NA	NA	NA

TABLE 2

Total confirmed cases in Southern and Western Asia by country from 1974 to 2017 based on peer-reviewed literature or reports from government organizations

Country	Total confirmed cases	Total deaths	Cases per year (range)	Years cases reported	References
Turkey	10,333	469	150–1,318	2002–2017	7,31
Iran	1,256	177	18–150	1999–2017	28–30
Pakistan	429	94	3–83	1976–2017	32,38,39,54–61
Iraq	377	39	0–55	1979–1980, 1990–2010, 2013, 2015	62,63
Afghanistan	334	88	1–237	1998–2017	21,33,64–67
Georgia	56	7	0–25	2009, 2012–2017	42,68,69
India	47	19	6–18	2011–2015	43,70–75
Oman	34	14	0–33	1995–2014	76–79
United Arab Emirates	24	14	0–11	1979, 1994–1995, 2010	6,80–82
Saudi Arabia	8	0	0–7	1989–1990	83
Kuwait	2	0	0–2	1980, 1982	46
Armenia	1	0	0–1	1974	45

Total deaths are those among confirmed cases only. Therefore, the case fatality rates were not calculated, as cases were more frequently reported confirmed or suspected than deaths. A conservative approach was used by limiting data to peer-reviewed literature, but this approach likely underestimates the true burden of Crimean-Congo hemorrhagic fever (CCHF). For instance, Pakistan reported 1,339 suspected CCHF cases from 2011 to March 2017, but only 429 cases were confirmed.

Epidemiology risks

- Rural areas engaged in animal husbandry
- Tick bites
- Direct contact with infected animal blood, body fluid or tissues
- 88% of human infections were subclinical
- Nosocomial infections in the hospital setting
- Imported cases were also described.
- ? sexual transmission
- Travel to endemic area

Crimean-Congo hemorrhagic fever (CCHF) in Turkey

- The first CCHF cases detected in Turkey in 2002
- Outbreaks have been seen in Central, Northern and Eastern Anatolia, and Eastern Black Sea regions of Turkey

The distinct geographic regions of Turkey:
- Eastern, southeastern and central Anatolia
- Mediterranean,
- Aegean,
- Marmara,
- Black Sea regions

Koksal et al. / Journal of Clinical Virology *47 (2010) 65–68*

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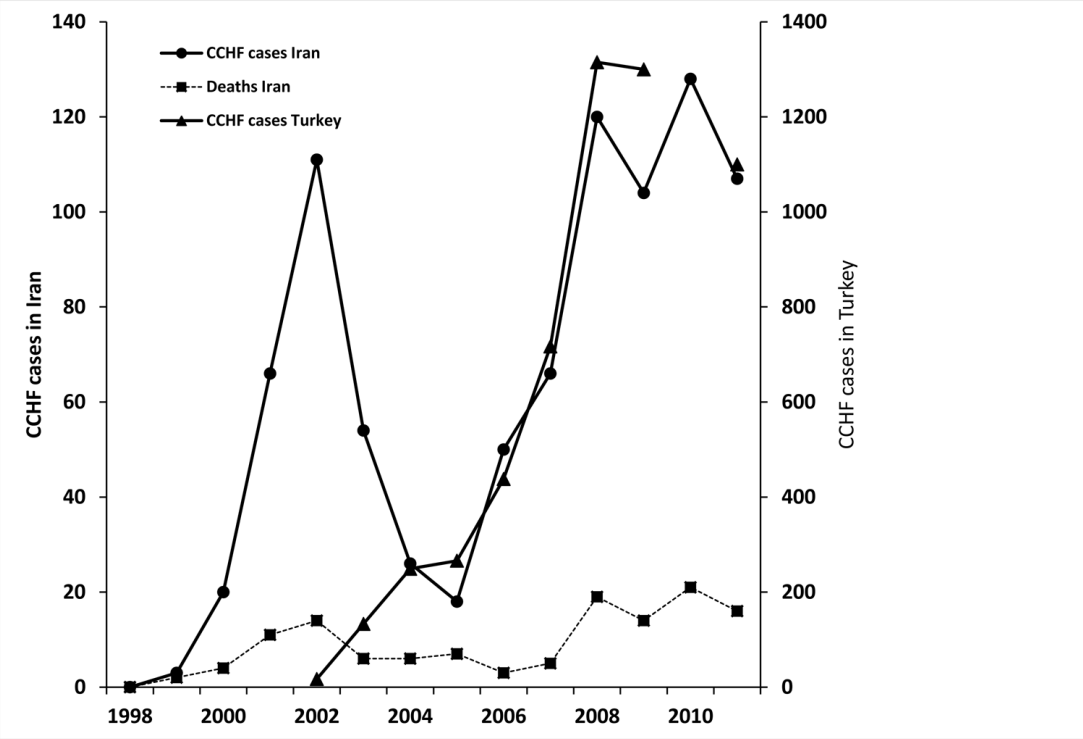
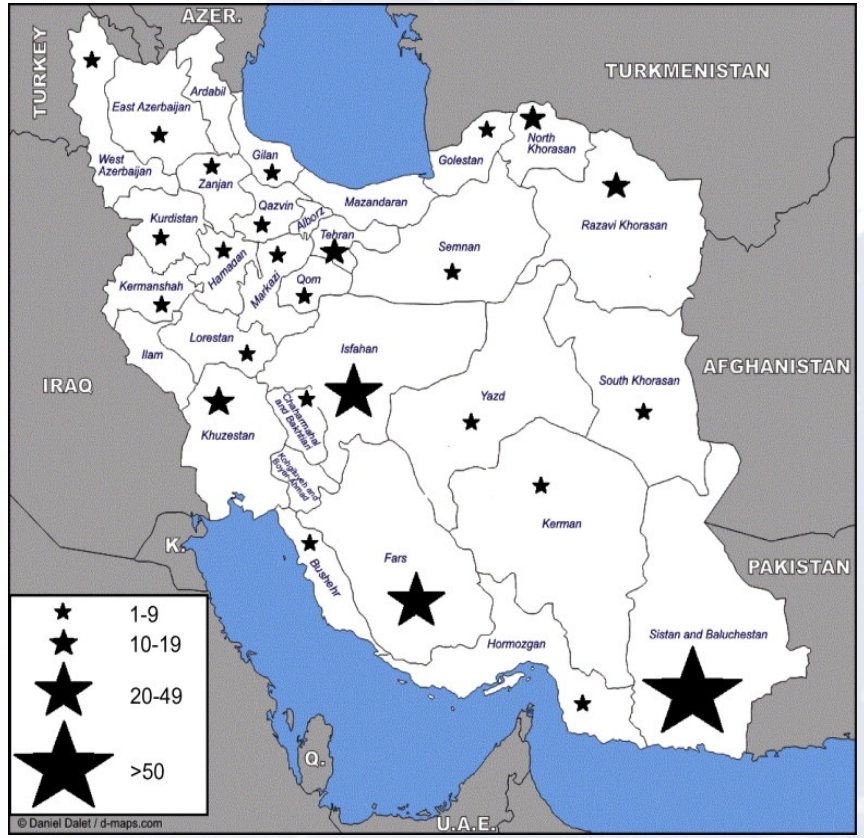
CCHF in Iran (First case diagnosed in 1999)





Total number of confirmed CCHF cases in Iran and Turkey reported each year for the period 2000–12, with the total number of fatal cases in Iran. Data were obtained from the Pasteur Institute of Iran and from (Chinikar, et al., 2012; Yilmaz et al., 2008; Ergonul, 2009; Maltezou et al., 2010; Burki, 2012).

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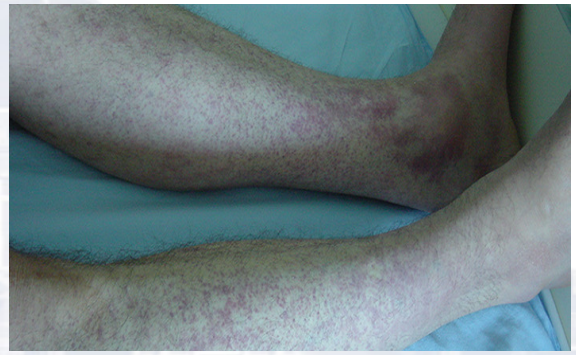
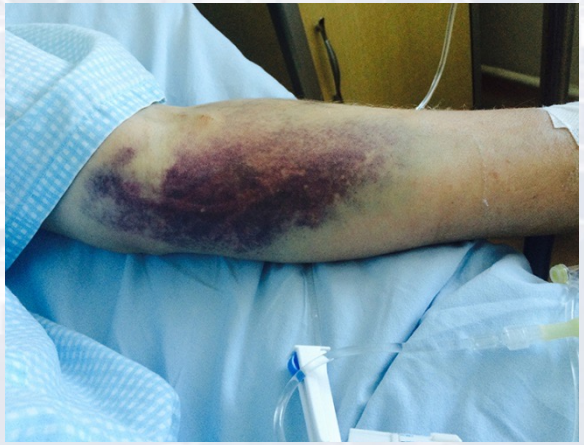
CCHF Clinical Features

- 3 - 12 day incubation period
- Sudden onset of fever, myalgia, stiffness, neck pain, dizziness, sore eyes, photophobia, diarrhea, nausea, vomiting, & generalized abdominal pain, restlessness, confusion, mood swings
- Hemorrhagic manifestations in severe cases
- Petechiae, ecchymoses, epistaxis, and gum bleeding
- Pulmonary hemorrhage, intra-abdominal bleeding, hematuria, melena, and vaginal bleeding
- Severe disease 2/2 proinflammatory cytokine response ("cytokine storm"), causing endothelial cell activation and increased vascular permeability, resulting in hypotension, shock, multiple organ failure, and death.





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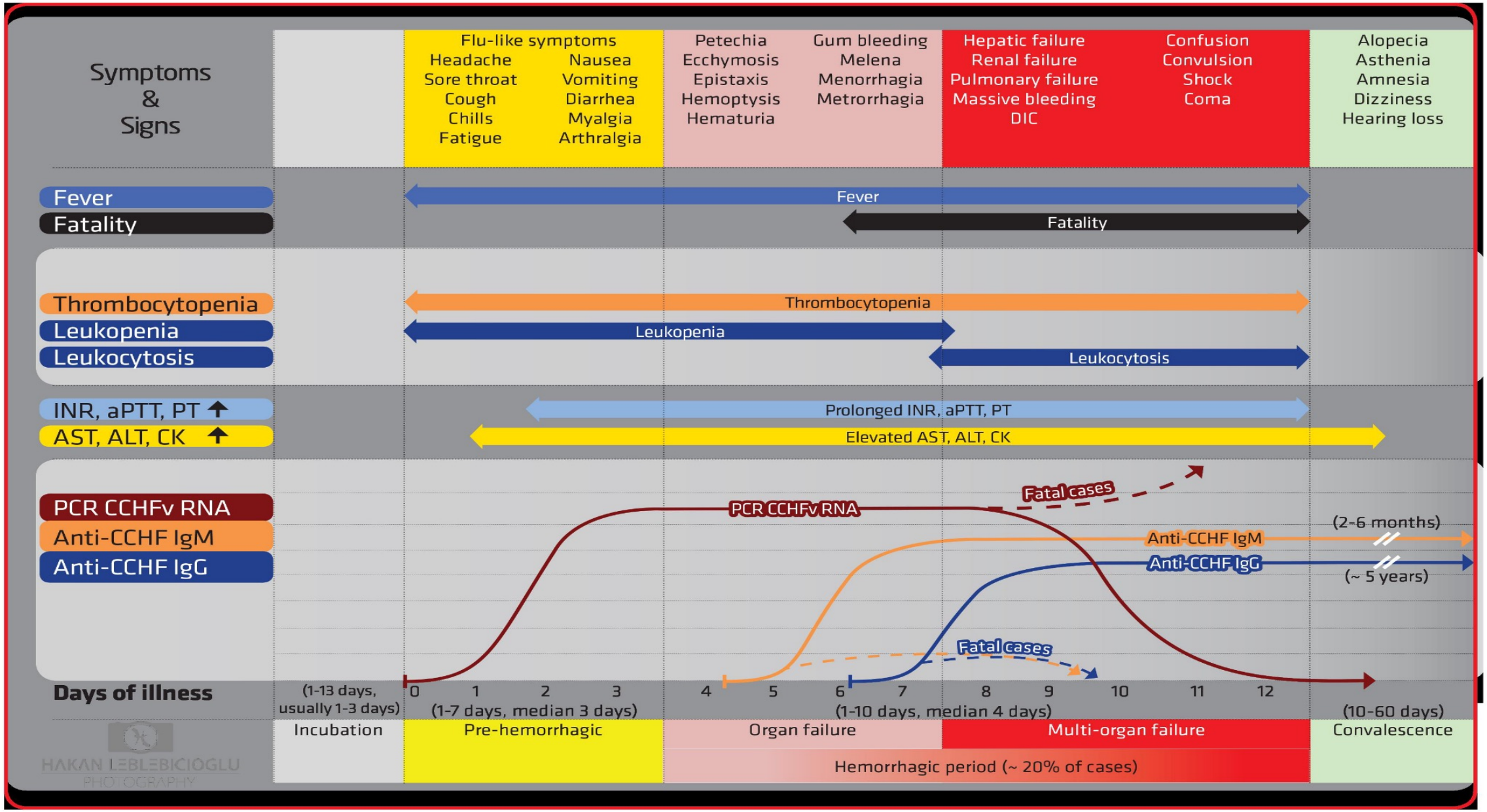

Symptoms & Signs
Flu-like symptoms
Headache
Sore throat
Cough
Chills
Fatigue
Nausea
Vomiting
Diarrhea
Myalgia
Arthralgia
Petechia
Ecchymosis
Epistaxis
Hemoptysis
Hematuria
Gum bleeding
Melena
Menorrhagia
Metrorrhagia
Hepatic failure
Renal failure
Pulmonary failure
Massive bleeding
DIC
Confusion
Convulsion
Shock
Coma
Alopecia
Asthenia
Amnesia
Dizziness
Hearing loss
Fever
Fatality
Thrombocytopenia
Leukopenia
Leukocytosis
INR, aPTT, PT
AST, ALT, CK
Prolonged INR, aPTT, PT
Elevated AST, ALT, CK
PCR CCHFv RNA
Anti-CCHF IgM
Anti-CCHF IgG
Fatal cases
(2-6 months)
(~ 5 years)
Days of illness
(1-13 days, usually 1-3 days)
(1-7 days, median 3 days)
(1-10 days, median 4 days)
(10-60 days)
Incubation
Pre-hemorrhagic
Organ failure
Multi-organ failure
Convalescence
Hemorrhagic period (~ 20% of cases)
HAKAN LEBLEBICIOGLU PHOTOGRAPHY

CCHF Laboratory findings

- Thrombocytopenia, leukopenia, hyperbilirubinemia with elevated transaminases, and prolongation of international normalized ratio, prothrombin time, and activated partial thromboplastin time
- Anemia is observed in some cases.
- Multiorgan failure: elevated blood urea nitrogen, creatinine, CK
- DIC: decreased fibrinogen levels, increased fibrin-degradation products.

Diagnosis

- RT-PCR
- Specific immunoglobulin (Ig)M and IgG by ELISA
- Specific IgM and IgG antibodies usually detectable five days from disease onset
- IgG antibodies can remain detectable for at least five years
- Specific IgM positivity in a single sample indicates current infection
- seroconversion or fourfold rise of CCHFV IgG antibody levels in paired sera confirms recent or current infection
- CCHFV can be cultured in cell culture (biosafety level 4 laboratories, used for research purposes)

Treatment

- No approved antiviral
- Ribavirin has been studied in vitro, and in animal models
- It has not been shown to reduce viral load or mortality in humans and its clinical efficacy is controversial
- Most studies evaluating the effectiveness of ribavirin are limited by methodology flaws.
- Appears to be beneficial in post-exposure prophylaxis and early treatment for healthcare workers at risk for CCHFV infection.

- Koksal I et al. ,2010, Celikbas et al., 2014; Guner et al., 2014; Guven et al., 2017
- Serretiello E. Travel Medicine and Infectious Disease 37 (2020) 101871

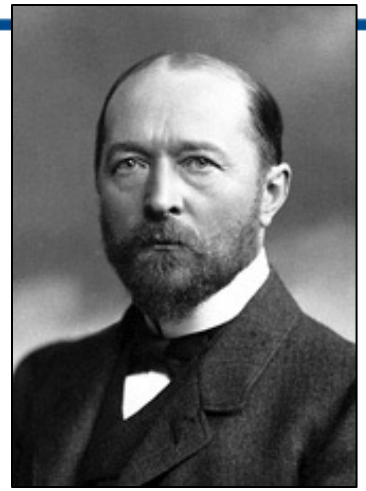
Treatment

- Supportive care:
  - in severe cases, blood product replacement is warranted
  - Electrolyte and fluid balance
  - Mechanical ventilaiton, hemodialysis, vasopressors, Inotropic agents
  - Acetaminophen for fever and pain
  - Avoid Ibuprofen and aspirin due to affects on normal clotting.
- Insufficient data to support routine use of steroids, intravenous immunoglobulin, or plasma exchange
- Hyperimmunoglobulin (prepared from convalescent sera) requires further study.
- Hyperimmunoglobulin can decrease viral load via direct neutralization, although viral strain variability may be an important determination in the use of this therapy.

Supportive Care:

- Platelet transfusion is warranted to maintain platelet count >50,000/mm^3 in the setting of bleeding; platelet count <20,000/mm^3 in the absence of bleeding.
- The need for blood transfusion based on the hemoglobin level and hemodynamic status
- Avoided unnecessary interventional procedures
- Nonsevere cases: symptoms usually resolve in 7 to 10 days
- In the absence of bleeding, transaminases and platelet counts tend to return to normal levels after 5 to 10 days.

Antibody-based therapies for infectious diseases



The mechanism of immunity
in animals to diphtheria
and tetanus

1890 • Emil von Behring and Shibasaburo Kitasato

von Behring, Emil, and Kitasato, Shibasaburo. 1890. Ueber das Zustandekommen der Diphtherie-Immunität und der Tetanus-Immunität bei Thieren. *Deutsche Medizinische Wochenschrift*, Vol. 16, pages 1113–1114.

Effective in a post-exposure setting

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Antibody-based therapies for CCHFV

Table 4. CCHFV therapeutic MCMs evaluated in humans and laboratory animals.

Class	MCM	Treatment Regimen	Route of Delivery	Animal Species/Strain	Post-Exposure Protection	% Protection	Target(s)	Mechanism of Protection	Human Efficacy Data	REF
Immunotherapeutic	CCHF-bulin #	3–9 mL, 1–5 d or longer	IM	humans	Y	>60(human)	antibody targets unidentified	human convalescent plasma	Y	[55]
	CCHF-venin#	30 mL combined with 30mL of CCHF-Bulin	IV	humans	Y	100(human)	antibody targets unidentified	human convalescent plasma	Y	[55]
	mAb-13G8	1 mg/dose, two doses	SC, IP	IFNAR$^{-/-}$, mAb 5A3 treated C57BL/6 mice	Y	70–100	GP38	may involve complement	N	[48]

- Convalescent plasma is a poorly defined product that is difficult to consistently produce in large amounts and to standardized levels.
- A non-neutralizing antibody mAb-13G8 could protect, even subsequent to virus infection.
- GP38 or a cleavage product that contains GP38 may be an important viral target against CCHFV

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Garrison, A.R., et al. Viruses. 11(590): doi:10.3390/v11070590

The algorithm used for case management of CCHF

VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 12, Number 9, 2012
© Mary Ann Liebert, Inc.
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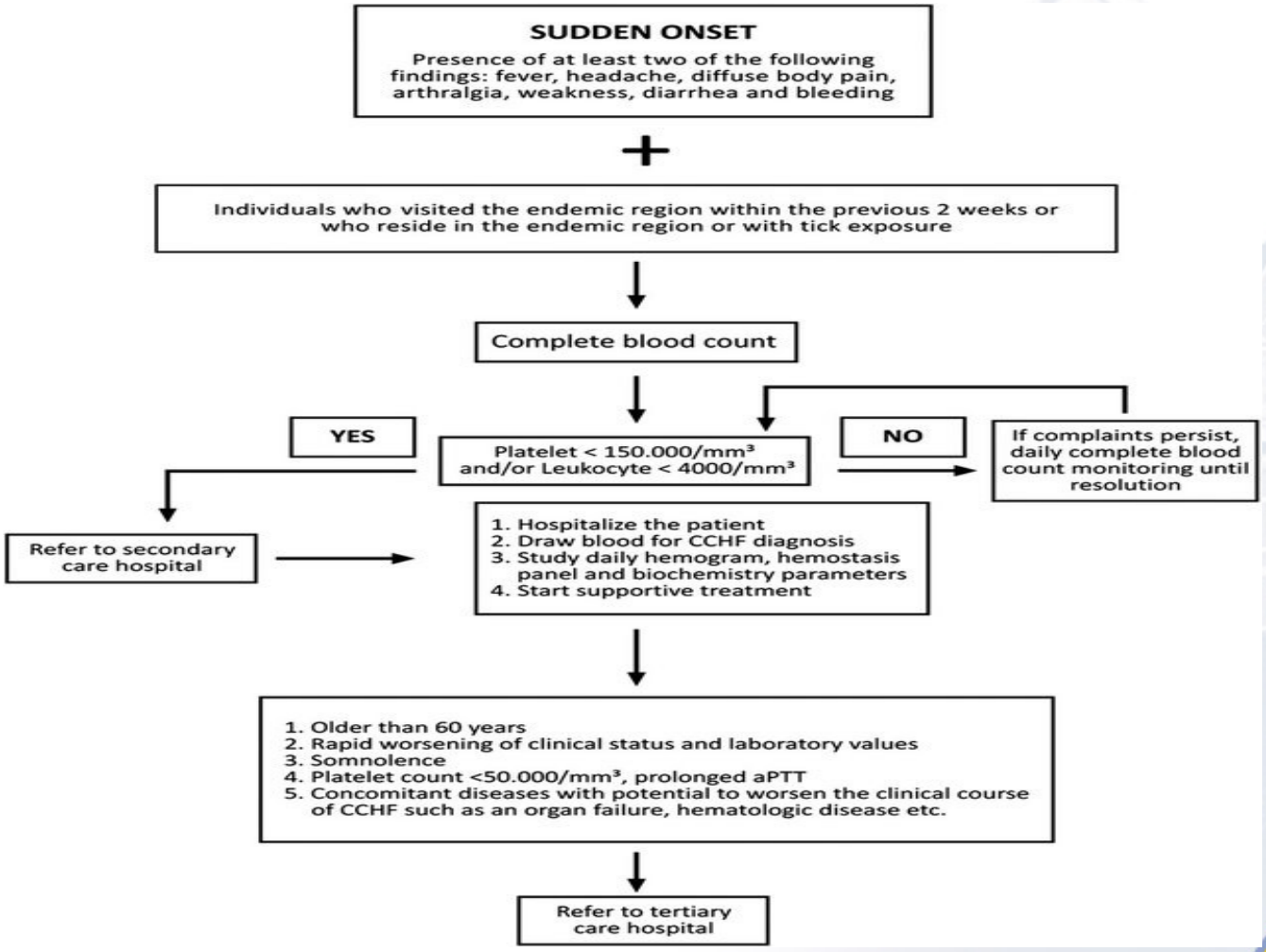
Case Management and Supportive Treatment for Patients with Crimean-Congo Hemorrhagic Fever

Hakan Leblebicioglu,[1] Hurrem Bodur,[2] Basak Dokuzoguz,[2] Nazif Elaldi,[3] Rahmet Guner,[4] Iftihar Koksal,[5] Halil Kurt,[6] and Gonul Cicek Senturk[7]



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Vaccines

- No approved vaccine
- The first CCHFV vaccine was developed and licensed in Bulgaria, in 1970, using a CCHFV strain V42/81, propagated in the brain of suckling Mouse.
- This vaccine was not approved for use in other countries with at-risk populations, as its effectiveness is much debated
- In 2012, studies in healthy vaccinated people highlighted that sequential doses of the Bulgarian CCHFV vaccine stimulate specific immune responses against the virus.

CCHF vaccines to date

Vaccine	Route of vaccination	Animal Species/strain	% Protection	Target(s)	Mechanism of protection	REFS
MVA-GP	IM	IFNAR$^{-/-}$(A129)	100	All glycoproteins	antibody appeared irrelevant	Buttigieg 2014
M-segment DNA vaccine	IM-EP	IFNAR$^{-/-}$(C57BL/6) and C57BL/6 (IS)*	70-100	All glycoproteins	neutralizing and total antibody titers do not correlate with protection	Garrison 2017
rVSV expressing M-segment ORF	IP	STAT-1	100	All glycoproteins	antibody against glycoproteins, and neutralizing antibody titers but mechansim is unclear	Rodriguez 2019
CCHF virus-like replicon particle with M-segment	SC	IFNAR$^{-/-}$	80-100	All glycoproteins	unknown	Scholte 2019
GN ectodomain or GC ectodomain subunit vaccines	IP	STAT-1	0	G_N, G_C	Not protective despite high neutralizing antibody response to G_C vaccine	Kortekaas 2015
G_N/G_C and N DNA vaccine and/or VLPs	IM	IFNAR$^{-/-}$(A129)	100	G_N, G_C and N	Unknown	Hinkula 2017
Bovine Herpesvirus N subunit vaccine	IM	IFNAGR$^{-/-}$	100	N	Unknown	Farzani 2019
Adenovirus N subunit vaccine	IM	IFNAR$^{-/-}$(C57BL/6)	33-78	N	prime/boost more protective	Zivcec 2018
MVA-NP	IM	IFNAR$^{-/-}$(A129)	0	N	Not protective	Dowall 2016
S-segment mRNA	IM	IFNAGR$^{-/-}$ (challenged) C57BL/6 (not challenged)	50 (single) 100 (2 doses)	N	Significant levels of IgG1 and IgG2a and IFN-gamma, significant weight loss in survivors	Farzani 2019
Formalin inactivated cell culture derived CCHFV mixed with alum	IP	IFNAR$^{-/-}$	60-80	Whole virus	antibody against glycoproteins, and neutralizing antibody titers but mechanism is unclear	Canakoglu 2015
mouse brain-derived inactivated CCHFV adsorbed on $Al(OH)_3$	SC	humans	Unknown	Whole virus	antibody against glycoproteins, and N but mechanism is unclear	Mousavi-Jazi

Animal Models to Evaluate Medical Countermeasures

Animal model	% lethality	Time to death [days]	Salient features
Neonatal mice	100	3 d	Do not predict immunotheraptuic protection behavior in adult rodents. Ribavirin protects against lethality.
STAT-1 KO mice	100	3-5 d	heptic injury, subunit vaccines may not protect well in this model
IFNAR KO mice	>90	4-8 d	Prototypical rodent model for CCHFV. BL6 or 129 background develop severe disease. Strain Hoti has a reduced MTD.
IFNARG KO mice	100%	4-6 d	Used to evaluate N subunit vaccines
C57BL/6, BALB/c, B6:129	0-100	5 d	No disease ensues unless IFN-I signaling is blocked by antibody (MAR1-5A3).
Rag2 KO mice	0-100	4-5 d after disruption of IFN-I signaling	Hepatitis in mice with active IFN-I signaling. Disruption of IFN-I signaling results in 100% lethality similar to normal mice
SGM3 Humanized mice	0-100	15-23 d	Neurological disease ensues absent of systemic (visceral) disease. Only strain Turkey produced severe disease.
STAT-1 KO mice	100%	5.6 d	heptic injury
Cynomolgus macaques	0-60	6-7 d	Disease model with fever, increased liver enzymes, thrombocytopenia, leukocytopenia. In some studies animals meet euthanasia criteria.

The problems:

1. No licensed drugs or vaccines to effectively treat CCHF
2. Immune correlates of protection are not well understood
3. Experimental vaccines tested on limited strains

Conclusion and future directions

- The increased reports and attention towards CCHF cases helped in changing the tools used to develop specific therapies and vaccines.
- Information about CCHFV are still very limited.
- An early diagnosis of the disease is fundamental for early treatment and to prevent nosocomial transmission.
- Cross-protection studies across numerous CCHFV clades are needed to determine if a single vaccine is practical for broad protection
- Correlates of protection still need to be defined for the advanced development of a CCHFV vaccine
- The newly developed NHP model will allow for the advanced development of the various CCHFV vaccine platforms in a more relevant animal model, but validation studies for this model are needed.

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