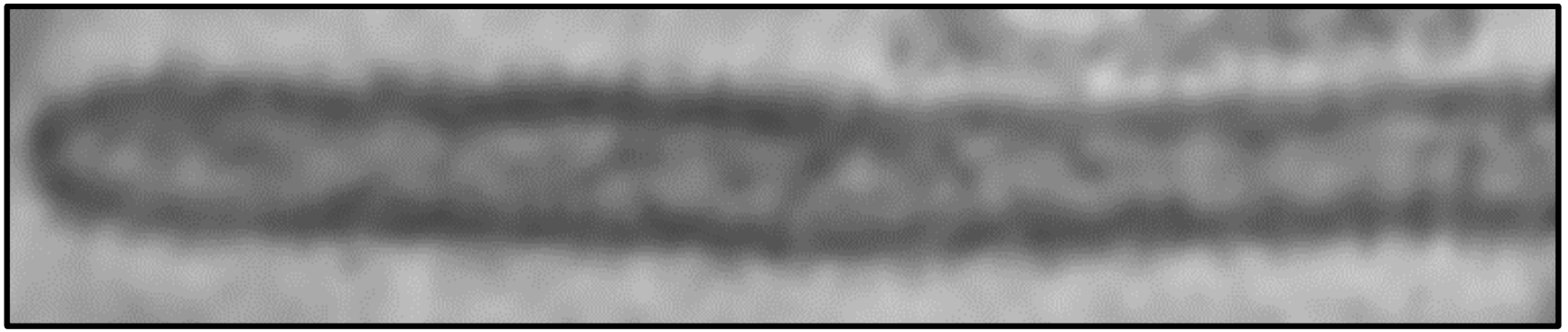
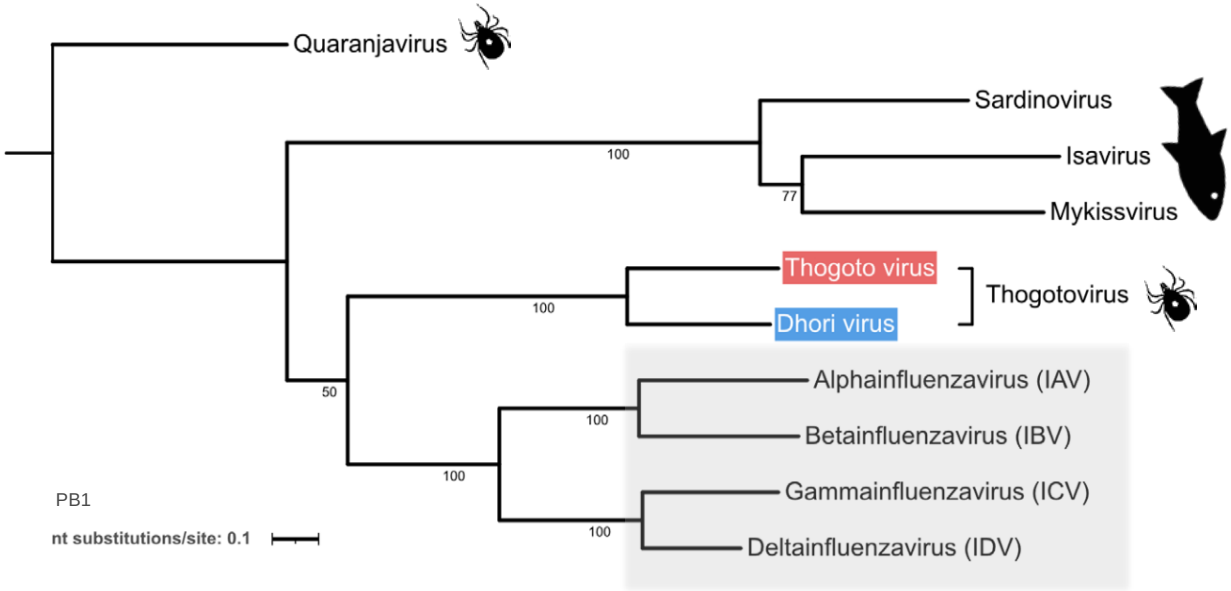
Thogotoviruses

(Re-)Emerging Tick Borne-Orthomyxoviruses



Elias Bendl

Thogotoviruses: **Phylogeny**



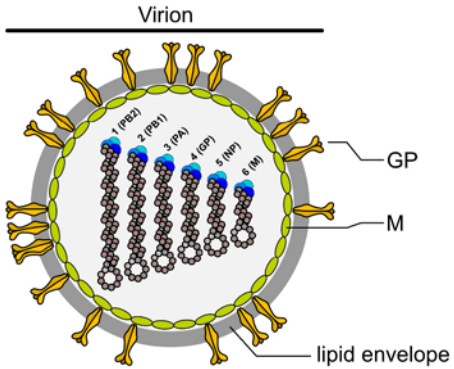
- Family: ***Orthomyxoviridae***
- Genus: **Thogotovirus**
- **Arboviruses (Ar**thropod **bo**rne-)
  - ticks!

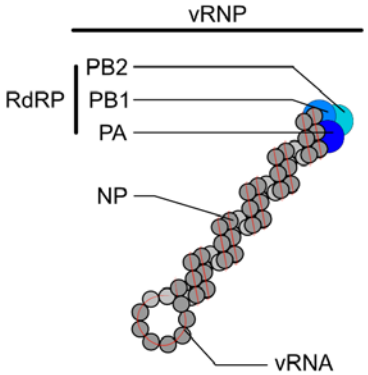


***Argulus sp.* on a stickleback**
photo by Michal Grabowski – own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=77271

Adapted fom Bendl et. al. 2023

Thogotoviruses: **Structure**





- Enveloped particles (**Ø 80-120 nm**)
- (-)ssRNA genome, vRNPs
- RNA not infectious
- 6 Segments
- **Glycoprotein** (Class III fusion protein)

THOV-GP



5XEA

AcMNPV-gp64



3DUZ

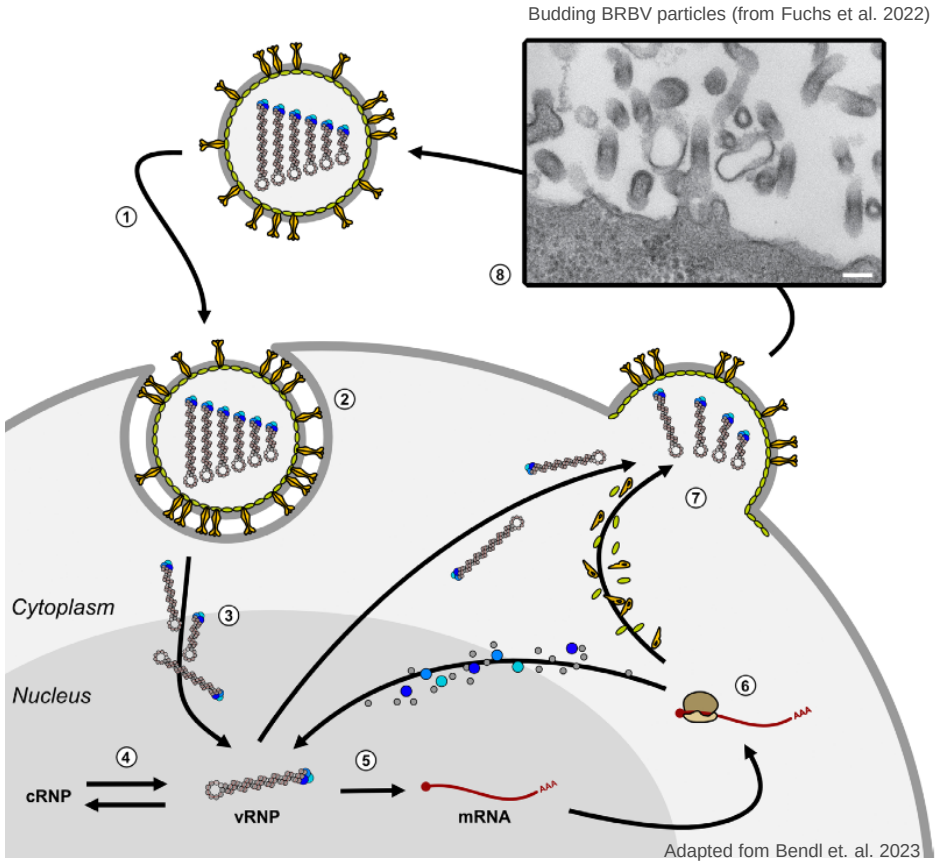
Adapted fom Bendl et. al. 2023

Thogotoviruses: **Pathogen safety**

- Enveloped particles (**Ø 80-120 nm**) **-> nanofilters**
- Expected sensitivity to desiccation, heat treatment, solvent disinfectants, formaldehyde and detergents
- Irradiation
- **Limited data** for Thogotoviruses
- **Influenza virus as a model** for THOV during processing of blood products
- Influenza (e.g. H5N1[1] and H1N1[2]) is **inactivated like other enveloped viruses** (e.g. HIV, PRV) during common plasma fractionation and pasteurization methods.

[1] *Kreil et al., Blood Components, 2007;* [2]*Jeong et al., Biologicals, 2010*

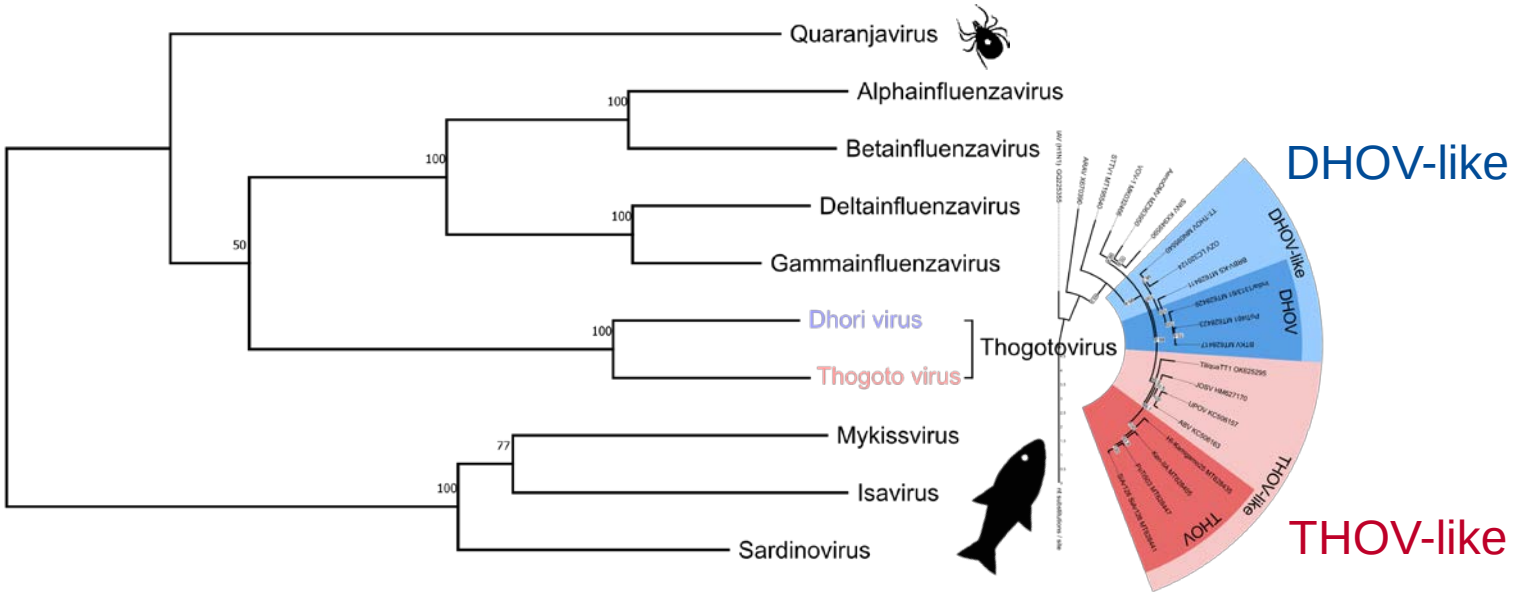
Thogotoviruses: **Replication**



- **Unknown host receptor**
- **Nuclear replication**
- RdRP (viral Polymerase) sensitive to antivirals Ribavirin and Favipiravir (T705)
- Reassortment ?
- Host response: **controlled by type-I interferon induced antiviral response**
- Used as a model to study host Myxovirus resistance (Mx) proteins

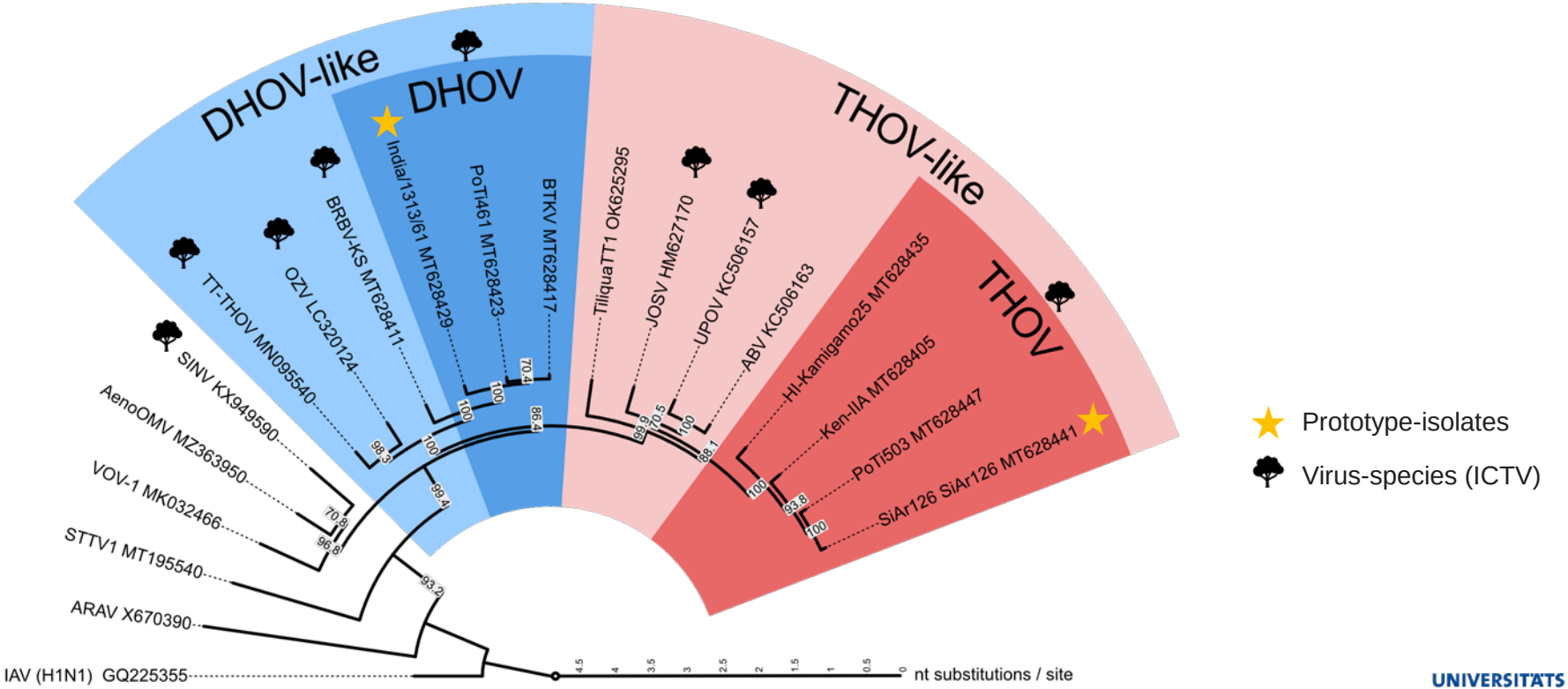
Thogotoviruses: **Phylogeny**

- Two clades:



Adapted fom Bendl et. al. 2023

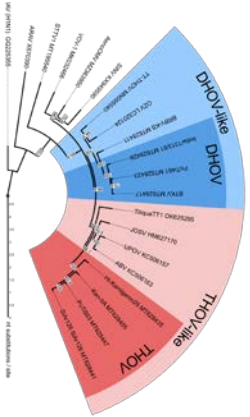
Thogotoviruses: **Phylogeny**



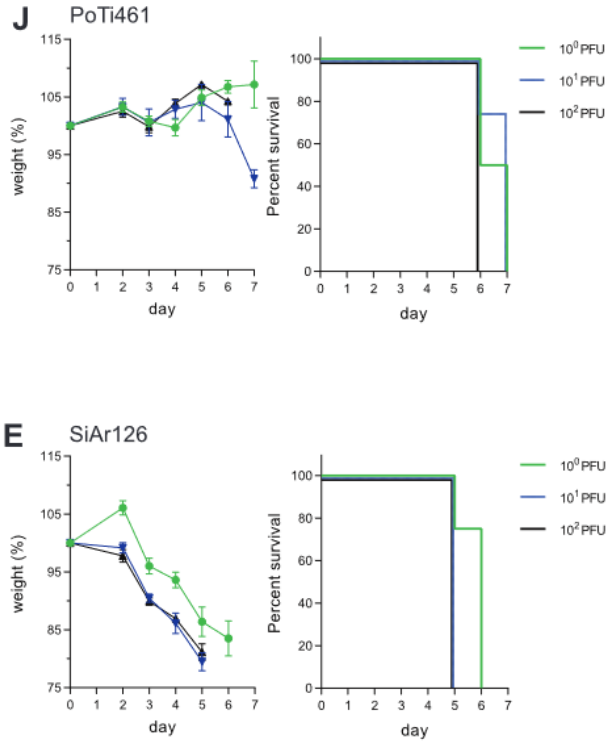
Adapted fom Bendl et. al. 2023

Thogotoviruses: **Phylogeny**

DHOV



THOV



viral replication of DHOV-like isolates peaked in the lung

hepatotropic THOV-like

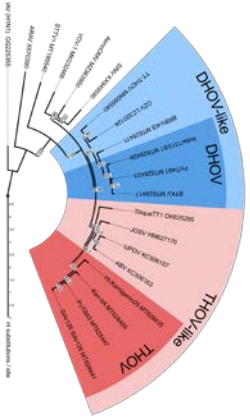
C57BL/6, i.p. 10-1000 pfu
Adapted from Fuchs et.al. 2022

- Two clades: THOV/DHOV
  - Different disease in mice

UNIVERSITÄTS KLINIKUM FREIBURG

Thogotoviruses: **Phylogeny**

DHOV

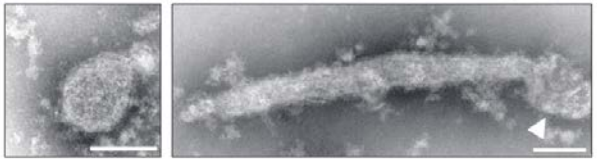


THOV

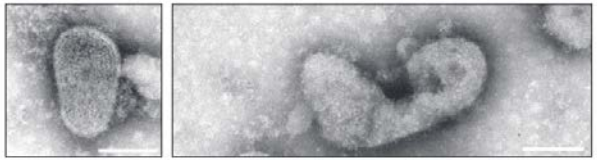
India/61 (DHOV-like)
Diameter: 100.7 ± 19.0 nm
Filamentous particles: 26%
Length: 1079.8 ± 685.5 nm



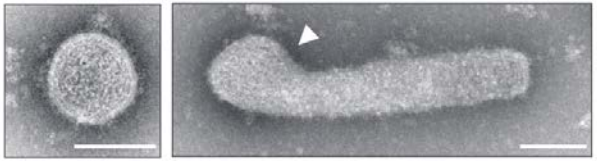
BRBV (DHOV-like)
Diameter: 101.3 ± 18.9 nm
Filamentous particles: 29%
Length: 714.6 ± 497.5 nm



SiAr126 (THOV-like)
Diameter: 101.5 ± 16.6 nm
Filamentous particles: 14%
Length: 316.2 ± 83.4 nm



JOSV (THOV-like)
Diameter: 100.7 ± 16.9 nm
Filamentous particles: 17%
Length: 413.3 ± 256.5 nm

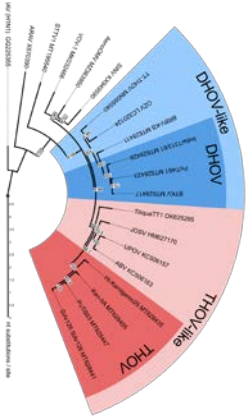


Supernatants of infected cells / 24 h.p.i. / negative staining
Adapted from Fuchs et.al. 2022

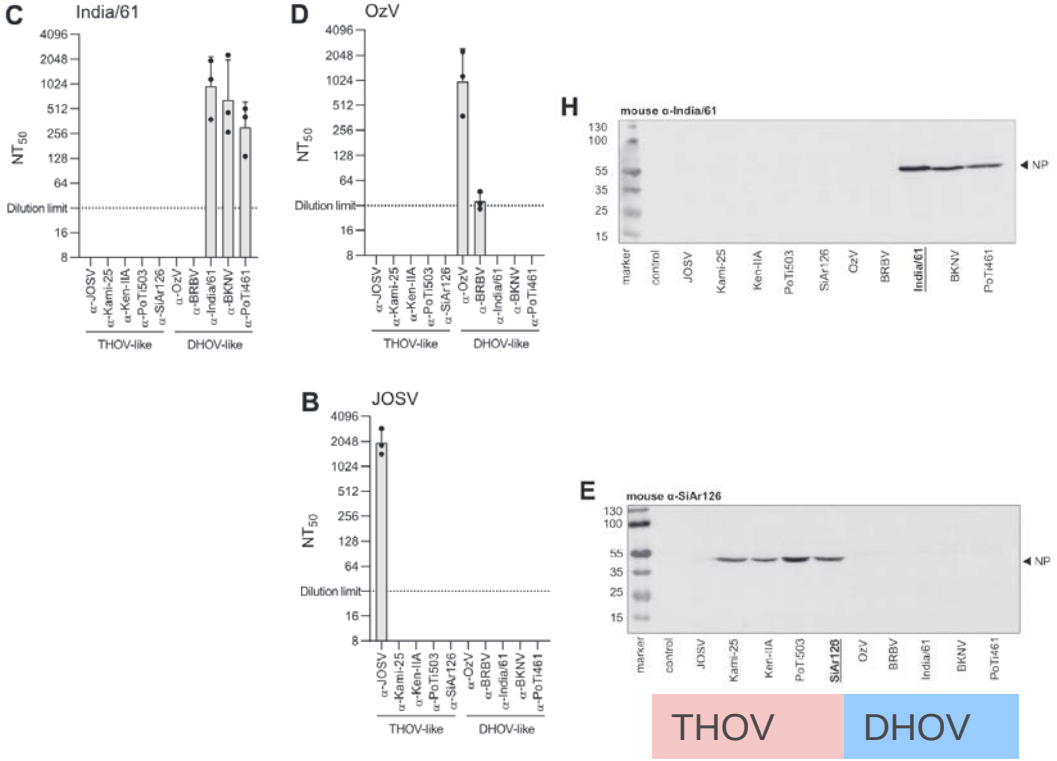
- Two clades: THOV/DHOV
  - Different disease in mice
  - Morphology

Thogotoviruses: **Phylogeny**

DHOV



THOV



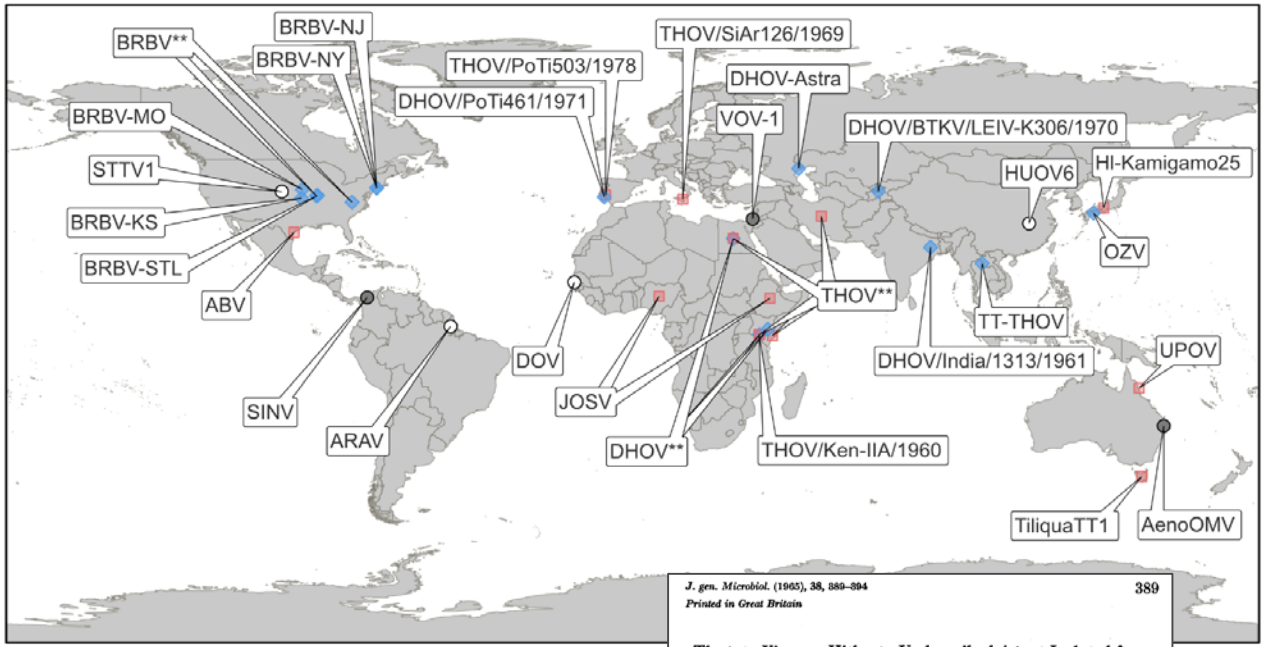
- Two clades: THOV/DHOV
  - Different disease in mice
  - Morphology
  - DHOV and THOV as well as more distantly related BRBV and OzV and JOSV form **distinct serotypes**

Neutralization capacity of postinfectious sera

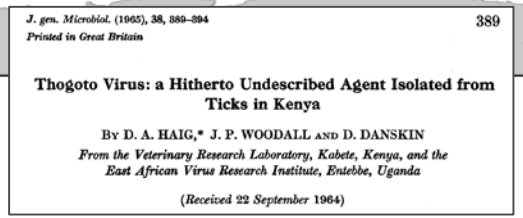
Adapted from Fuchs et.al. 2022

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Thogotoviruses: **Geographical Distribution**



Sites of THOV and DHOV detection or isolation.



J. gen. Microbiol. (1965), **38**, 389–394
Printed in Great Britain

389

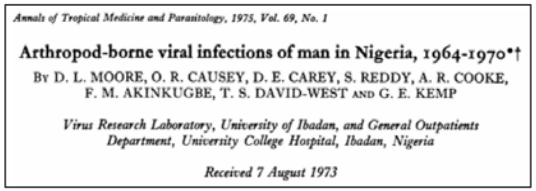
Thogoto Virus: a Hitherto Undescribed Agent Isolated from Ticks in Kenya

By D. A. HAIG,* J. P. WOODALL and D. DANSKIN

From the Veterinary Research Laboratory, Kabete, Kenya, and the East African Virus Research Institute, Entebbe, Uganda

(*Received* 22 *September* 1964)

- Global distribution
- First isolation from **Thogoto forest** near Nairobi, **Kenya**
- Sporadically cause **febrile illness** in humans and animals
- 5 laboratory infections with DHOV in the former USSR (Butenko et al. 1987) “fever and encephalitis”



Annals of Tropical Medicine and Parasitology, 1975, Vol. 69, No. 1

Arthropod-borne viral infections of man in Nigeria, 1964-1970*†

By D. L. MOORE, O. R. CAUSEY, D. E. CAREY, S. REDDY, A. R. COOKE, F. M. AKINKUGBE, T. S. DAVID-WEST and G. E. KEMP

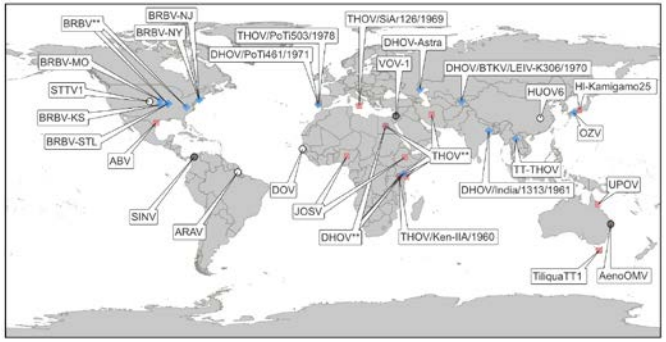
Virus Research Laboratory, University of Ibadan, and General Outpatients Department, University College Hospital, Ibadan, Nigeria

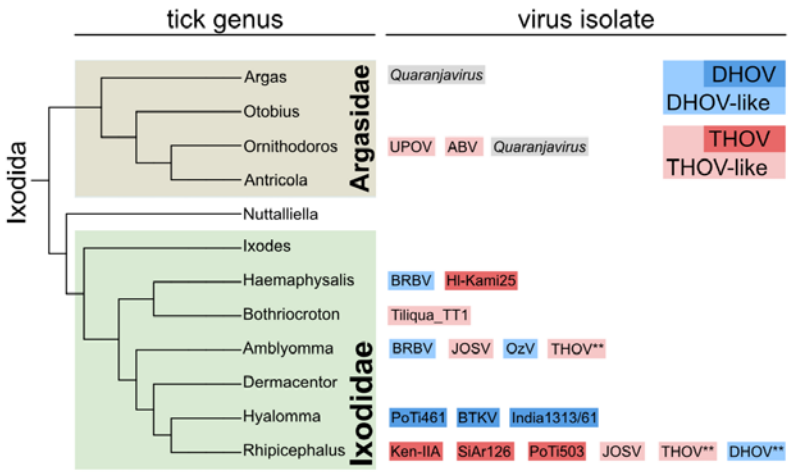
Received 7 August 1973

First described human cases (2)
„ febrile illness with neurological symptoms“

Adapted fom Bendl et. al. 2023

Thogotoviruses: **The Tick Vector**





- Mostly isolated from **hard-ticks**
- THOV -> *Rhipicephalus spp.*
- DHOV -> *Hyalomma spp.*
- Not (yet) found in *Ixodes* & *Dermacentor* ticks

Photo credit: Alan R Walker (https://commons.wikimedia.org/wiki/File:Ornithodoros-savignyi.jpg)

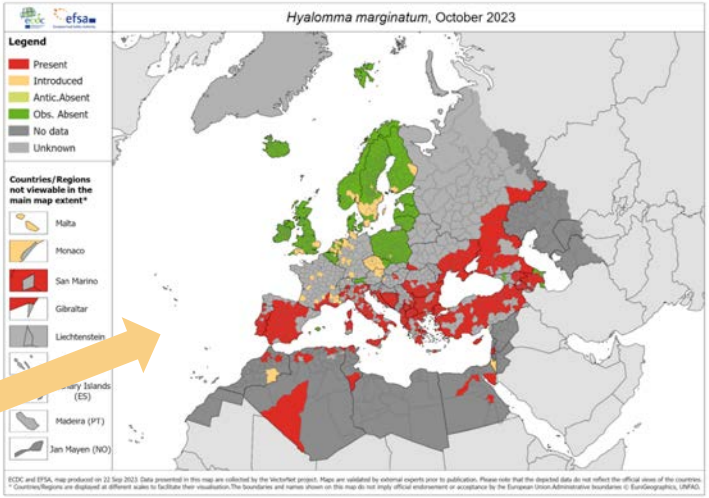


Ornithodoros savignyi

Photo credit: Acarologiste https://commons.wikimedia.org/wiki/File:Hyalomma_tick_female_dorsal.jpg



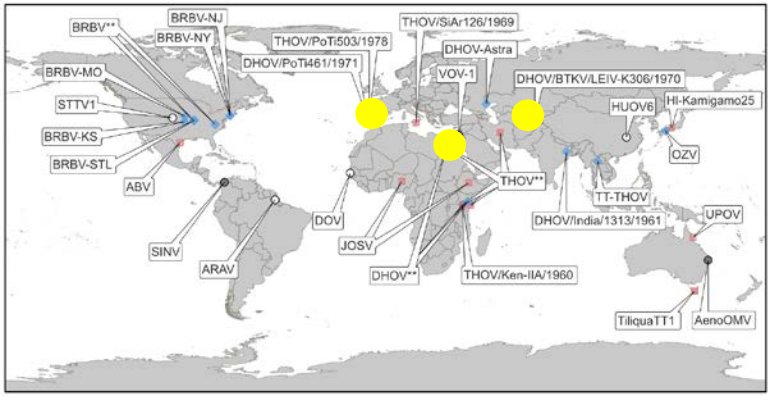
Hyalomma marginatum



Source: ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/tick-maps

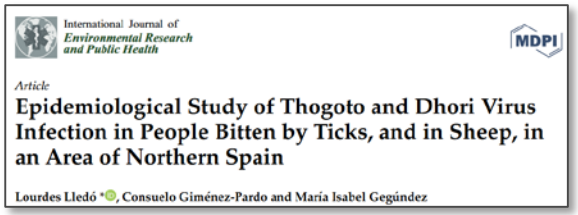
Adapted fom Bendl et. al. 2023

Thogotoviruses: **Seroprevalence**



Virus species	Location	Vertebrate species	Sero- positivity	References
THOV	Portugal and Spain	human	0.8–3.3%	[18, 94]
		sheep	20%	
	Kenya and Sicily, Italy	cattle	39,7% and 13%	[42, 99]
		sheep	6.4 and 11%	
	Egypt	human ← 1979	0.44%	[96]
		rat / sheep / goat	6.2% / 8.5% / 8.1%	
DHOV	Portugal and Spain	human	0.8–4%	[18, 94]
		goats, sheep, cattle	3,6%–26.8%	
BKNV	Kirghizia	human, cattle, sheep ← 1974	0.4–1.5%	[79]

- Thogoto-reactive sera found in **diverse mammals**
- Recent study found seroprevalence in humans:


International Journal of *Environmental Research and Public Health*

MDPI

Article

Epidemiological Study of Thogoto and Dhori Virus Infection in People Bitten by Ticks, and in Sheep, in an Area of Northern Spain

Lourdes Lledó *, Consuelo Giménez-Pardo and María Isabel Gegúndez

- 283 serum samples from 150 people bitten by ticks
- Palencia, Spain (2013-2020)
- THOV = 8 / DHOV = 6 (IgG IFA) positive
- **One worker with multiple tick bites & „asymptomatic acute in fection“ (IgM for THOV + DHOV)**

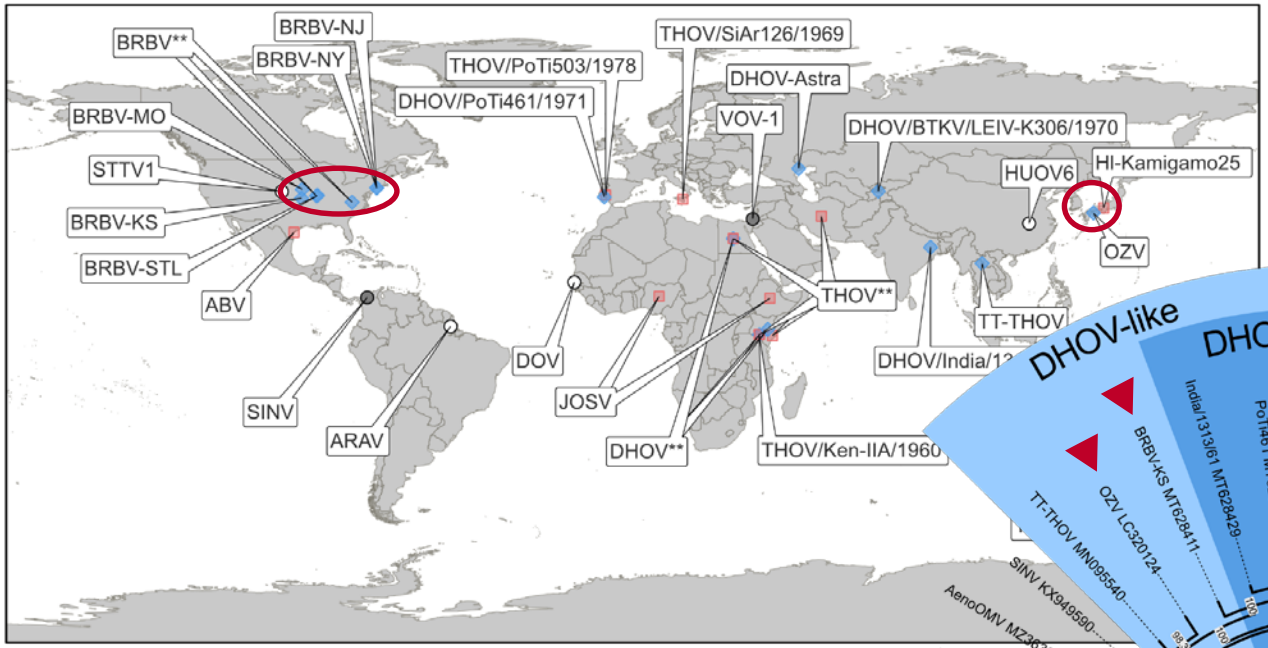
Filipe et al., 1985, Acta Virol.

- Human: 2/258 positives (PRNT) each for THOV and DHOV
- Goat: 5/141 pos. for DHOV

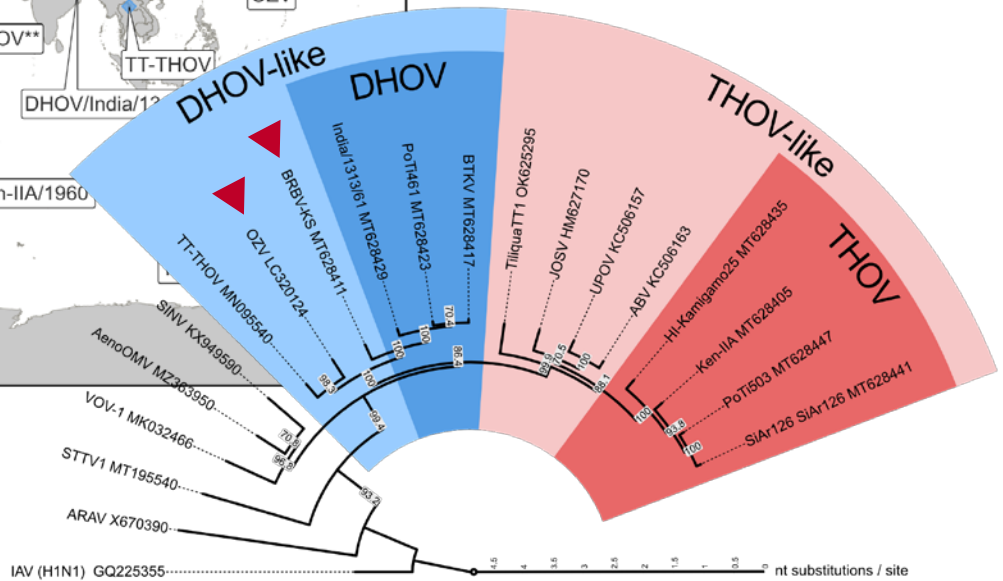
[..] References as listed in review: Bendl, Fuchs, Kochs, JoGV 2023

Adapted fom Bendl et. al. 2023

Thogotoviruses: **Emerging Thogotoviruses**



Sites of THOV and DHOV RNA detection or virus isolation.

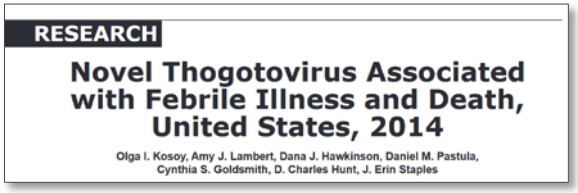


- Emergence of **Bourbon virus** and **Oz virus**

Adapted fom Bendl et. al. 2023

The (Re-)Emergence of Zoonotic Thogotoviruses

BRBV

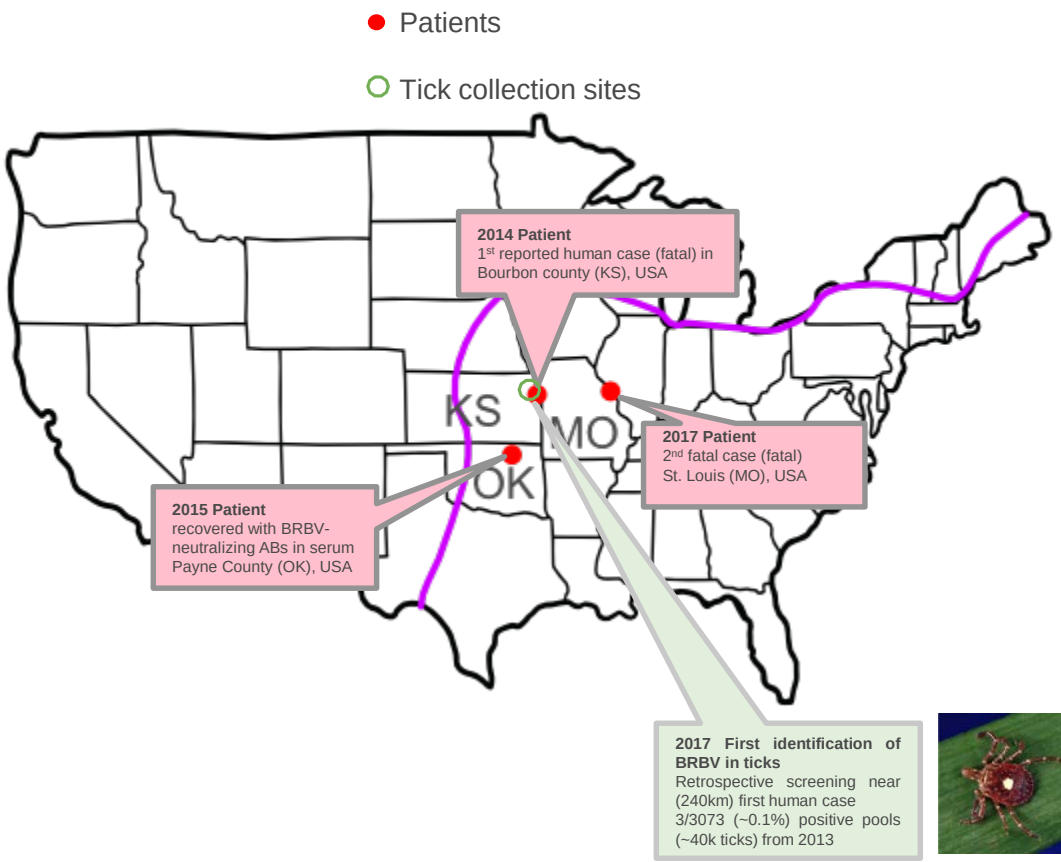

RESEARCH

Novel Thogotovirus Associated with Febrile Illness and Death, United States, 2014

Olga I. Kosoy, Amy J. Lambert, Dana J. Hawkinson, Daniel M. Pastula, Cynthia S. Goldsmith, D. Charles Hunt, J. Erin Staples

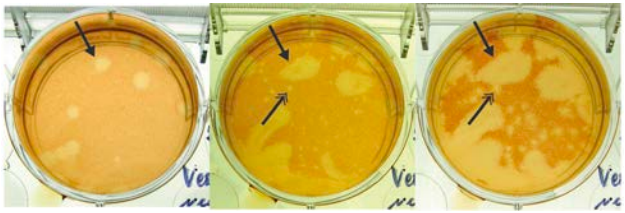
2014 Patient
1st reported human case (fatal) in Bourbon county (KS), USA

2013 ´14 ´15 ´16 ´17 ´18 ´19 ´20 ´21 ´22 ´23 ´24



Case 1 (2014)

- 50 year old patient
- tick bite history
- nausea, weakness, diarrhea; progressive heart failure
- died 11 days after symptoms onset
- Unresponsive to ABX
- Screening for HRTV antibodies at CDC
- Isolate from serum sample at 9 day post symptom onset -> **Viremia**



Adapted from Kosoy et al., 2014

Amblyomma americanum

„Lone star tick“

Photo Credit: James Gathany Content Providers(s): CDC/ Michael L. Levin, Ph. D.

The (Re-)Emergence of Zoonotic Thogotoviruses



Amblyomma americanum

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BRBV

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2015 Patient
recovered with BRBV-neutralizing ABs in serum
Payne County (OK), USA

2017 Patient
2nd fatal case (fatal)
St. Louis (MO), USA

2017 First identification of BRBV in ticks
Retrospective screening near (240km) first human case 3/3073 (~0.1%) positive pools (~40k ticks) from 2013

2019 in vivo/ in vitro characterization of BRBV in vitro / in vivo (Bricker et al. 2019; Fuchs et al. 2019)

2019 Serostudy (Jackson et al.)
High BRBV seroprevalence in raccoons (50%) / white-tailed deer (86%) suggest widespread circulation

2020 Serostudy (Komar et al.) confirmed high seroprevalence in white-tailed deer from 2014 in North Carolina (56%)

2020 Experimental verification of vector competence of *A. americanum* for BRBV (Jackson et al.)

2022 Serostudy (Bamunuarachchi et al.) found 3/440 (0.7%) BRBV-positive human sera
from St. Louis (MO), USA

2022 First detection in invasive *Haemaphysalis longicornis*
(Cumbie et.al)
- vector spillover?

2023
Detection of BRBV-RNA in a tick from a 2019 from Long Island (NY), USA (Dupuis et al.)
- **New clade of BRBV!** (Lange et.al. 2023; Egizi et.al. 2023)

2024 First detection in Arkansas (Panella et.al.)
> Widening known distribution of BRBV

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OzV

2015 First Thogotovirus reported in east asia **THOV-HI-Kamigamo-25** strain from *Haemaphysalis longicornis* (Yoshii et.al. 2015)

2018 (Ejiri et.al.) first isolated OzV from Japanese *Amblyomma testudinarium* from 2013

2022 Serostudy (Tran et.al.) OzV neutralizing human sera (**2/24 hunters** from 2015) and several wild mammals

human (hunters)	8.3% (2/24)
sika deer and boars	8.3–55.8%
macaques	6.3–47.5%

2023 Patient
1st reported human case (fatal) of OzV infection: 70 yo woman with engorged tick (fatal) in Ibari Prefecture, Japan

Characterization of a novel thogotovirus isolated from *Amblyomma testudinarium* ticks in Ehime, Japan: A significant phylogenetic relationship to Bourbon virus

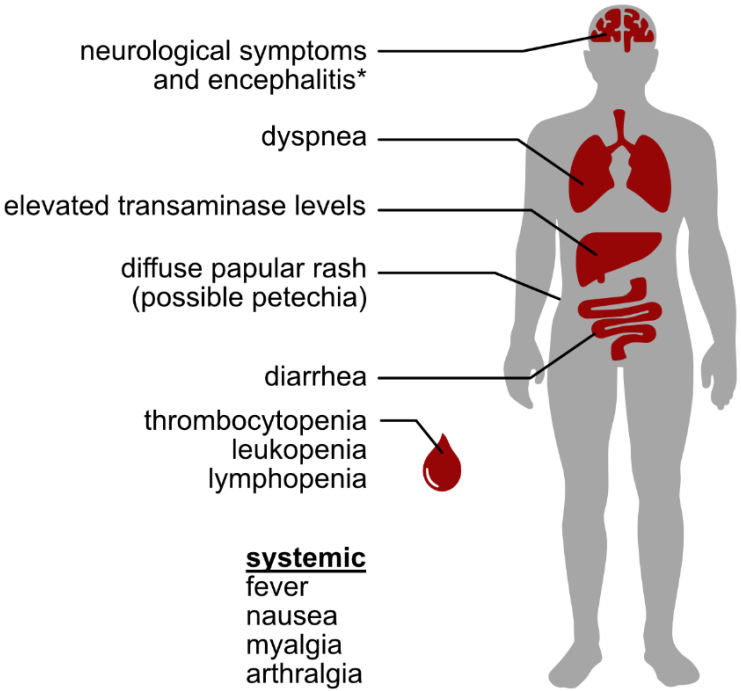
Hiroko Ejiri[a,b,1], Chang-Kweng Lim[c,1], Haruhiko Isawa[a,*], Ryosuke Fujita[a,d,e], Katsunori Murota[a,d], Tomomi Sato[a], Daisuke Kobayashi[a], Miki Kan[f], Masashi Hattori[f], Toshiya Kimura[f], Yukie Yamaguchi[f], Mutsuyo Takayama-Ito[c], Madoka Horiya[c], Guillermo Posadas-Herrera[c], Shohei Minami[g], Ryusei Kuwata[g], Hiroshi Shimoda[g], Ken Maeda[g], Yukie Katayama[h], Tetsuya Mizutani[h], Masayuki Saijo[c], Koki Kaku[b], Hiroto Shinomiya[f], Kyoko Sawabe[a,i,*]

IASR

初めて診断されたオズウイルス感染症患者

The (Re-)Emergence of Zoonotic Thogotoviruses: **BRBV**

Symptoms associated with BRBV infection



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- tick bite history
- symptoms: nausea, weakness, diarrhea; progressive heart failure
- died 11 days after symptoms onset

2017 Patient
2nd fatal case (fatal)
St. Louis (MO), USA

Case 2 (2017)

- 53 year old patient
- tick bite history
- symptoms: fever, rash, headache, nausea, vomiting
- died 3 weeks after symptoms appeared

- **Biosafety level 3** pathogen in Germany



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Adapted fom Bendl et. al. 2023

The (Re-)Emergence of Zoonotic Thogotoviruses



Amblyomma americanum

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2015 First Thogotovirus reported in east asia **THOV-HI-Kamigamo-25** strain from *Haemaphysalis longicornis* (Yoshii et.al. 2015)

2018 (Ejiri et.al.) first isolated OzV from Japanese *Amblyomma testudinarium* from 2013

2022 Serostudy (Tran et.al.) OzV neutralizing human sera (**2/24 hunters** from 2015) and several wild mammals

human (hunters)	8.3% (2/24)
sika deer and boars	8.3–55.8%
macaques	6.3–47.5%

2023 Patient
1st reported human case (fatal) of OzV infection: 70 yo woman with engorged tick (fatal) in Ibari Prefecture, Japan

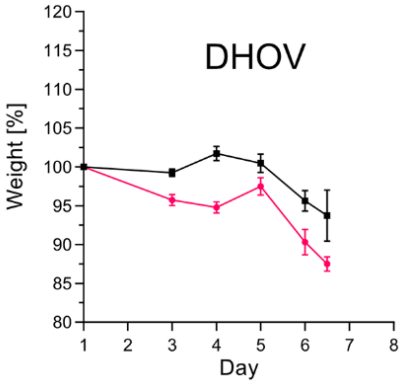
Characterization of a novel thogotovirus isolated from *Amblyomma testudinarium* ticks in Ehime, Japan: A significant phylogenetic relationship to Bourbon virus

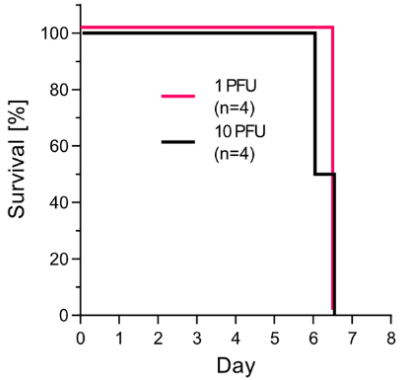
Hiroko Ejiri[a,b,1], Chang-Kweng Lim[c,1], Haruhiko Isawa[a,*], Ryosuke Fujita[a,d,e], Katsunori Murota[a,d], Tomomi Sato[a], Daisuke Kobayashi[a], Miki Kan[f], Masashi Hattori[f], Toshiya Kimura[f], Yukie Yamaguchi[f], Mutsuyo Takayama-Ito[c], Madoka Horiya[c], Guillermo Posadas-Herrera[c], Shohei Minami[g], Ryusei Kuwata[g], Hiroshi Shimoda[g], Ken Maeda[g], Yukie Katayama[h], Tetsuya Mizutani[h], Masayuki Saijo[c], Koki Kaku[b], Hiroto Shinomiya[f], Kyoko Sawabe[a,i,*]

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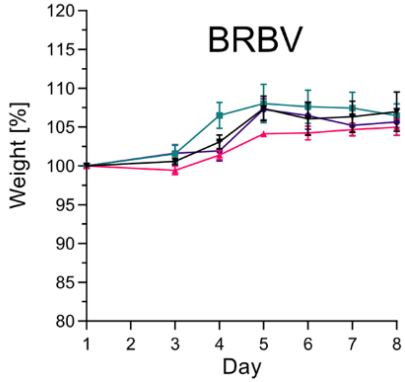
初めて診断されたオズウイルス感染症患者

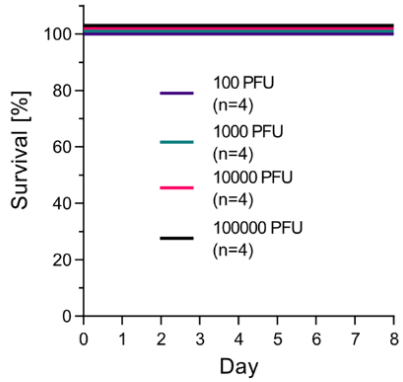
The (Re-)Emergence of Zoonotic Thogotoviruses: **BRBV**

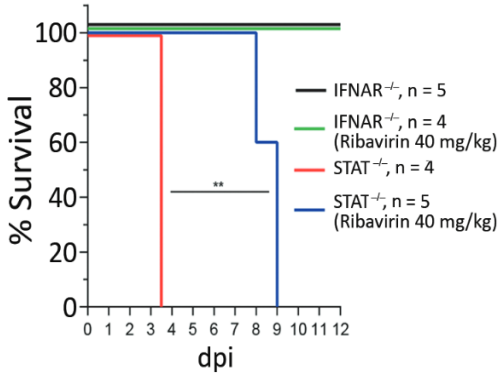




2019 in vivo/ in vitro characterization of BRBV in vitro / in vivo (Bricker et al. 2019; Fuchs et al. 2019)







- BRBV is **apathogenic in wildtype mice**
- **Highly pathogenic** in mice with defective IFN signalling
- severe BRBV cases might have been due to transient immunosuppression / undiscovered defect in innate immunity

C57BL/6 wt, i.p. infection route

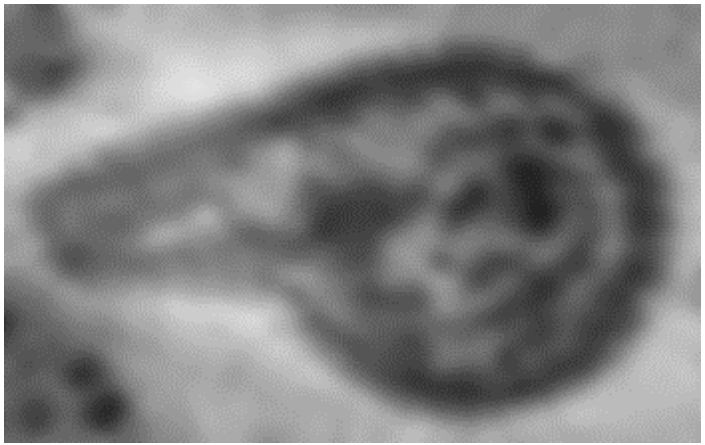
Adapted from Fuchs et al. 2019

Thogotoviruses:

- are widespread and **regularly infect wild mammals**, occasionally humans
- show **potential for zoonotic transmission and disease in humans** with recent cases of **BRBV and OzV** infection raising concerns

To better assess zoonotic threat, more research is needed on:

- **prevalence** of thogotoviruses in their respective **tick vectors** and **mammalian hosts**
- **EU/US seroprevalence** estimation through **Immunoglobulin lots** *(Collaboration with Takeda Path. Safety, Vienna)*
- **molecular determinants** of their enzootic transmission cycle and the efficiency of zoonotic spillover events / pathogenicity



DHOV-PoTi-461 particle

Thank you!

Prof. Dr. Georg Kochs
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Dr. Jonas Fuchs

Dr. Thomas Kreil, Takeda Pathogen Safety
Dr. Maria Farcet, Takeda, Pathogen Safety

Dr. Petr Chlanda & Moritz Wachsmuth-Melm, Heidelberg, CryoEM images

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