

West Nile virus, Usutu virus, TBE virus

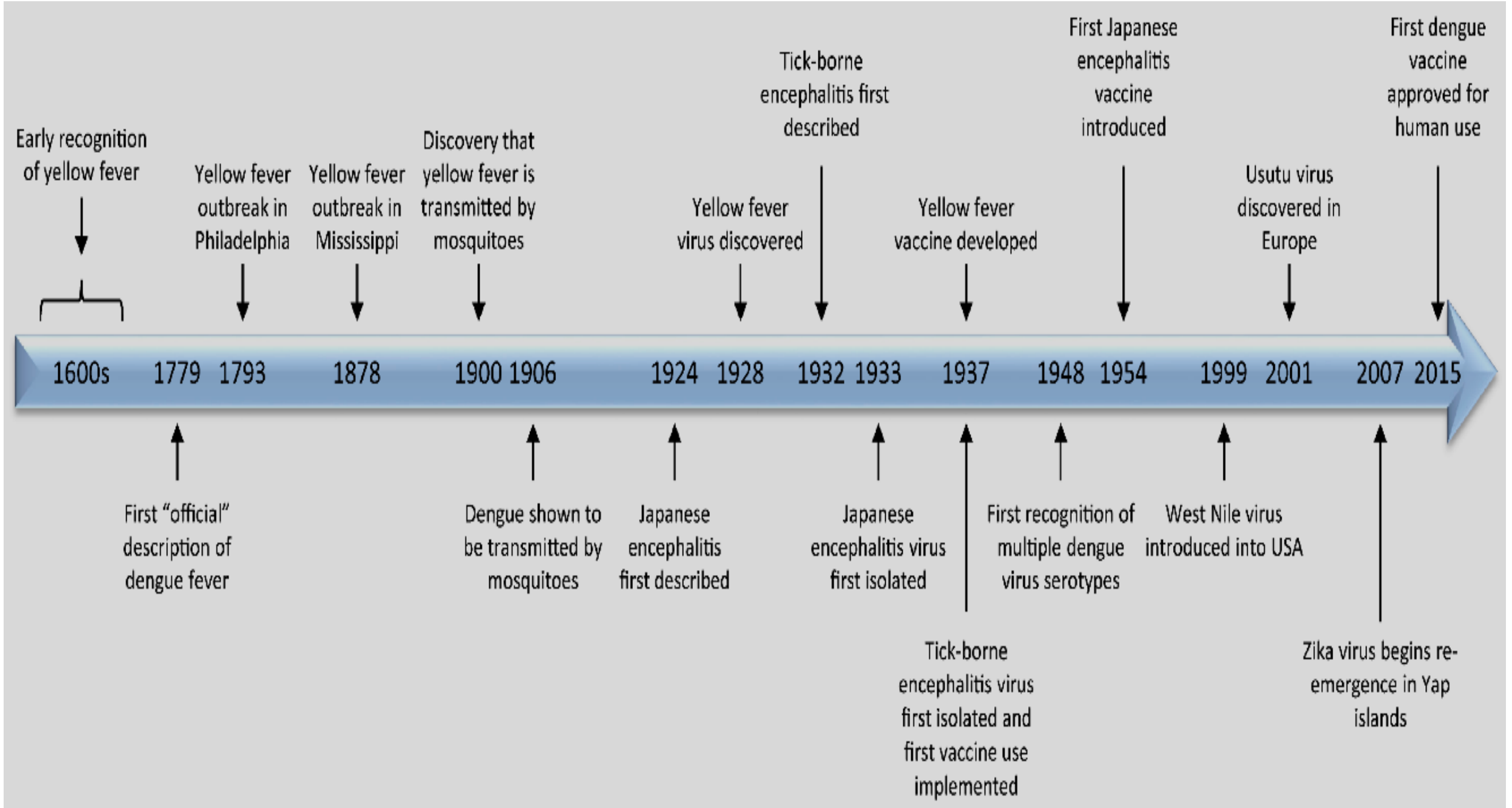
Mehdi Fazlalipour

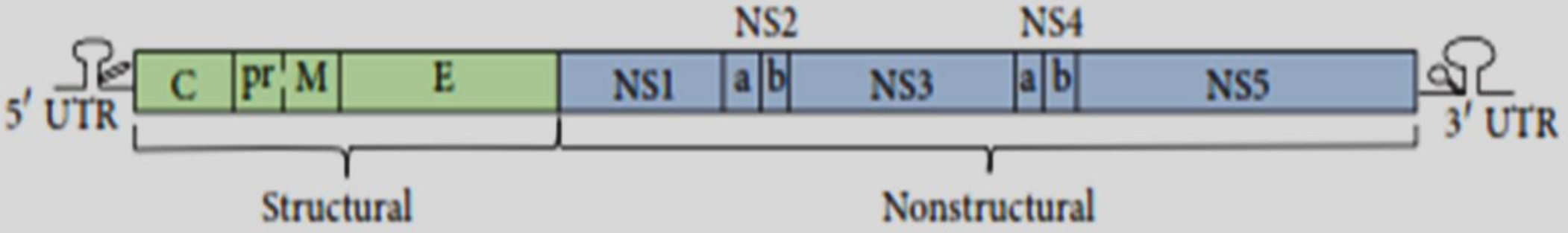
PhD, Virology

Importance of WNV

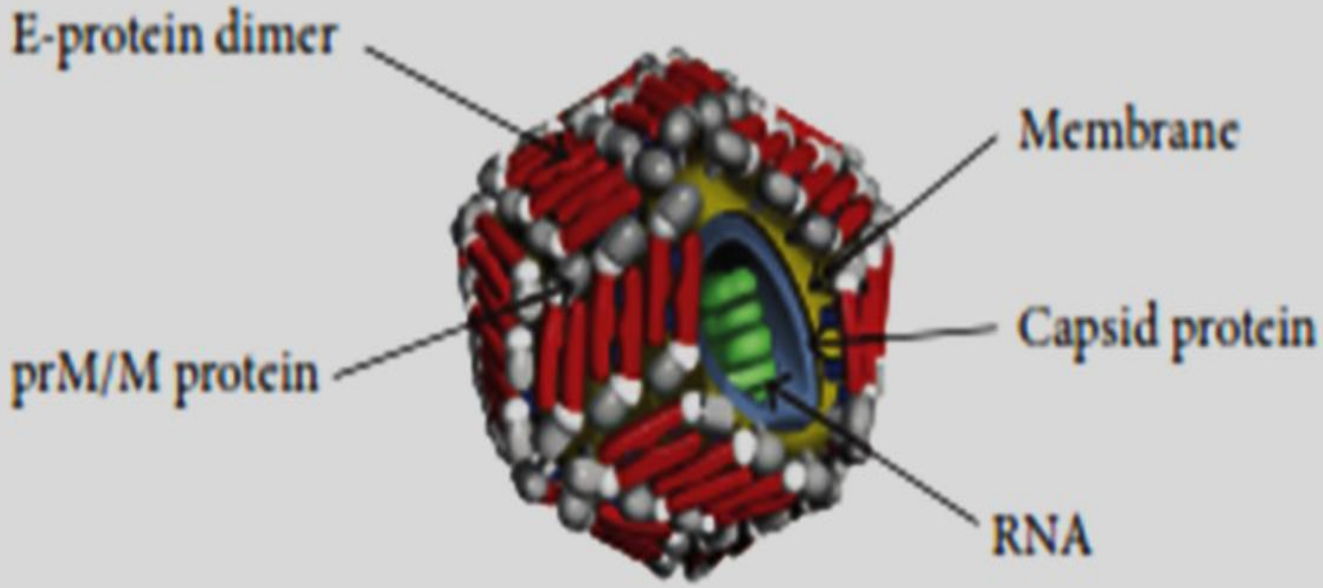
Family *Flaviviridae*
Genus *Flavivirus*
Representative species:
Yellow fever virus
West Nile virus
Dengue virus
Japanese encephalitis virus

- Common size (40–65 nm),
- Symmetry (enveloped, icosahedral),
- Nucleic acid (positive-sense, single-stranded RNA around 10,000–11,000 bases)

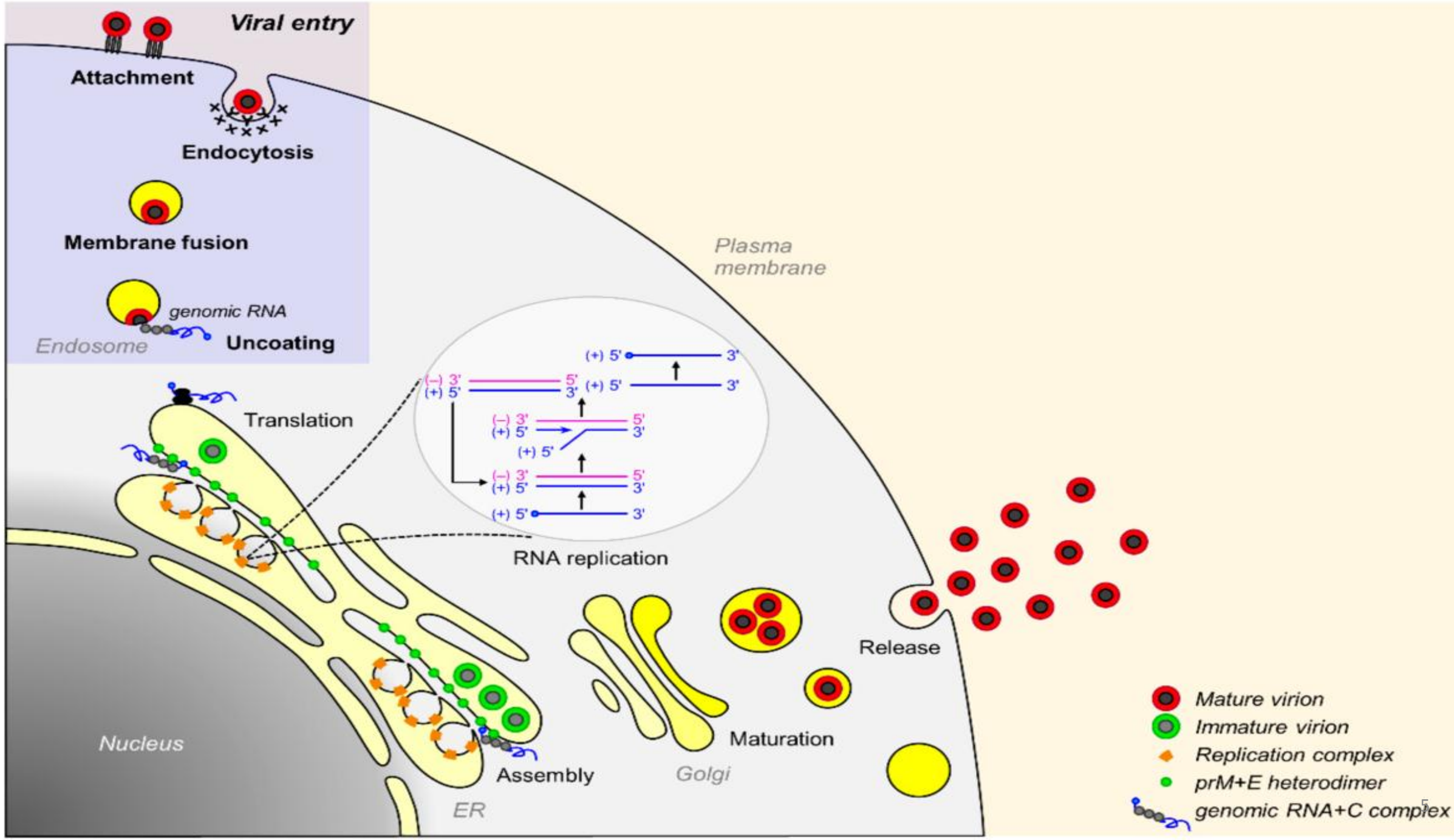

Early recognition of yellow fever
Yellow fever outbreak in Philadelphia
Yellow fever outbreak in Mississippi
Discovery that yellow fever is transmitted by mosquitoes
Yellow fever virus discovered
Tick-borne encephalitis first described
Yellow fever vaccine developed
First Japanese encephalitis vaccine introduced
Usutu virus discovered in Europe
First dengue vaccine approved for human use
1600s
1779
1793
1878
1900
1906
1924
1928
1932
1933
1937
1948
1954
1999
2001
2007
2015
First "official" description of dengue fever
Dengue shown to be transmitted by mosquitoes
Japanese encephalitis first described
Japanese encephalitis virus first isolated
Tick-borne encephalitis virus first isolated and first vaccine use implemented
First recognition of multiple dengue virus serotypes
West Nile virus introduced into USA
Zika virus begins re-emergence in Yap islands


NS2
NS4
5′ UTR
C
pr
M
E
NS1
a
b
NS3
a
b
NS5
3′ UTR
Structural
Nonstructural

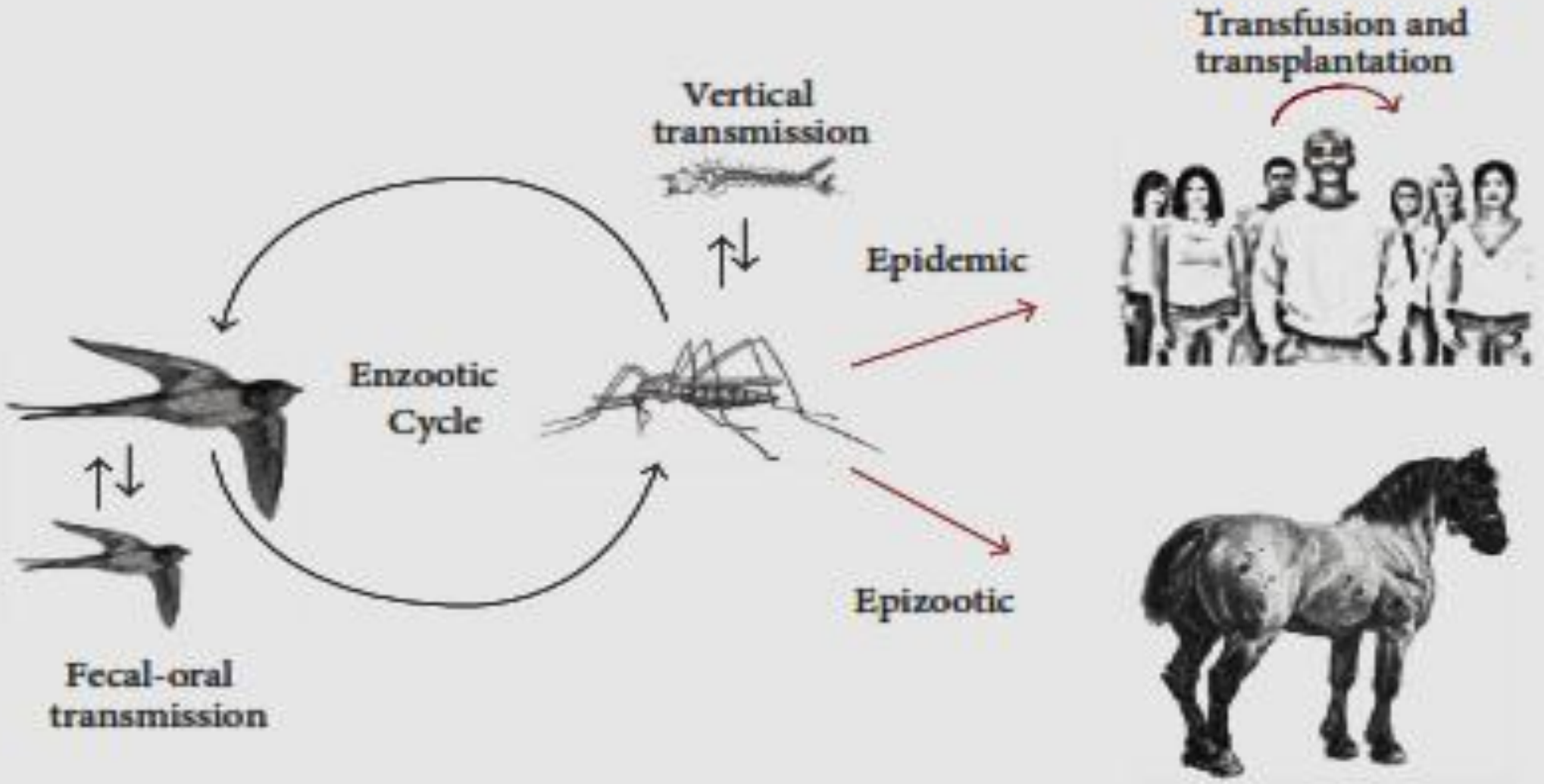
(a)


E-protein dimer
Membrane
prM/M protein
Capsid protein
RNA

(b)


Viral entry
Attachment
Endocytosis
Membrane fusion
genomic RNA
Endosome
Uncoating
Plasma membrane
Translation
(+) 5'
3'
(–) 3'
5'
RNA replication
Release
Nucleus
Assembly
ER
Golgi
Maturation
Mature virion
Immature virion
Replication complex
prM+E heterodimer
genomic RNA+C complex

Importance of WNV



Symptoms

- Incubation: 2 to 14 days
- Most infections resolve in 2 to 6 days
- Most people infected with the West Nile virus have no signs or symptoms.
- About 20 percent of people develop a mild infection called West Nile fever. Common signs and symptoms of West Nile fever include:
  - Fever
  - Headache
  - Muscle aches
  - Backache
  - Lack of appetite
  - Nausea, vomiting and diarrhea
  - Skin rash

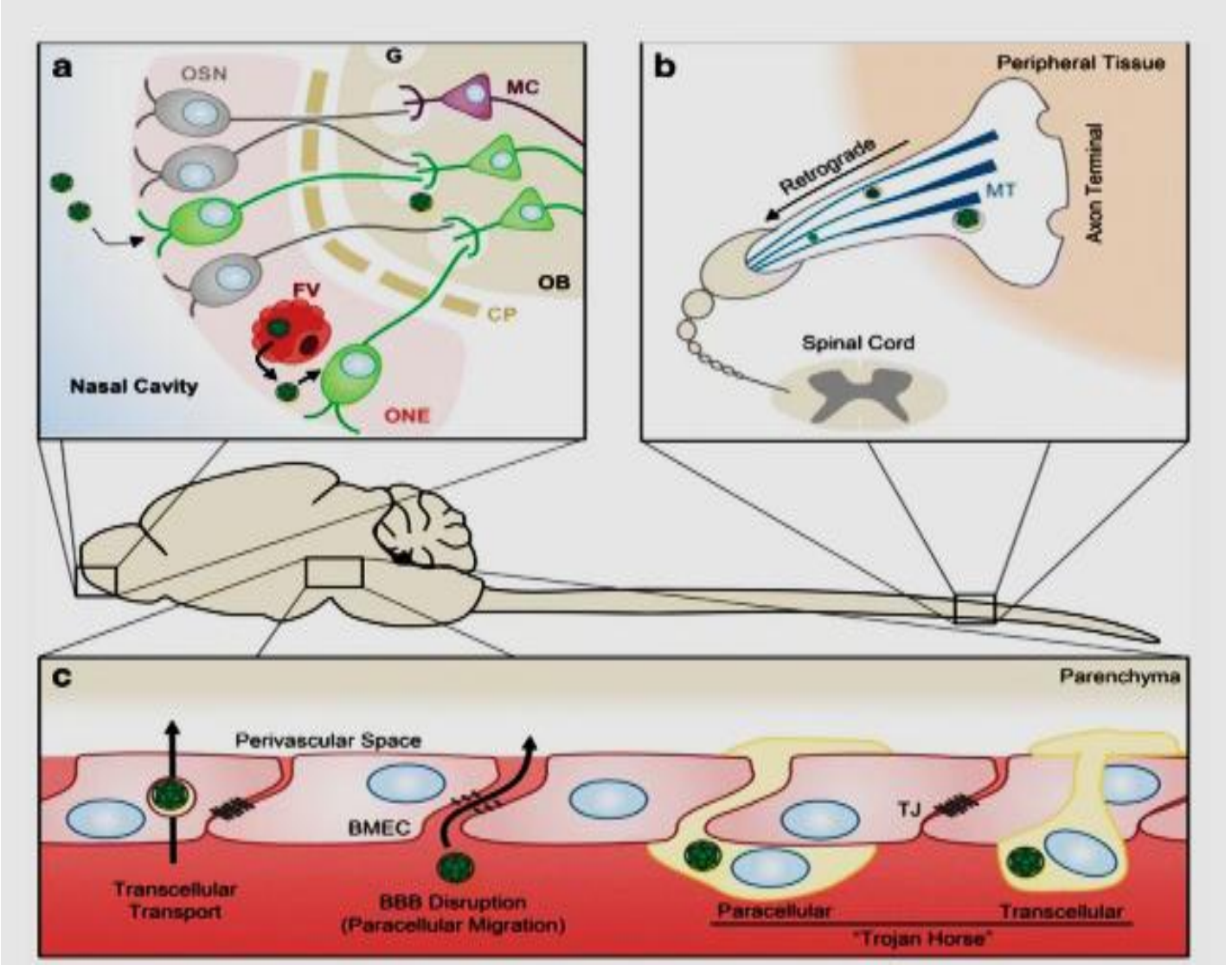
Symptoms

- In less than 1 percent of infected people, the virus causes a more serious neurological infection,
  - inflammation of the brain (encephalitis) or of the brain and surrounding membranes (meningoencephalitis), and paralysis.

- Signs and symptoms of these diseases include:
  - High fever
  - Severe headache
  - Stiff neck
  - Disorientation or confusion
  - Stupor or coma
  - Tremors or muscle jerking
  - Signs and symptoms of Parkinson's disease
  - Convulsions
  - Partial paralysis

Disease in Humans

- **West Nile neuroinvasive disease**
  - Occurs rarely
    - Progression of West Nile fever
  - Can be severe and life-threatening
  - Three syndromes
    - Encephalitis
    - Meningitis
    - Acute flaccid paralysis
  - Persistent neurological dysfunction



Diagnosis in Humans

- Serology
  - Serum or CSF
  - IgM/IgG, ELISA
    - Cross reactions possible
  - Plaque neutralization test
- Detection of virus by culture
- Detection of nucleic acids
  - RT-PCR

Importance of WNV

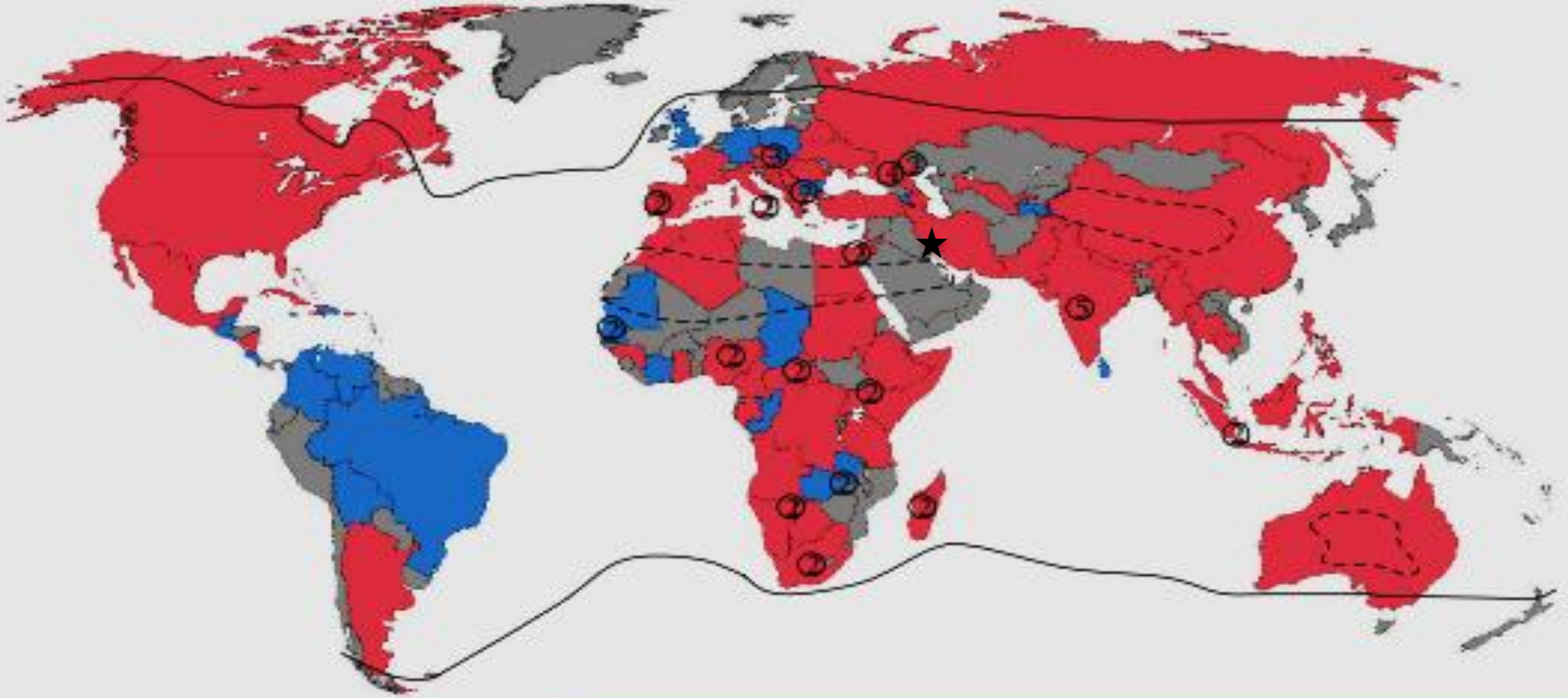


FIGURE 1: Global distribution of WNV by country: Red—human cases or human seropositivity; Blue—nonhuman/mosquito cases or seropositivity; Gray—no data or no positives reported. Black lines represent worldwide distribution of the main WNV mosquito vectors, excluding areas of extreme climate denoted by dashed lines. Circled numbers indicate the reported presence of WNV lineages other than lineage 1 in that specific area. For Japan, South Korea, Finland, and Sweden, seropositivity for WNV has been detected only in nonresident birds, which was not considered indicative of local transmission. Kading et al. [182] reported infections in gorillas living near the border of the Democratic Republic of the Congo and Rwanda, which were sampled in the D.R.C., but may have been infected in Rwanda.

THE PREVALENCE OF HUMAN INFECTION WITH WEST NILE VIRUS IN IRAN

S. Saidi, R. Tesh,
E. Javadian, A. Nadim

ABSTRACT

A total of 698 blood and serum specimens from residents of 13 Iranian communities were examined by plaque reduction neutralization test for antibodies to West Nile virus. In general, infection rates in the North of the country were low, while higher rates were observed among residents of Central and Southwestern Iran. The highest prevalence of infction was found among residents of Khuzestan Province, indicating that this region is one of endemic West Nile virus activity. The symptoms of West Nile virus infection in man are also discussed.

1971-1975

Table 1 summarizes neutralization test results obtained from the 13 communities sampled. Of a total of 698 sera and bloods examined, 186 (26.6%) had antibodies against West Nile virus. With the exception of Rasht, infection rates in communities located in the North of the country (Tabriz, Ali-Abad, Neishabor and Mashad) were low, while higher rates were observed in Central and Southwestern Iran. The highest prevalence of antibodies was found in the Dezful-Deigi area. In Deigi, 69 of 72 persons (95.8%) had West Nile neutralizing antibodies. As shown in Table 2, the three negative sera were from children 1 to 4 years af age. By the age of 5 years, 100% of the population sampled in this community had West Nile antibodies.

None of 24 selected West Nile positive sera neutralized yellow fever or Japanese encephalitis viruses, indicating that the antibodies were specific and were not due to cross-reaction with heterologous Group B arbovirus antibodies.

WNV in Iran

PREVALENCE OF WEST NILE VIRUS NEUTRALIZING ANTIBODIES IN 13 IRANIAN COMMUNITIES

Community-Province	Number /Total positive*/tested	Percentage positive
Tabriz (U)**, East Azerbaijan	0/50	0.0
Rasht (U), Gilan	6/51	1.8
Ali-Abad (R)**, Khorassan	2/50	4.0
Neishabor (U), Khorassan	0/50	0.0
Mashad (U), Khorassan	1/50	2.0
Tehran (U), Tehran	13/103	12.6
Varamin (R), Tehran	3/48	6.3
Isfahan (U), Isfahan	6/49	12.2
Kermanshah (U), Kermanshah	15/32	46.9
Deiqi (R), Khuzestan	69/72	95.8
Dezful (U), Khuzestan	34/38	89.5
Khorousi (R), Khuzestan	31/49	63.3
Abadan (U), Khuzestan	6/56	10.7

Figure 1 Map of Iran showing the location of 13 communities sampled for West Nile virus antibodies

Prevalence of West Nile virus in Mashhad, Iran: A population–based study

Zahra Meshkat[1], Sadegh Chinikar[2*], MohammadTaghi Shakeri[3], Lida Manavifar[4], Maryam Moradi[2], Hessam Mirshahabi[5], Tahmineh Jalali[2], Sahar Khakifirouz[2] and Nariman Shahhosseini[2]

[1]*Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, IR Iran*
[2]*Arboviruses and Viral Hemorrhagic Fevers Laboratory (National Ref. Lab), Pasteur Institute of Iran*
[3]*Department of Biostatistics, Public Health School, Mashhad University of Medical Sciences, Mashhad, Iran*
[4]*Department of Laboratory Sciences, School of Paramedical and Rehabilitation, Mashhad University of Medical Sciences, Mashhad, Iran*
[5]*Department of Microbiology and Immunology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*

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ABSTRACT

Objective: To evaluate the prevalence of West Nile virus seropositivity in the general population of Mashhad, Northeast of Iran. **Methods:** One hundred and eighty two individuals living in the city of Mashhad were studied using cluster sampling method. Both IgM and IgG antibodies against WNV were detected by ELISA method. **Results:** In this study, the overall IgG seroprevalence of positive West Nile virus was 11%; however, IgM antibody was not found in the participants. **Conclusions:** Our study suggested that the prevalence rate of West virus is considerable in Mashhad city. It seems necessary for clinicians and health care workers to be aware of WNV infection in the Northeast Iran.

2011-2012

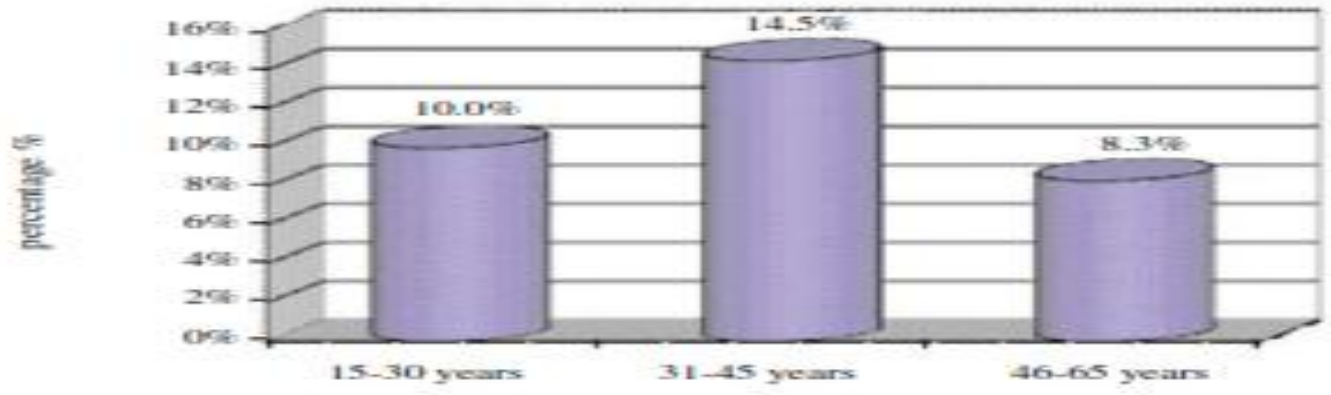


Figure 1. Frequency of WNV IgG antibody according to the age groups.

Detection of West Nile virus genome and specific antibodies in Iranian encephalitis patients

S. CHINIKAR[1]†, A. JAVADI[2]†, B. ATAEI[2]*, H. SHAKERI[2], M. MORADI[1], E. MOSTAFAVI[1] AND S. M. GHIASI[1]

[1] *Laboratory of Arboviruses and Viral Haemorrhagic Fevers (National Reference Laboratory), Pasteur Institute of Iran, Tehran, Iran*
[2] *Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*

(*Accepted 17 September 2011*)

SUMMARY

West Nile virus (WNV) is a mosquito-borne flavivirus which circulates in birds, horses and humans. An estimated 80% of WNV infections are asymptomatic. Fewer than 1% of infected persons develop neuroinvasive disease, which typically presents as encephalitis, meningitis, or acute flaccid paralysis. This study was conducted from January 2008 to June 2009 in Isfahan, Iran. Patients attending the emergency department with fever and loss of consciousness were consecutively included. Cerebrospinal fluids (CSF) were initially analysed through bacteriology and biochemistry examinations, resulting in those with evidence of meningitis being excluded. Patients' CSF and serum were diagnosed by serological and molecular assays. A total of 632 patients with fever and loss of consciousness were tested by CSF analyses. Samples of the remaining patients (39·4%) were referred for WNV investigation. Three (1·2%) of the patients were positive for both serum and CSF by RT–PCR, and six (2·4%) were positive only for IgG antibodies. History of insect bite, and blood transfusion and transplantation were risk factors for being positive by RT–PCR ($P=0·048$) and being IgG positive ($P=0·024$), respectively. The results of this study showed that the prevalence of West Nile fever is low in patients with encephalitis.

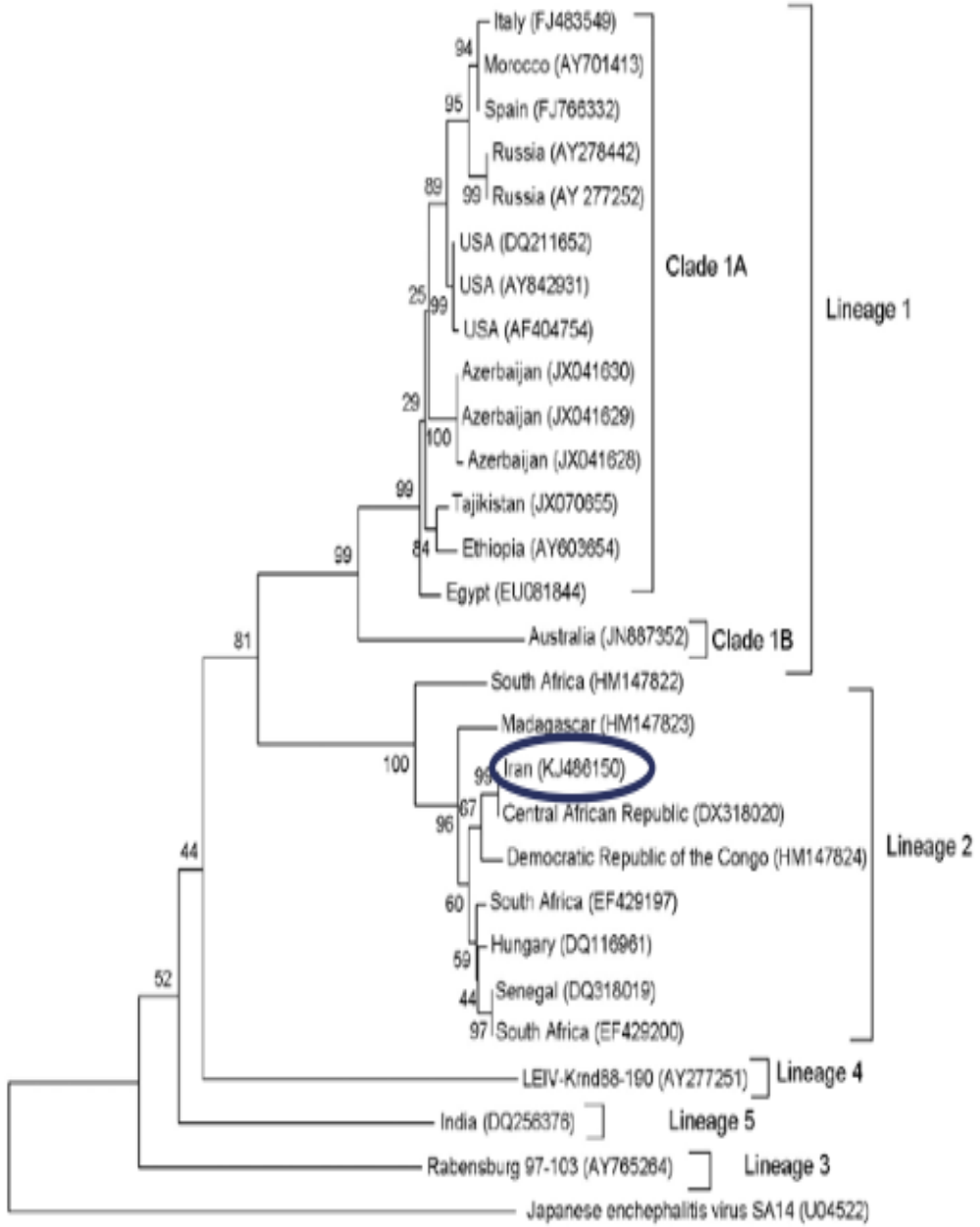
Key words: West Nile virus, encephalitis, Isfahan, Iran.

2008-2009

Phylogenetic Analysis of West Nile Virus Genome, Iran

2009

WNV was obtained from a blood sample from an Iranian patient who had encephalitis and was hospitalized in 2009 in Isfahan in the central highlands of Iran. The patient reported no history of animal contact, insect bites, blood transfusions, transplantations, and travel. He exhibited fever, headache, hypertension, and vomiting. On initial examination, he had a



2005

Original Article

A Study of West Nile Virus Infection in Iranian Blood Donors

Zohreh Sharifi PhD*, Mahmood Mahmoodian Shooshtari PhD*, Ali Talebian MD*

Background: West Nile virus is a mosquito transmitted virus that can cause disease in humans and horses. A majority of people infected with WNV will have no symptoms or may only experience mild symptoms, such as headaches. About 20% of infected humans develop a flu-like illness characterized by fever; while in the elderly and immunocompromised West Nile virus can cause a more serious neurologic disease and may be fatal. West Nile virus infection is endemic in the Middle East. West Nile virus can also be transmitted by transfusion through infected blood components.

The objective of this study is to find the West Nile virus-RNA incidence and anti-West Nile virus prevalence amongst Iranian blood donors in order to determine whether this emerging infection is a possible risk for the blood supply in Iran.

Methods: Serum samples from 500 blood donors who donated blood at the Tehran Blood Transfusion Center were collected between May and October 2005. Serum samples were examined for IgM and IgG antibodies to West Nile virus using the ELISA method. The samples were tested for the presence of West Nile virus RNA by the real-time RT-polymerase chain reaction assay. All data were analyzed statistically using the Chi-Square test.

Results: All 500 donors were negative for West Nile virus-specific IgM antibody at the time of donation. No WNV RNA-positive samples were detected. The percentage of seropositivity of IgG antibodies to WNV was 5% at donation.

Conclusion: No evidence of WNV-specific IgM antibody and WNV RNA in blood donor samples was found. In order to increase the safety of blood donation, it is essential to continue surveillance of this emerging infection in order to protect the blood supply in the future.

Spread of West Nile virus in Iran: a cross-sectional serosurvey in equines, 2008–2009

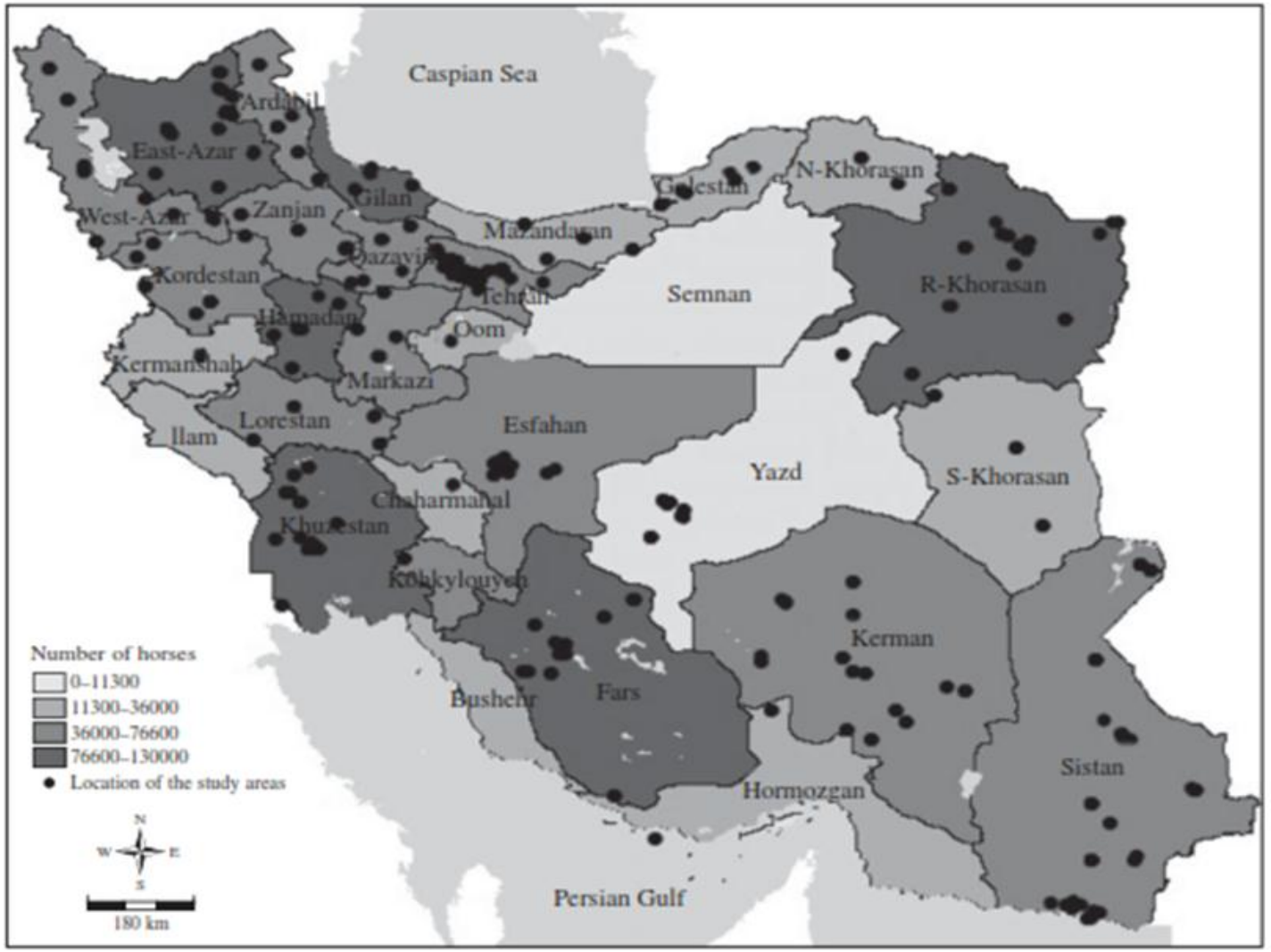
F. AHMADNEJAD[1,2*], V. OTAROD[3], M. H. FALLAH[4], S. LOWENSKI[5], R. SEDIGHI-MOGHADDAM[6], A. ZAVAREH[1], B. DURAND[5], S. LECOLLINET[5] AND P. SABATIER[2]

2008-2009

SUMMARY

We report the first large-scale serosurvey for West Nile virus (WNV) conducted in the equine population in Iran. Blood samples were obtained in 2008–2009 from 1054 equines collected from 260 districts located in 27 provinces. The overall seroprevalence rate for WNV neutralizing antibodies was 23·7%. Marked geographical variations were observed as province-specific seroprevalence rates ranged from 1% to 88%, the highest values being observed in the southern and western parts of the country. The presence of IgM-positive animals ($n=9$) indicated a recent circulation of WNV in several provinces. Logistic modelling confirmed this result with a significant effect of age on seropositivity. This study revealed extensive circulation of WNV in Iran particularly in southwestern provinces where the virus probably circulates every year.

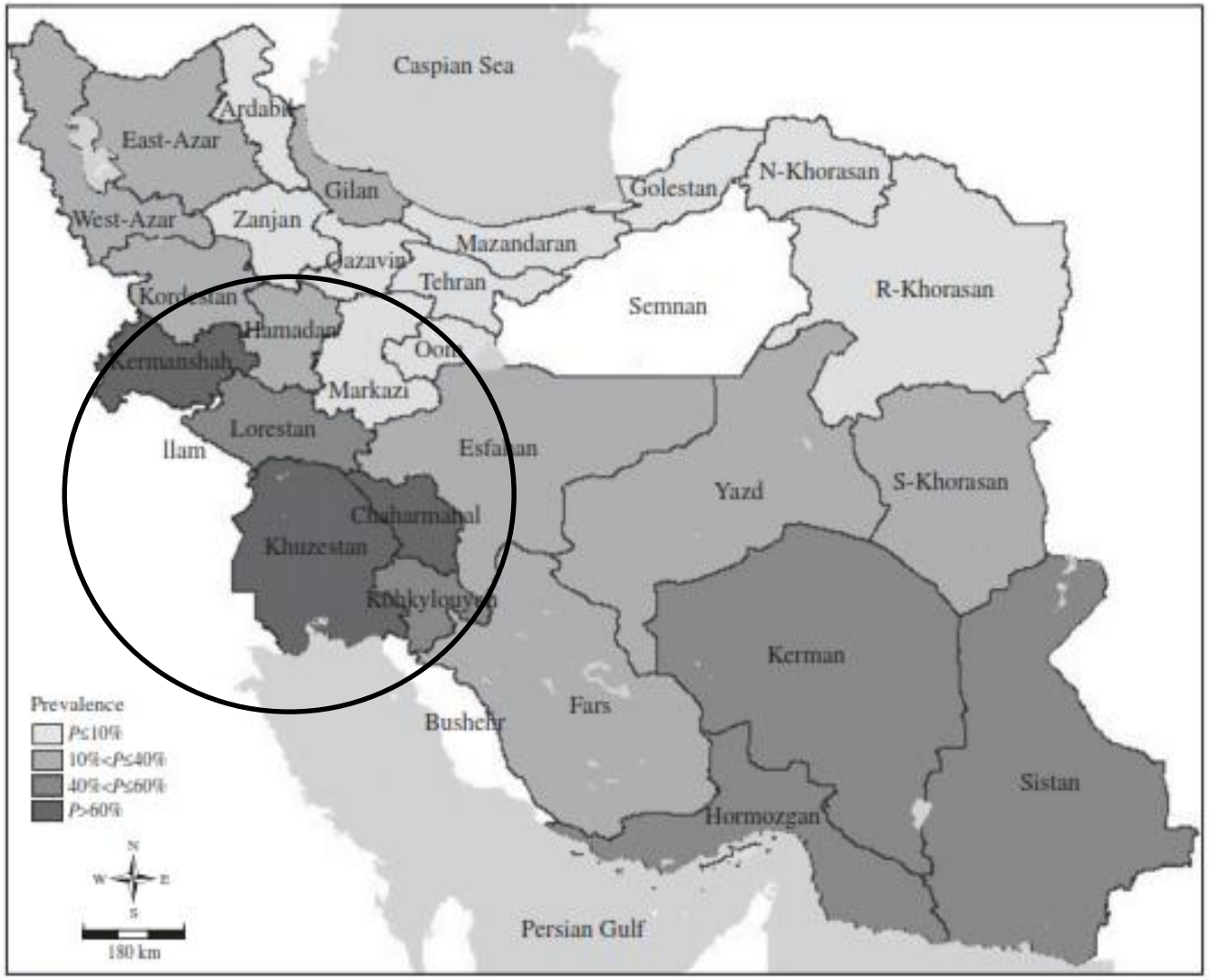
Sample collection equine



Iran

Fig. 1. Geographic distribution of equine population by province and geographic location of collected samples in 27 out of 30 provinces, Iran, 2008–2009.

WNV IgG positive



Iran

Fig. 2. Anti-WNV seroprevalence determined by plaque reduction neutralization test in equines in 27 out of 30 provinces, Iran, 2008–2009.

Seroprevalence of West Nile Virus in Iran*

Sadegh Chinikar,[1] Nariman Shah-Hosseini,[1] Ehsan Mostafavi,[2] Maryam Moradi,[1] Sahar Khakifirouz,[1] Tahmineh Jalali,[1] Mohammad Mehdi Goya,[3] Mohammad Reza Shirzadi,[3] Mohammad Zainali,[3] and Anthony R. Fooks[4]

Abstract

This study was undertaken to determine the seroprevalence of West Nile virus (WNV) in human and equine sera in Iran. Blood samples were tested from 300 human samples and 315 equine samples in five geographic zones of north and central parts of Iran between 2010 and 2012. All samples were tested for the immunoglobulin G (IgG) antibody to WNV by using an enzyme-linked immunosorbent assay (ELISA). Of all samples, 4 (1.3%) human and 9 (2.8%) equines were considered to be seropositive for WNV. These results suggest circulation and exposure of the human and equine populations to WNV in Iran.

TABLE 1. SUMMARY OF PATIENTS WITH A POSITIVE IMMUNOGLOBULIN G WEST NILE VIRUS LABORATORY TEST

Animal contact	*Province*	*Region*	*Professions*	*Age*	*Sex*	*No.*
Yes	Golestan	Northeast Iran	Driver	25	Male	1
No	Golestan	Northeast Iran	Self- employed	27	Male	2
Yes	Qom	Central Iran	Ironworker	35	Male	3
Yes	Gilan	Northwest Iran	Self- employed	30	Male	4

2010-2012

TABLE 2. SUMMARY OF EQUINES WITH IMMUNOGLOBULIN G–POSITIVE WEST NILE VIRUS LABORATORY TEST

Province	*Region*	*Age*	*Sex*	*Nombe*
Isfahan	Central Iran	11	M	1
Isfahan	Central Iran	6	S	2
Isfahan	Central Iran	8	M	3
Gilan	Northwest Iran	5	M	4
Gilan	Northwest Iran	7	S	5
Golestan	Northeast Iran	4	S	6
Golestan	Northeast Iran	5	S	7
Golestan	Northeast Iran	3	M	8
Golestan	Northeast Iran	8	M	9

M, mares; S, stallions.

WNV IgG positive

Iran

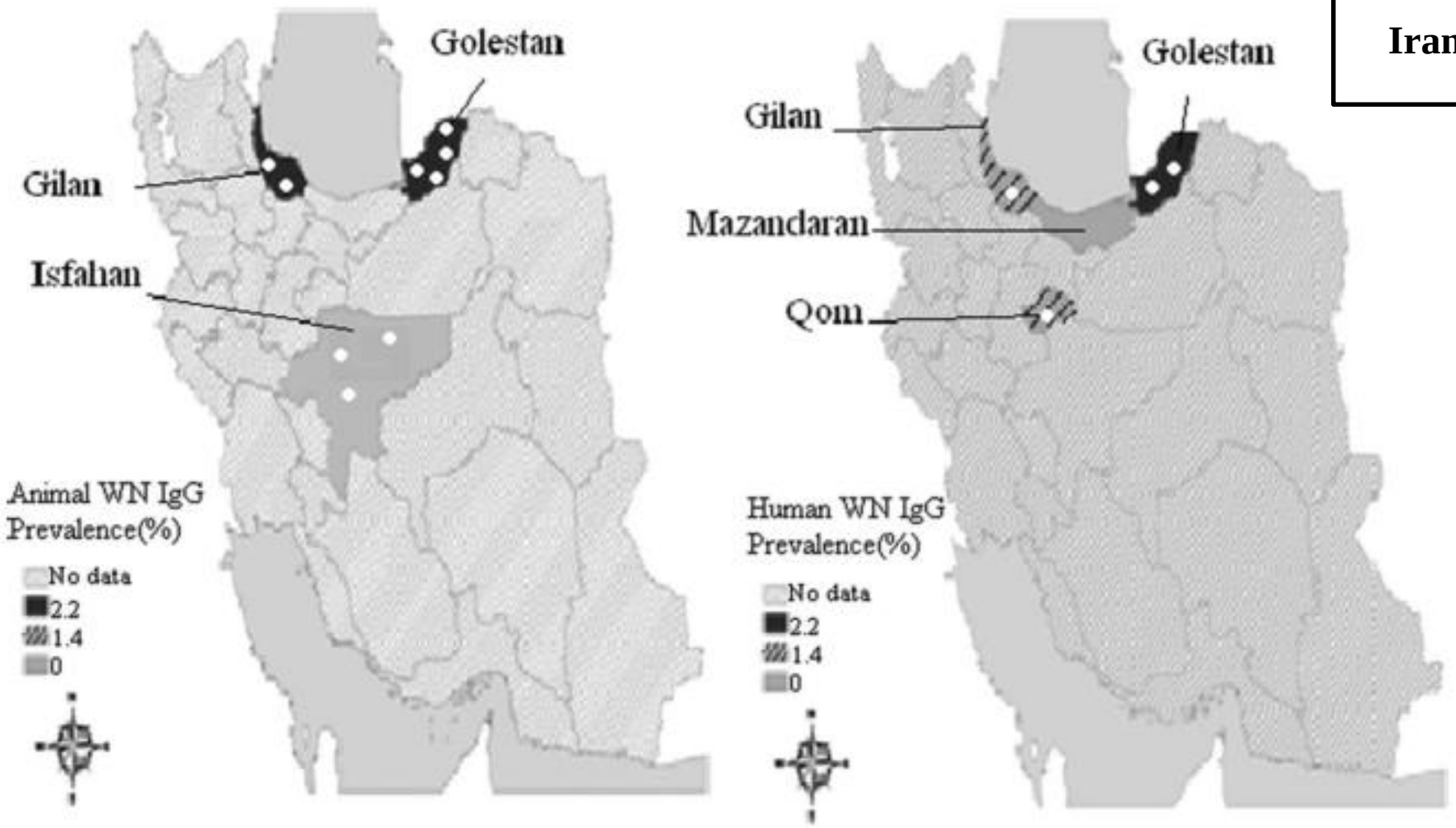


FIG. 1. Geographic location of human seropositive (*right*) and animal seropositive (*left*). For each province, the shading styles (see key) indicate the prevalence of serological positive cases. The number of human and animal positive sera samples is marked in each province (white dot).

West Nile Virus in Mosquitoes of Iranian Wetlands

2014-2015

ABSTRACT

The West Nile virus (WNV) transmission cycle includes a wide range of migratory wetland birds as reservoirs, mosquitoes as biological vectors, and equines and humans as dead-end hosts. Despite the presence of potential vector species, there is no information about the existence of WNV in mosquito vectors in Iran. The Iranian West Azerbaijan Province is located in the northwestern part of Iran and has borders with Turkey, Iraq, Armenia, and the Republic of Azerbaijan. The current study was conducted to identify the wetland mosquitoes of the West Azerbaijan Province and their infection with WNV. In this study, 2143 specimens were collected, comprising 1541 adults and 602 larvae. Six species belonging to four genera were collected and identified: *Anopheles maculipennis* sensu lato (s.l.), *Culex (Cx.) hortensis*, *Cx. pipiens* s.l., *Cx. theileri*, *Culiseta longiareolata*, and *Aedes (Ae.) (Ochlerotatus) caspius*. In total, 45 pools of mosquitoes were examined. Two of the adult pools collected from the same location showed the presence of WNV in *Ae. (Och.) caspius*, from Sangar, Makoo County, as confirmed by PCR and sequencing. Due to the discovery of WNV in the mosquito population of the region, and the presence of wetlands and significant populations of migratory birds, the health sector should carefully monitor the factors involved in the cycle of this disease.

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Seroprevalence of West Nile virus in Khuzestan province, southwestern Iran, 2016–2017

Masoumeh Kalantari[1], Mostafa Salehi-Vaziri[2–3], Mohammad Hassan Pouriayevali[3], Vahid Baniasadi[3], Shokrollah Salmanzadeh[4], Morteza Kharat[5] & Mehdi Fazlalipour[3]

[1]*Biology Department, Payame Noor University, Tehran;* [2]*Research Center for Emerging and Reemerging Infectious Diseases, Pasteur Institute of Iran;* [3]*Department of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory), Pasteur Institute of Iran, Tehran;* [4]*Health Research Institute, Infectious and Tropical Diseases Research Center, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz;* [5]*Health Research Institute, Laboratory Health Center, Faculty of Medicine, Ahvaz, Iran*

ABSTRACT

Background & objectives: West Nile virus (WNV) is a neurotropic *Flavivirus* transmitted to humans through mosquito bites. As there is no specific antiviral treatment or approved vaccine against WNV, control and prevention of the infection is the best strategy to reduce the burden of WNV-related diseases. The circulation of WNV has been indicated in several regions of Iran including the Khuzestan province. Considering the complex ecology of WNV, the latest data are necessary for the implementation of preventive measures. Therefore, the present study was designed to provide updated information on the seroepidemiology of WNV in Khuzestan province.

Methods: A total of 408 sera were taken from volunteers living in Khuzestan. The presence of specific immunoglobulin G (IgG) antibody against WNV was tested by the enzyme-linked immunosorbent assay (ELISA) method. All the data and participants' demographic information were analyzed by SPSS and Esri's ArcMap GIS software programs.

Results: Anti-WNV IgG antibody was detected in 97 (23.8%) out of the 408 tested sera. The highest seropositivity rate was observed in cases aged between 20–29 yr and the lowest seropositivity rate was seen in those <19 yr of age ($p = 0.001$). There was no statistically significant association between WNV infection and gender, occupation, and educational level. The majority of positive cases were from the city of Ahvaz (47 cases, 48.4%) and Andimeshk (32 cases, 33%).

Interpretation & conclusion: This study supports the earlier findings suggesting the circulation of WNV in Khuzestan province. Therefore, the implementation of an integrated surveillance system and training of health care workers and general population regarding the infection would be valuable to reduce the burden of possible outbreaks.

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ORIGINAL ARTICLE

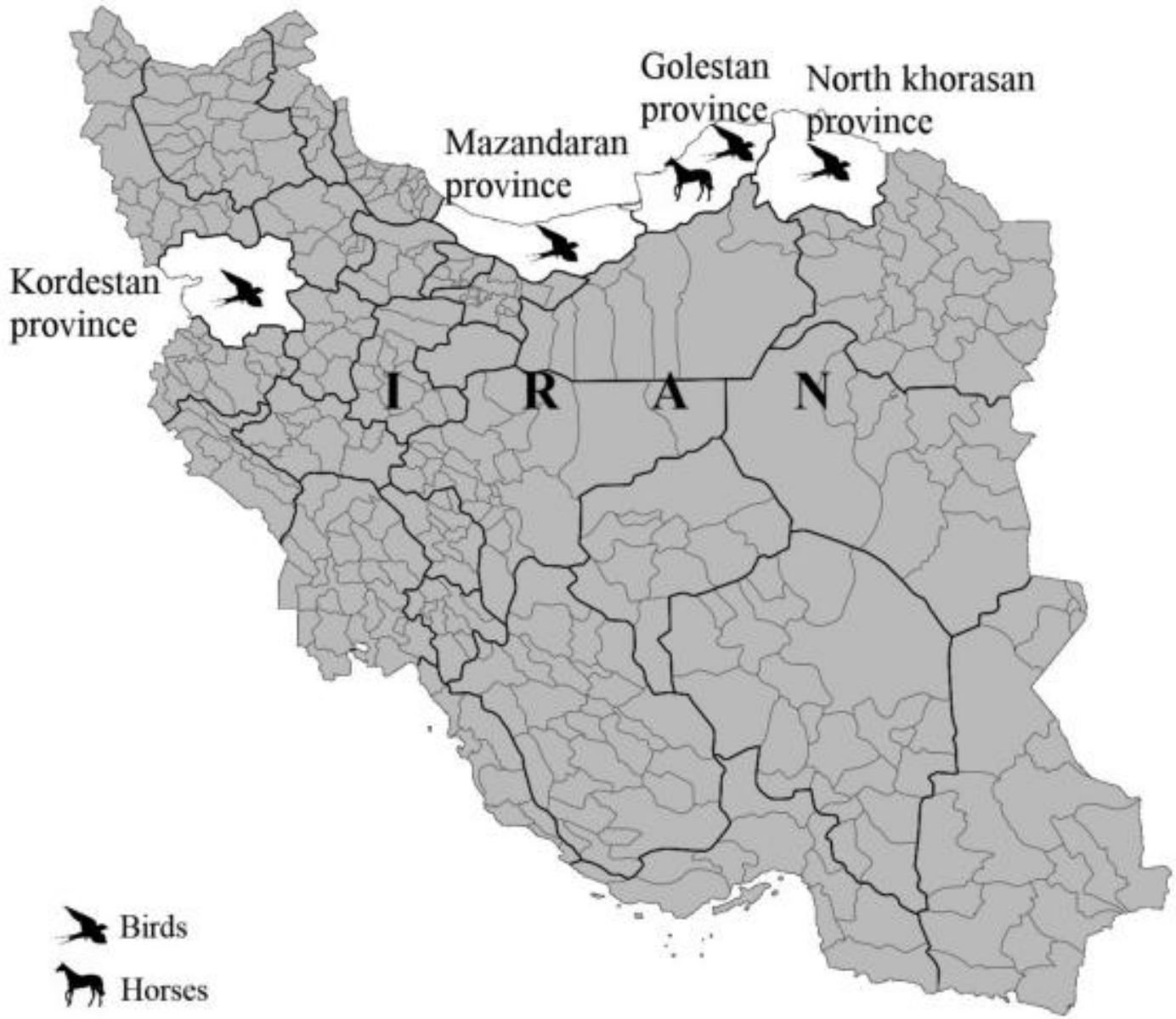
WILEY

Serological evidence of West Nile virus infection among birds and horses in some geographical locations of Iran

Hasan Bakhshi[1] | Cécile Beck[2] | Sylvie Lecollinet[2] | Maëlle Monier[2] | Laurence Mousson[3] | Sedigheh Zakeri[1] | Abbasali Raz[1] | Kourosh Arzamani[4] | Leila Nourani[1] | Navid Dinparast-Djadid[1] | Anna-Bella Failloux[3]

Abstract

Recent expansion of arboviruses such as West Nile (WNV), Usutu (USUV), and tick-borne encephalitis (TBEV) over their natural range of distribution needs strengthening their surveillance. As common viral vertebrate hosts, birds and horses deserve special attention with routine serological surveillance. Here, we estimated the seroprevalence of WNV, USUV and TBEV in 160 migrating/resident birds and 60 horses sampled in Mazandaran, Golestan, North Khorasan, Kordestan provinces and Golestan province of Iran respectively. ELISA results showed that of 220 collected samples, 32 samples (14.54%), including 22 birds and 10 horses, were positive. Microsphere immunoassay results showed that 16.7% (10/60) of horse blood samples collected in Golestan province were seropositive against WNV (7; 11.7%), *Flavivirus* (2; 3.3%) and seropositive for USUV or WNV (1; 1.7%). Furthermore, micro virus neutralization tests revealed that four of seven ELISA-positive bird blood samples were seropositive against WNV: two Egyptian vultures, and one long-legged buzzard collected in Golestan province as well as a golden eagle collected in North Khorasan province. No evidence of seropositivity with TBEV was observed in collected samples. We showed that WNV, responsible for neuroinvasive infection in vertebrates, is circulating among birds and horses in Iran, recommending a sustained surveillance of viral infections in animals, and anticipating future infections in humans.



Provinces	ELISA results
Kordestan	0/24 (0%)
Mazandaran	15/99 (15.1%)
Golestan	5/27 (18.5%) 10/60 (16.7%)
North Khorasan	2/10 (20%)

Virus Family	Viral Genus	Virus		Vector Species
	Orthobunyavirus	California serogroup viruses		Mosquito (Aedes sp.)
	Phlebovirus	Rift Valley Fever virus		Mosquito (various)
Bunyaviridae	Phlebovirus	Toscana virus		Sandfly (Phelbotomus sp.)
	Phlebovirus	Phlebotomus fever virus		Sandfly (phelbotomus)
	Phlebovirus	Sandfly Fever Naples virus		Sandfly (phelbotomus)
	Phlebovirus	Sandfly Fever Sicilian virus		Sandfly (phelbotomus)
	Phlebovirus	Heartland virus		Tick (A. americanum)
	Phlebovirus	Severe fever with thrombocytopenia syndrome virus		Tick (H. longicornis)
	Nairovirus	Crimean Hemorrhagic Fever virus		Tick (Hyalomma sp.)
Flaviviridae	Flavivirus	Dengue Virus		Mosquito (Aedes sp.)
	Flavivirus	Zika virus		Mosquito (Aedes sp.)
	Flavivirus	Yellow fever virus		Mosquito (Aedes sp.)
	Flavivirus	West Nile Virus		Mosquito (Culex sp.)
	Flavivirus	St. Louis Encephalitis virus		Mosquito (Culex sp.)
	Flavivirus	Japanese encephalitis virus		Mosquito (Culex sp.)
	Flavivirus	Murray Valley encephalitis virus		Mosquito (Culex sp.)
	Flavivirus	Usutu		Mosquito (various)
	Flavivirus	Omsk Hemorrhagic fever virus		Tick (dermacentor)
	Flavivirus	Kyasanur Forest Diesease virus		Tick (Haemaphysalis sp.)
	Flavivirus	Tick-borne encephalitis virus		Tick (Ixodes and Haemaphysalis sp.)
	Flavivirus	Powassan virus		Tick (Ixodes sp.)
Orthomyxoviridae	Thogotovirus	Bourbon virus		Tick (A. americanum)
Reoviridae	Coltivirus	Colorado tick fever		Tick (dermacentor)
Rhabdoviridae	Vesiculovirus	Vesicular Stomatitis (New Jersey) virus		Sandflies (Lutz. Sp) | Mosquitos (various)
	Vesiculovirus	Chandipura		Sandfly (Phlebotomus Sp.)
Togaviridae	Alphavirus	Barmah Forest Virus		Mosquito (Aedes and Culex sp.)
	Alphavirus	Chikungunya virus		Mosquito (Aedes sp.)
	Alphavirus	Venezuelan equine encephalitis virus		Mosquito (Culex sp.)
	Alphavirus	Sindbis virus		Mosquito (Culex sp.)
	Alphavirus	Equine encephalitis virus		Mosquito (Culex sp.)
	Alphavirus	Mayaro virus		Mosquito (Haemagogus sp.)

Usutu virus

- USUV was first identified in Culex mosquitoes in South Africa in 1959, (Usutu River) and has caused outbreaks in birds across Europe since 1996
- The first human case outside Africa was reported in Italy in 2009, where an immunocompromised patient was infected, causing encephalitis.
- Based on NS5: 8 genetic lineage(1-3 Africa and 1-5 Europe)

Usutu virus

Since 2009, both systems recorded the circulation of Usutu virus (USUV), a flavivirus closely related to WNV with a not yet defined pathogenic capacity

Vectors: mosquitoes especially *Culex* (*Culex pipiens*)

Reservoirs: wild birds

Possible mass mortality in birds (blackbird)(Austria 2001).

WNV differential diagnosis (it can be cross-reactive also in PCR)

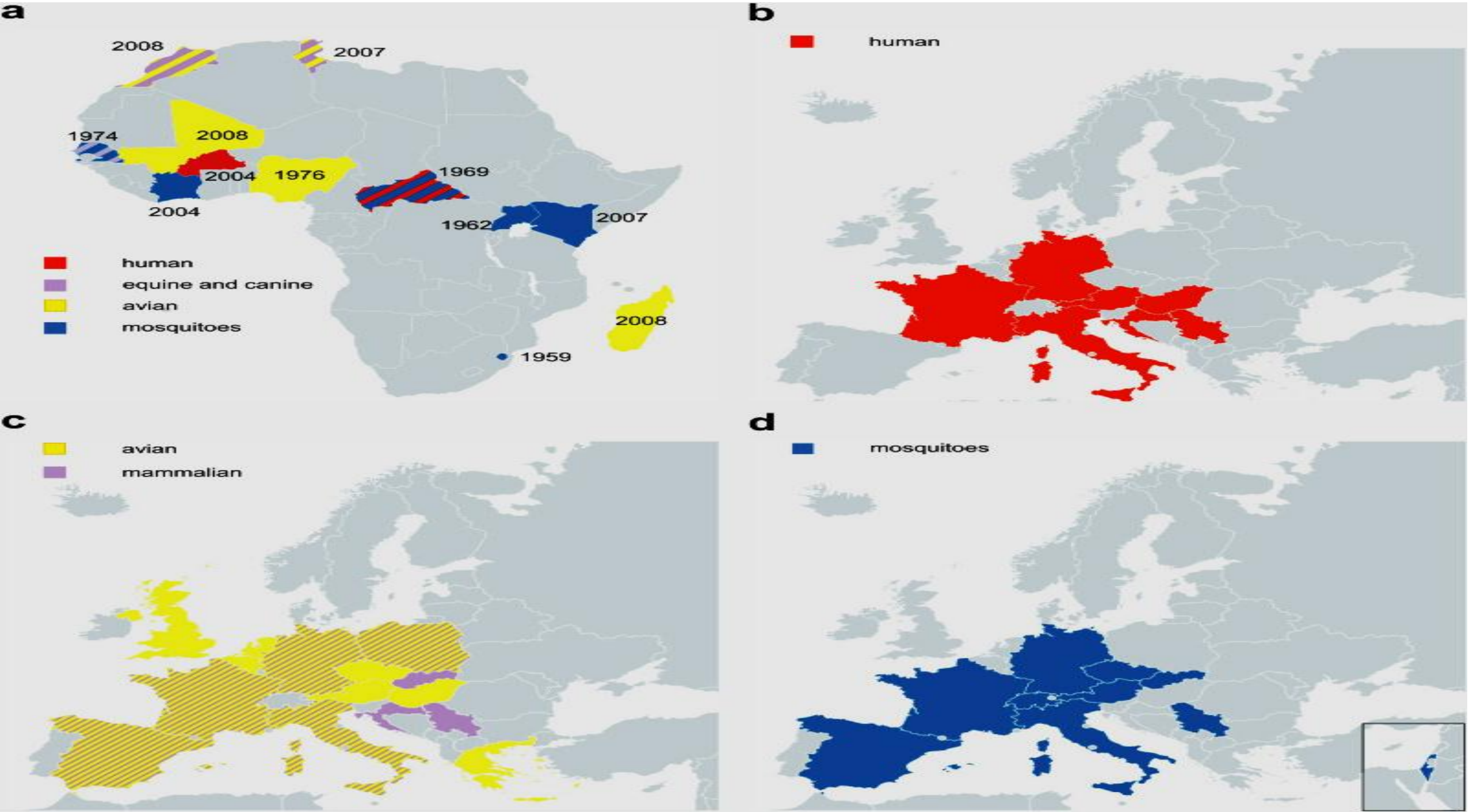
Human disease: 2 cases of encephalitis reported in immunosuppressed individuals in Italy in 2009 (Pecorari et al. 2009, Cavrini et al. 2009)

Anti-USUV response in healthy persons were recorded in ER, 4/359 in 2009; 14/6000 in 2010-11 (Gaibani et al. 2012, Pierro et al. 2013)

Usutu virus

- Two cases of symptomatic infection in humans have been reported from Africa, with fever and skin rash but no neurological symptoms.
- Skin rash, fever, jaundice, thrombotic thrombocytopenic purpura, hepatitis
- In Europe, 46 infections were detected up to 2019; these were mainly asymptomatic, but neurological symptoms including encephalitis and meningoencephalitis have been observed.

- The virus is highly pathogenic in birds, causing central nervous system symptoms, enlargement of the liver and spleen, and infiltration by inflammatory cells in a wide range of organs


a
2008
2007
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2008
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1969
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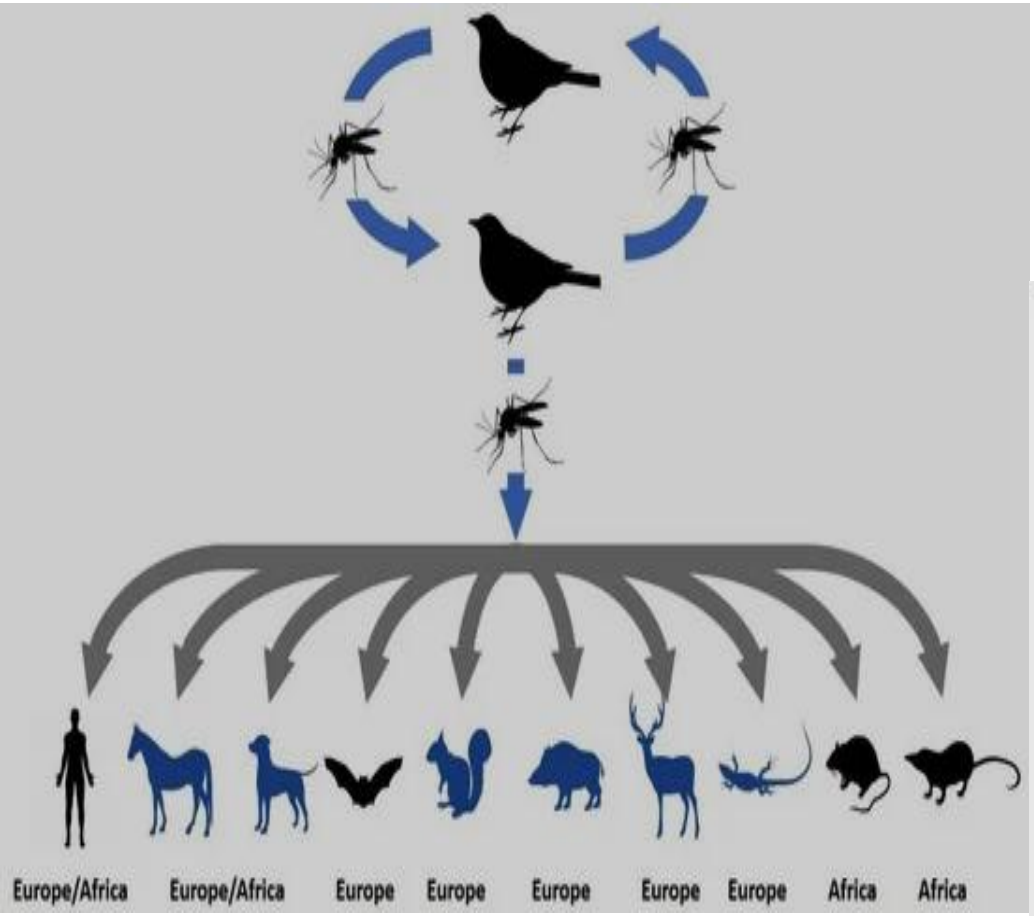
mosquitoes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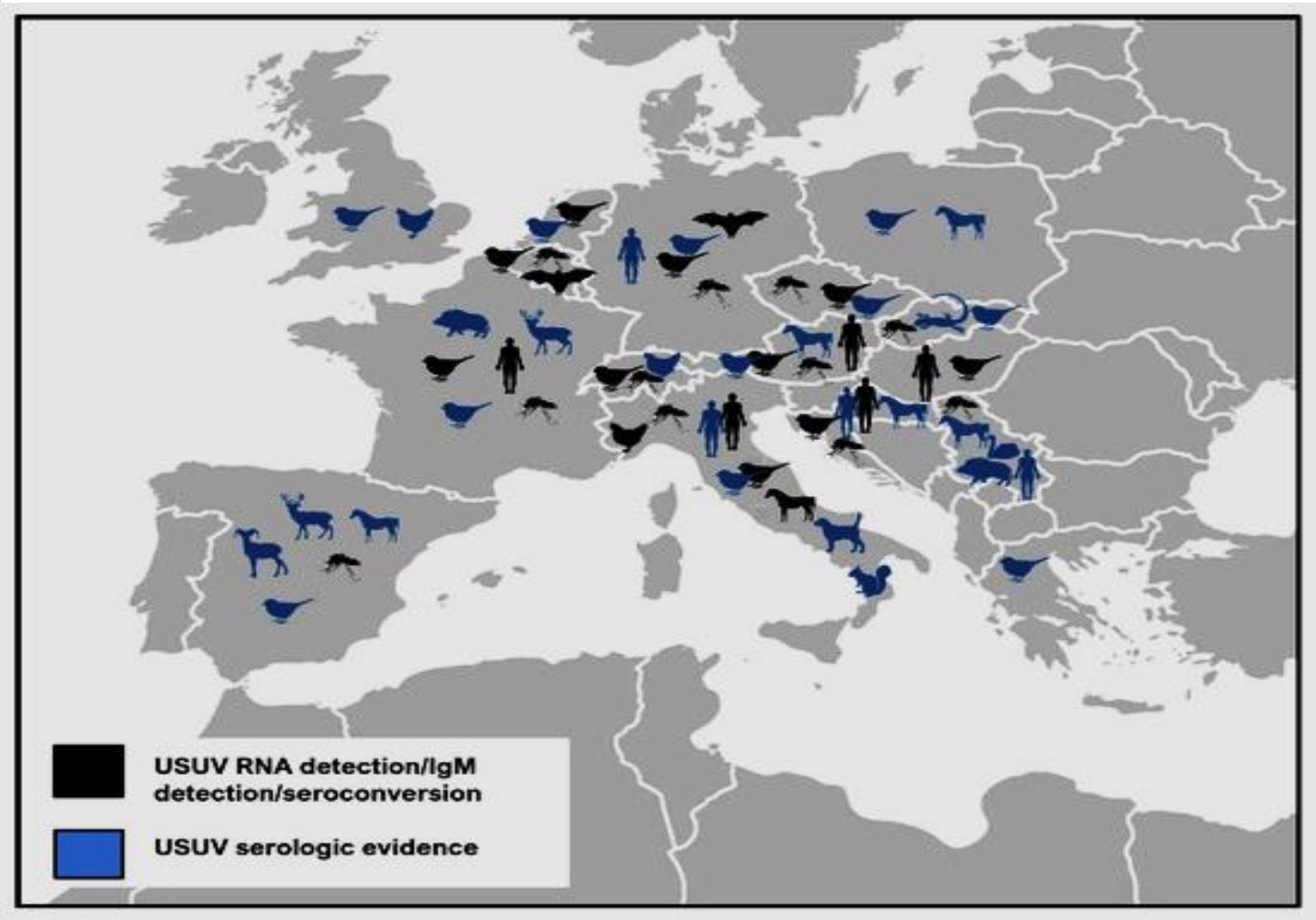

USUV RNA detection/IgM detection/seroconversion
USUV serologic evidence

DOI: 10.1002/vms3.342

ORIGINAL ARTICLE

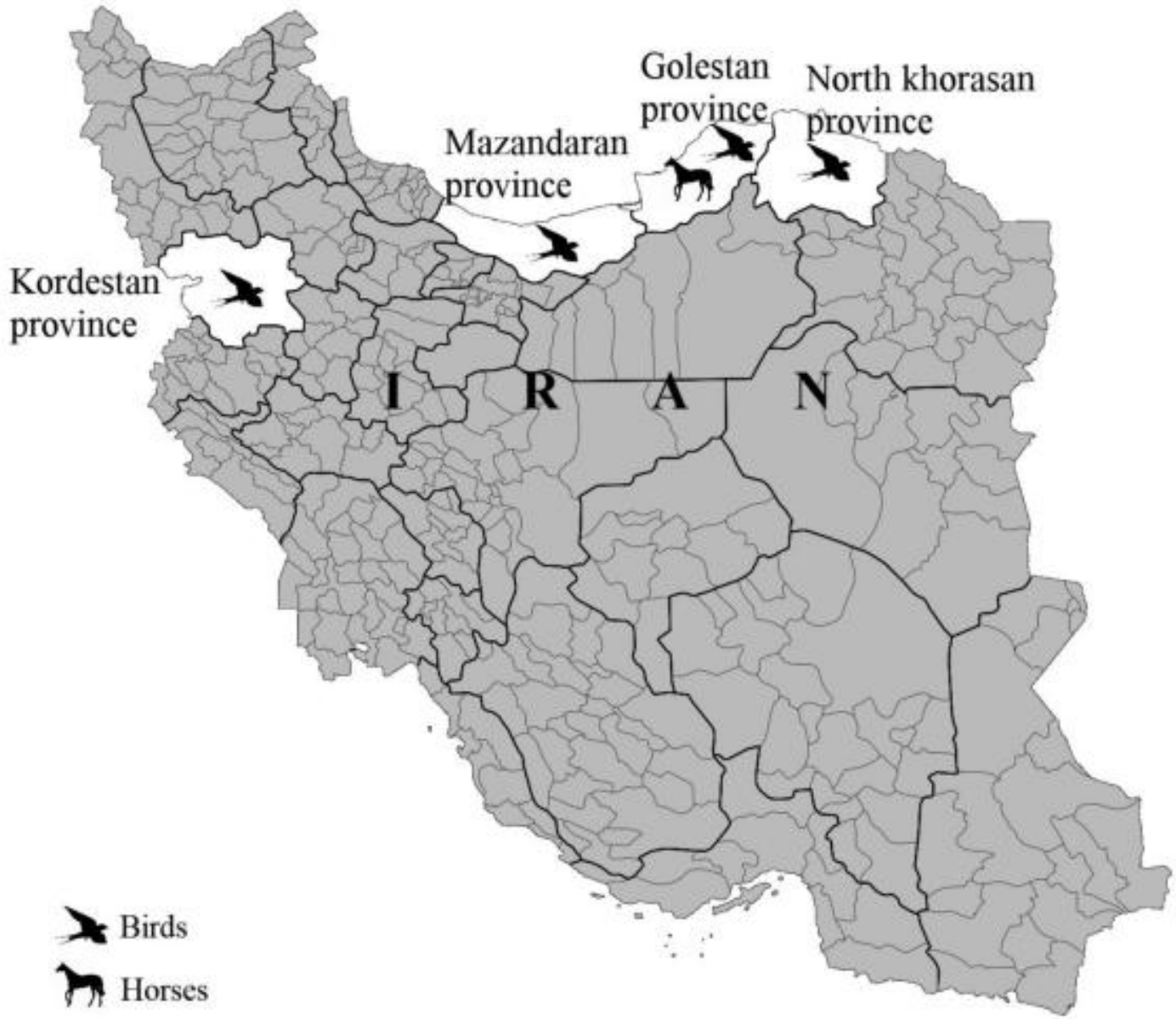
WILEY

Serological evidence of West Nile virus infection among birds and horses in some geographical locations of Iran

Hasan Bakhshi[1] | Cécile Beck[2] | Sylvie Lecollinet[2] | Maëlle Monier[2] | Laurence Mousson[3] | Sedigheh Zakeri[1] | Abbasali Raz[1] | Kourosh Arzamani[4] | Leila Nourani[1] | Navid Dinparast-Djadid[1] | Anna-Bella Failloux[3]

Abstract

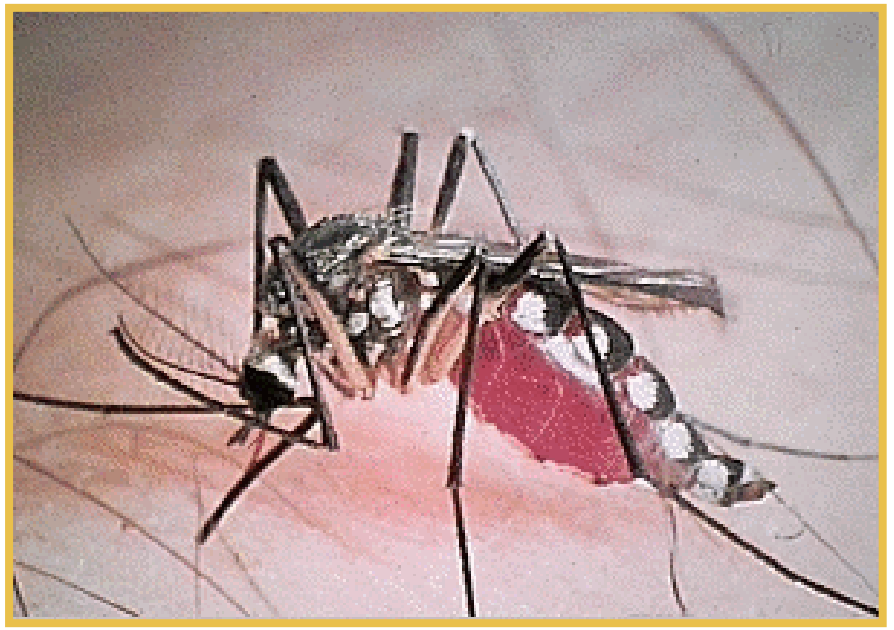
Recent expansion of arboviruses such as West Nile (WNV), Usutu (USUV), and tick-borne encephalitis (TBEV) over their natural range of distribution needs strengthening their surveillance. As common viral vertebrate hosts, birds and horses deserve special attention with routine serological surveillance. Here, we estimated the seroprevalence of WNV, USUV and TBEV in 160 migrating/resident birds and 60 horses sampled in Mazandaran, Golestan, North Khorasan, Kordestan provinces and Golestan province of Iran respectively. ELISA results showed that of 220 collected samples, 32 samples (14.54%), including 22 birds and 10 horses, were positive. Microsphere immunoassay results showed that 16.7% (10/60) of horse blood samples collected in Golestan province were seropositive against WNV (7; 11.7%), *Flavivirus* (2; 3.3%) and seropositive for USUV or WNV (1; 1.7%). Furthermore, micro virus neutralization tests revealed that four of seven ELISA-positive bird blood samples were seropositive against WNV: two Egyptian vultures, and one long-legged buzzard collected in Golestan province as well as a golden eagle collected in North Khorasan province. No evidence of seropositivity with TBEV was observed in collected samples. We showed that WNV, responsible for neuroinvasive infection in vertebrates, is circulating among birds and horses in Iran, recommending a sustained surveillance of viral infections in animals, and anticipating future infections in humans.



Provinces	ELISA results
Kordestan	0/24 (0%)
Mazandaran	15/99 (15.1%)
Golestan	5/27 (18.5%) 10/60 (16.7%)
North Khorasan	2/10 (20%)

Mosquito Management

- Surveillance
- Source reduction
- Personal protection
- Biological control
- Larvicide
- Adulticide



Surveillance

- Dead bird testing
- Sentinel chicken flocks
- Mosquito collection
  - Test for pathogens
  - Account for species
- Larval and adult mosquitoes
  - Map habitats
  - Record keeping

Immunohistochemistry (IHC), ELISA, PCR for detection

Source Reduction

- Eliminating larval habitats
  - Tires, bird baths, containers, rain gutters, unused swimming pools

Source Reduction

- Use repellents and wear long trousers and long-sleeved shirts when outdoors;
- Use mosquito nets on the windows;
- Frequently empty flowerpots or other containers (e.g., buckets) with stagnant water;
- Change water bowls for animals frequently;
- Keep paddling pools in an upright position when not in use.

Bird surveillance

Active surveillance on sinathropic species (target of control plans), particularly corvids:

- European Magpie (*Pica pica*)
- Hooded Crow (*Corvus cornix*)
- Eurasian Jay (*Garrulus glandarius*)

Passive surveillance: birds found dead in the field or dead in Wildlife Rehabilitation Centres

Magpie

Jay

Virus Family	Viral Genus	Virus		Vector Species
Bunyaviridae	Orthobunyavirus	California serogroup viruses		Mosquito (Aedes sp.)
	Phlebovirus	Rift Valley Fever virus		Mosquito (various)
	Phlebovirus	Toscana virus		Sandfly (Phelbotomus sp.)
	Phlebovirus	Phlebotomus fever virus		Sandfly (phelbotomus)
	Phlebovirus	Sandfly Fever Naples virus		Sandfly (phelbotomus)
	Phlebovirus	Sandfly Fever Sicilian virus		Sandfly (phelbotomus)
	Phlebovirus	Heartland virus		Tick (A. americanum)
	Phlebovirus	Severe fever with thrombocytopenia syndrome virus		Tick (H. longicornis)
	Nairovirus	Crimean Hemorrhagic Fever virus		Tick (Hyalomma sp.)
Flaviviridae	Flavivirus	Dengue Virus		Mosquito (Aedes sp.)
	Flavivirus	Zika virus		Mosquito (Aedes sp.)
	Flavivirus	Yellow fever virus		Mosquito (Aedes sp.)
	Flavivirus	West Nile Virus		Mosquito (Culex sp.)
	Flavivirus	St. Louis Encephalitis virus		Mosquito (Culex sp.)
	Flavivirus	Japanese encephalitis virus		Mosquito (Culex sp.)
	Flavivirus	Murray Valley encephalitis virus		Mosquito (Culex sp.)
	Flavivirus	Usutu		Mosquito (various)
	Flavivirus	Omsk Hemorrhagic fever virus		Tick (dermacentor)
	Flavivirus	Kyasanur Forest Diesease virus		Tick (Haemaphysalis sp.)
→	Flavivirus	Tick-borne encephalitis virus		Tick (Ixodes and Haemaphysalis sp.)
	Flavivirus	Powassan virus		Tick (Ixodes sp.)
Orthomyxoviridae	Thogotovirus	Bourbon virus		Tick (A. americanum)
Reoviridae	Coltivirus	Colorado tick fever		Tick (dermacentor)
Rhabdoviridae	Vesiculovirus	Vesicular Stomatitis (New Jersey) virus		Sandflies (Lutz. Sp) | Mosquitos (various)
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	Alphavirus	Equine encephalitis virus		Mosquito (Culex sp.)
	Alphavirus	Mayaro virus		Mosquito (Haemagogus sp.)

INFECTIOUS AGENT

- Tick-borne encephalitis, or TBE, is a human viral infectious disease involving the central nervous system.
- TBE is caused by the tick-borne encephalitis virus (TBEV), a member of the family *Flaviviridae* (single-stranded RNA virus), *and was initially isolated in 1937.*

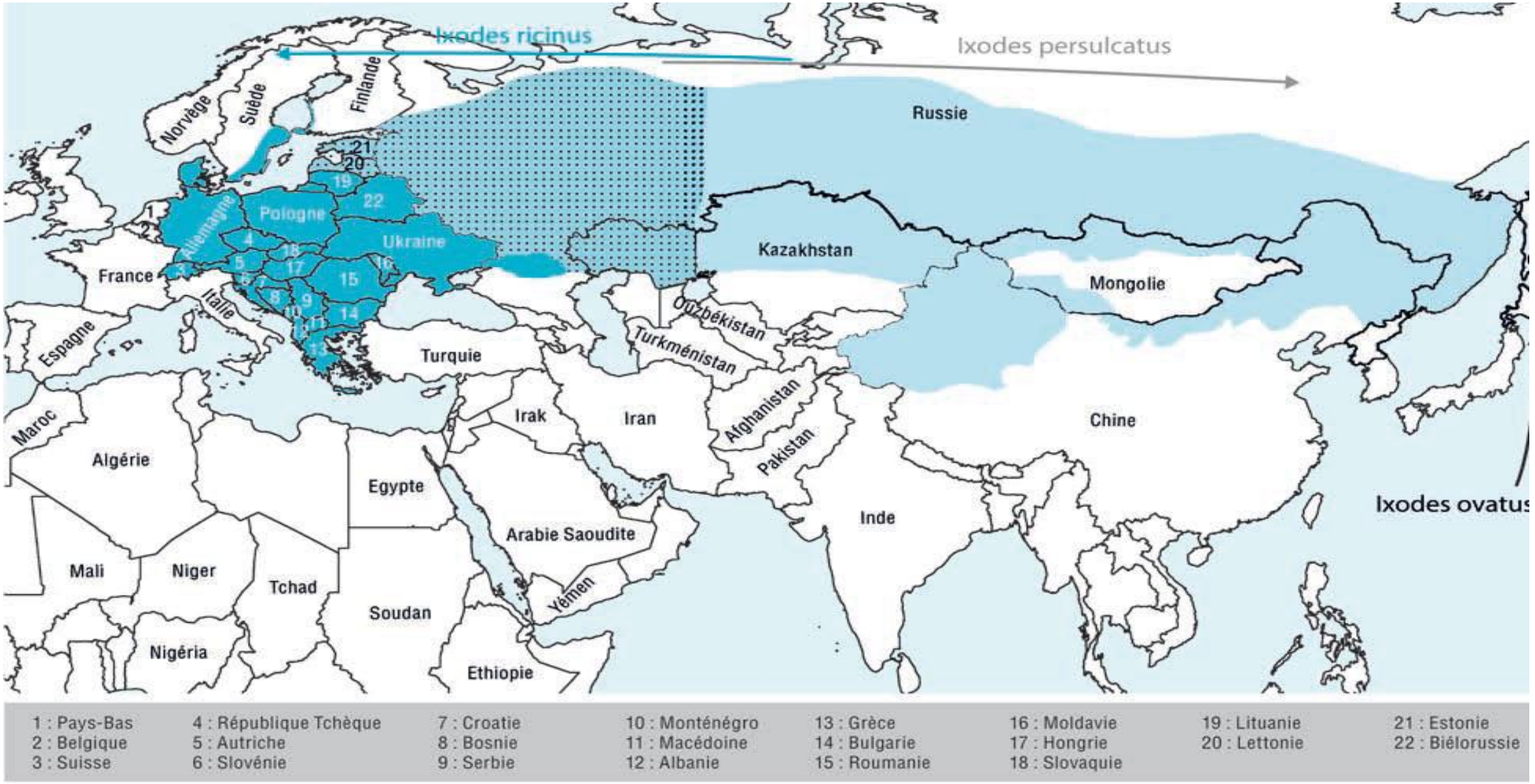
TBEV has 3 subtypes:

1. European or Western tick-borne encephalitis virus
2. Siberian tick-borne encephalitis virus
3. Far Eastern Tick-borne encephalitis virus (formerly known as Russian Spring Summer encephalitis virus, RSSEV)

- European TBE is mainly transmitted by *Ixodes ricinus*,
- Siberian and Far Eastern viruses are transmitted mainly by *I. persulcatus*.

EPIDEMIOLOGY

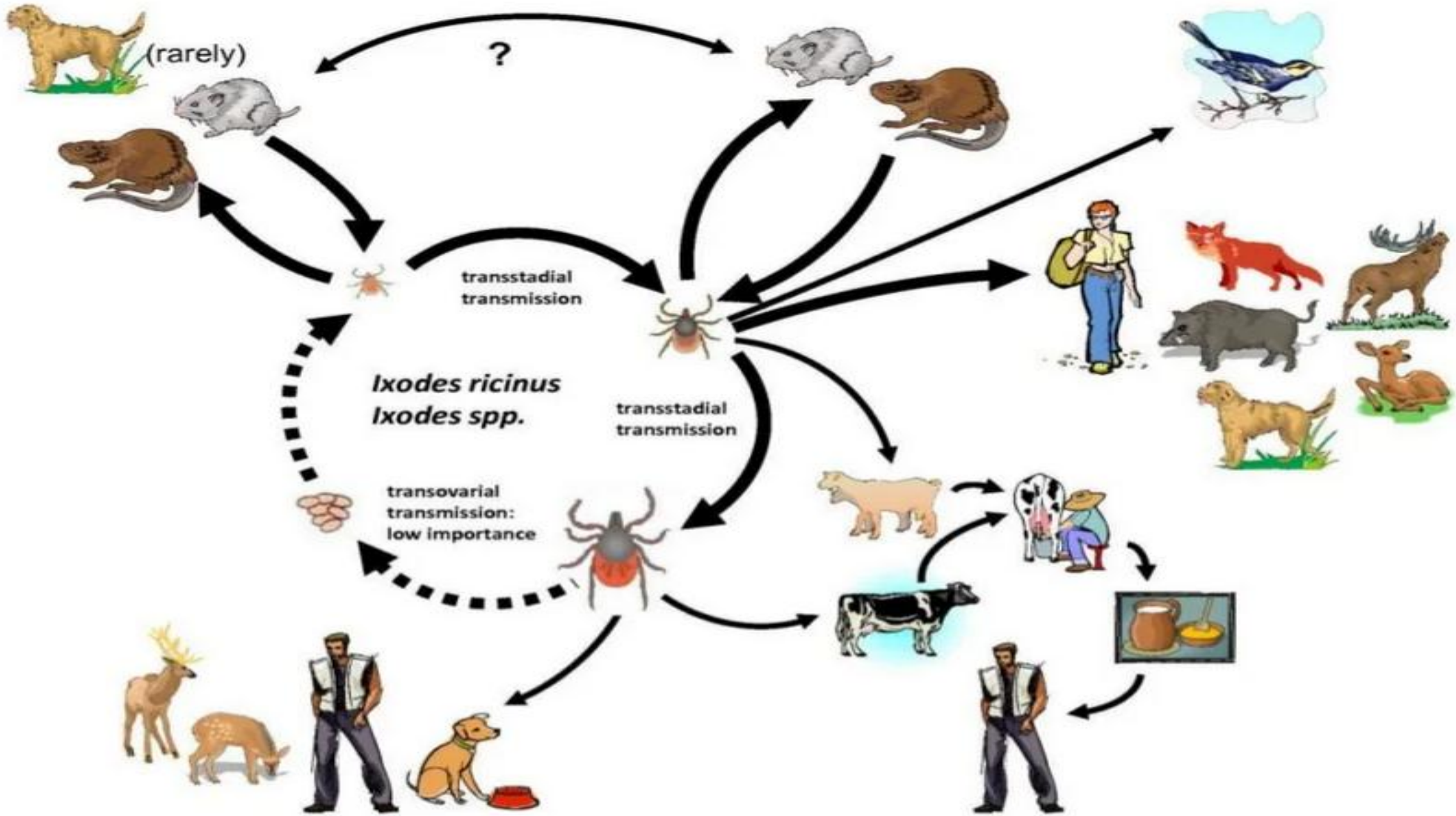
- TBE is endemic in focal areas of Europe and Asia (from eastern France to northern Japan and from northern Russia to Albania).
- From 1990 through 2009, an average of 8,500 cases per year (range, 5,352–12,733 cases) were reported from 19 European countries,
- Russia has the largest number of reported TBE cases, and western Siberia has the highest incidence of TBE in the world.
- Most cases occur from April through November
- incidence and severity of disease are highest in people aged ≥50 years

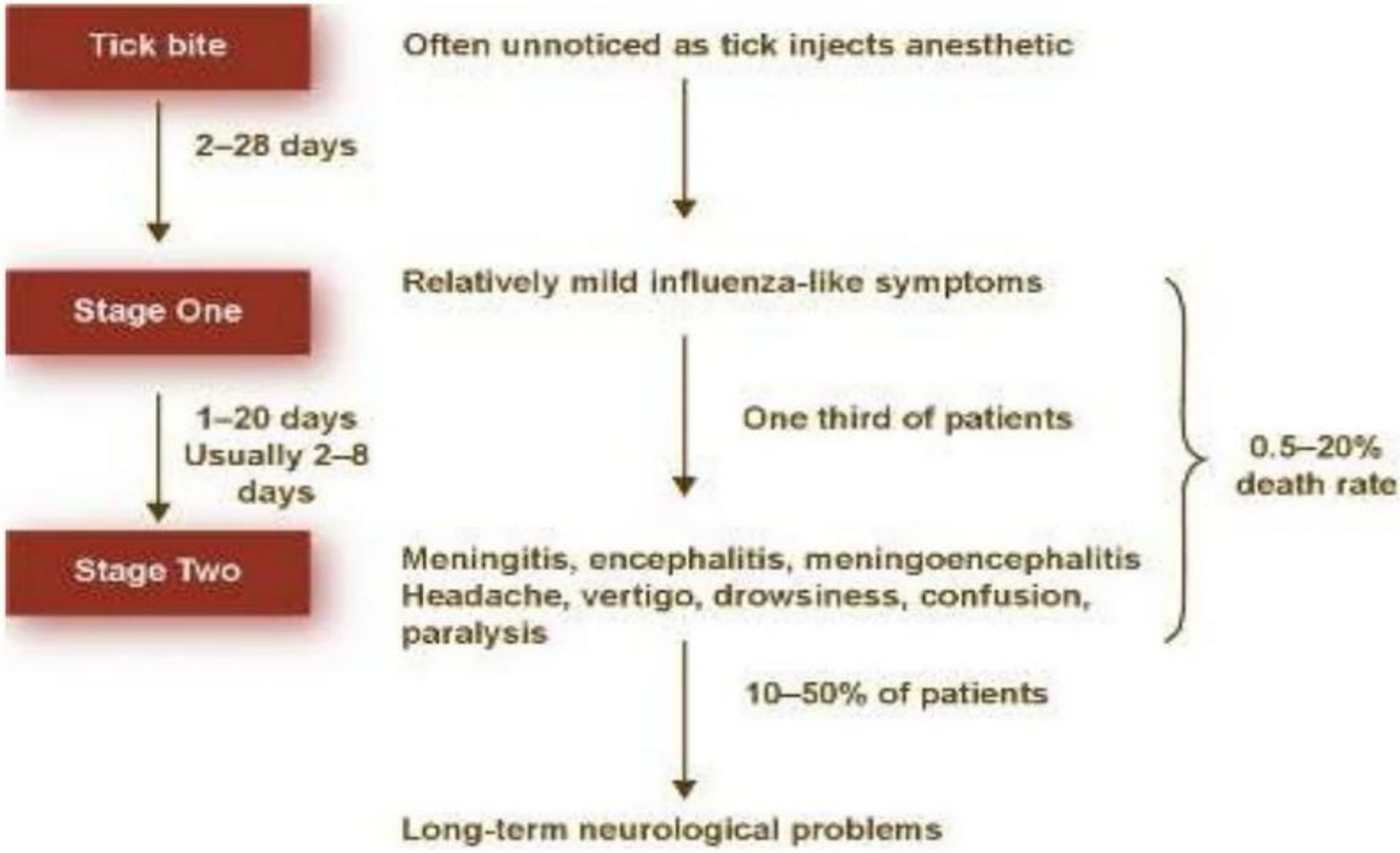


Countries with an Eastern subtype of tick-borne encephalitis are marked in light blue, countries with a Western subtype are marked in blue, and countries where the subtypes co-exist are marked in speckled blue.

Transmission

- Ticks, specifically hard ticks of the family *Ixodidae, act as both the vector and reservoir for TBEV.*
- *The main hosts are small rodents, with humans being accidental hosts.*
- *Large animals serve as feeding hosts for the ticks, but do not play a role in maintenance of the virus.*
- The virus can chronically infect ticks and is transmitted both transtadially and transovarially.


(rarely)
?
transstadial
transmission
Ixodes ricinus
Ixodes spp.
transstadial
transmission
transovarial
transmission:
low importance

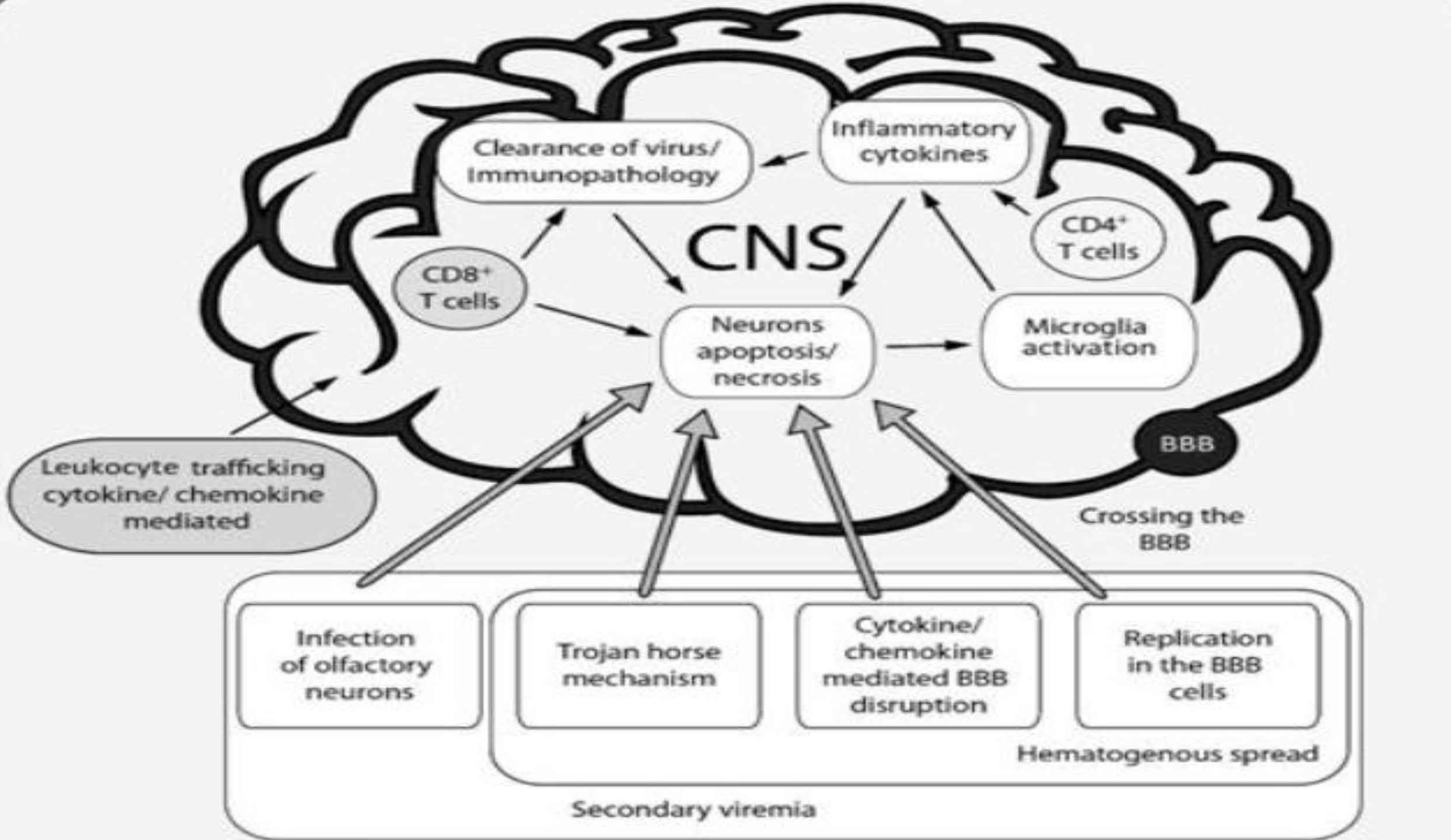

Tick bite
Often unnoticed as tick injects anesthetic
2–28 days
Stage One
Relatively mild influenza-like symptoms
One third of patients
1–20 days
Usually 2–8
days
Stage Two
Meningitis, encephalitis, meningoencephalitis
Headache, vertigo, drowsiness, confusion,
paralysis
0.5–20%
death rate
10–50% of patients
Long-term neurological problems

Signs and Symptoms

- The incubation period of TBE is usually between 7 and 14 days and 2/3rd is asymptomatic.
- Shorter incubation times have been reported after milk-borne exposure.

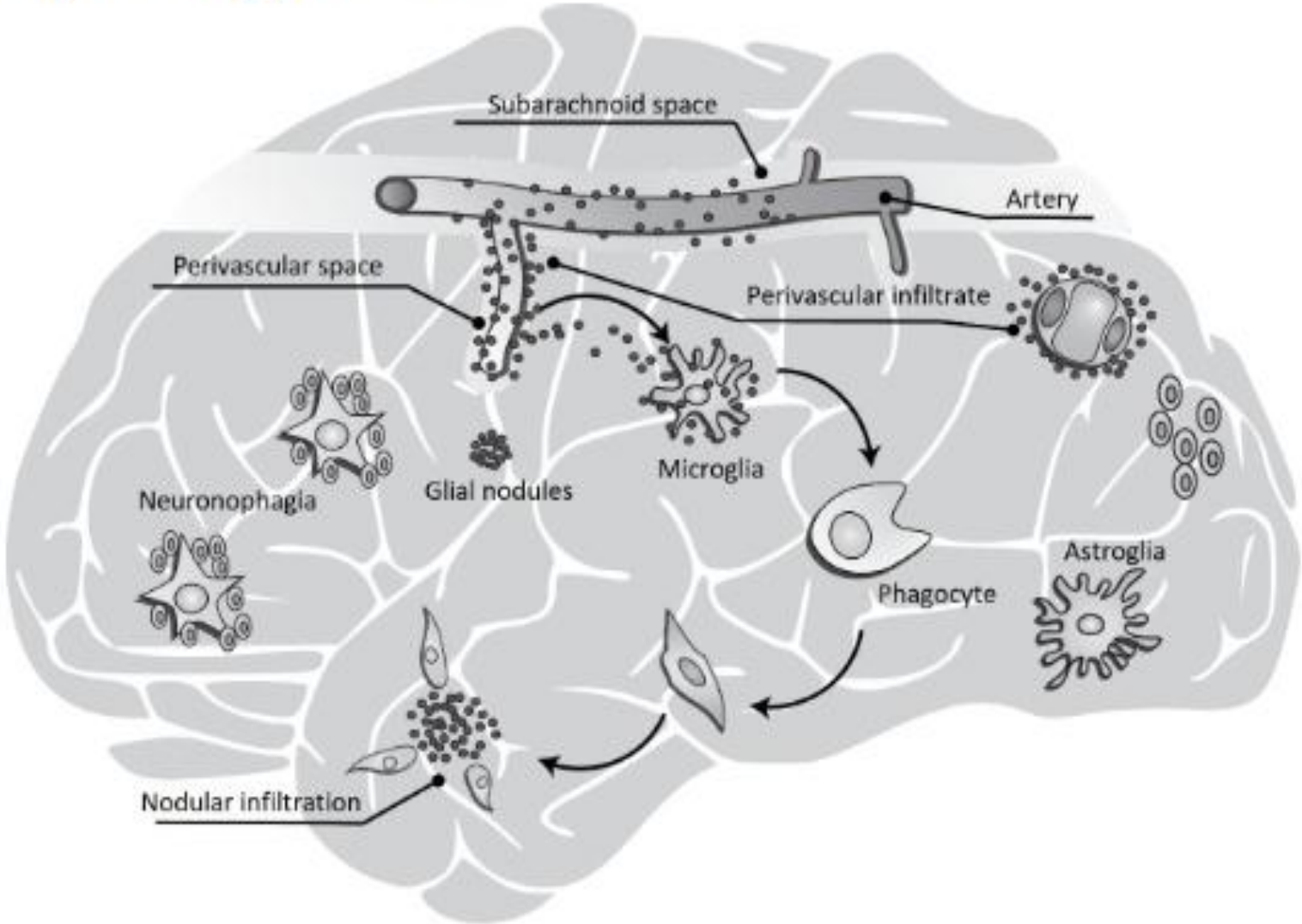
After a new phase of replication in DC, macrophages and, possibly, T lymphocytes, viremia occurs and the virus spreads to other organs, in particular the reticuloendothelial system (spleen, liver and thymus). High virus titers are necessary for the virus to pass through the blood–brain barrier.
The cytotoxic T-lymphocyte response is essential for virus clearance but may also lead to immune pathogenesis by targeting infected neurons. In human lethal cases, meningitis and encephalomyelitis are localized in the spinal cord, brainstem and cerebellum and are associated with inflammatory and lymphocytic cell infiltrations.

- **First phase**: nonspecific febrile illness with headache, myalgia, and fatigue.
  - Usually lasts for several days and may be followed by an afebrile and relatively asymptomatic period.
  - two-thirds of patients may recover without any further illness.
- **Second phase**: central nervous system involvement resulting in aseptic meningitis, encephalitis, or myelitis. Findings include meningeal signs, altered mental status, cognitive dysfunction, ataxia, tremors, cranial nerve palsies, and limb paresis.

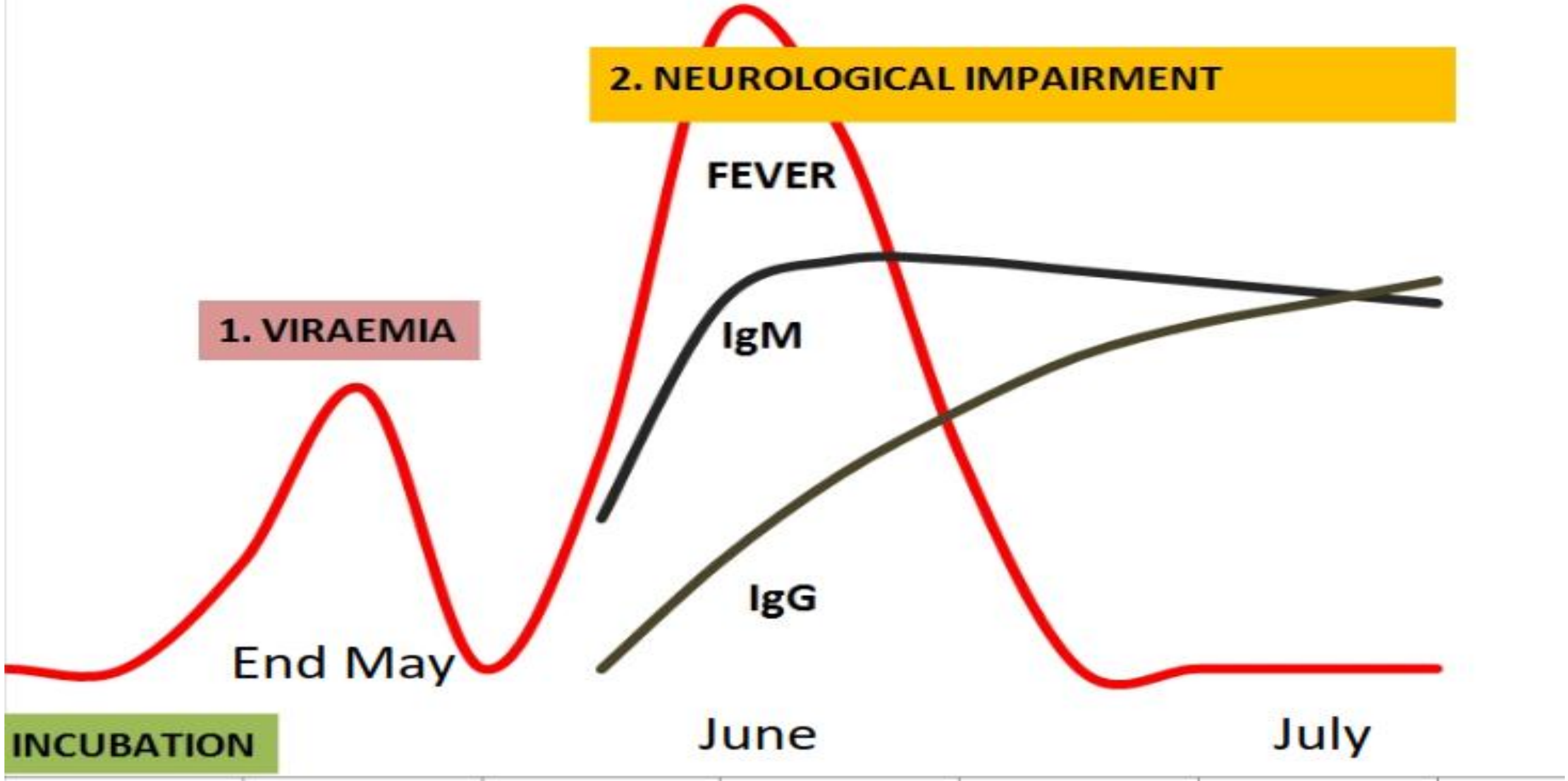

Clearance of virus/
Immunopathology
Inflammatory
cytokines
CNS
CD8+
T cells
CD4+
T cells
Neurons
apoptosis/
necrosis
Microglia
activation
BBB
Leukocyte trafficking
cytokine/ chemokine
mediated
Crossing the
BBB
Infection
of olfactory
neurons
Trojan horse
mechanism
Cytokine/
chemokine
mediated BBB
disruption
Replication
in the BBB
cells
Hematogenous spread
Secondary viremia

Astrocytes and microglia - production of TNF-a, IFN-a, IL-1b, IL-6, IL-8, IL-12, IFN-g, IP-10

Neuronophagia
Endothelial activation
Perivascular infiltrates
Activated microglia
Histiocyte nodules
Cytotoxic T cell infiltration



BIPHASIC COURSE OF TICK-BORNE ENCEPHALITIS



The Seroprevalence of Tick-Borne Encephalitis in Rural Population of Mazandaran Province, Northern Iran (2018 - 2019)

Mostafa Salehi-Vaziri [1, 2], Mohammad Hassan Pouriayevali [2], Sanam Azad-Manjiri [2], Abbas Ahmadi Vasmehjani [3], Vahid Baniasadi [2] and Mehdi Fazlalipour [2, *]

[1] Research Centre for Emerging and Reemerging Infectious Diseases, Pasteur Institute of Iran, Tehran, Iran
[2] Department of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory), Pasteur Institute of Iran, Tehran, Iran
[3] Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[*] *Corresponding author*: Department of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory), Pasteur Institute of Iran, Tehran, Iran. Email: mfp.virology@gmail.com

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Abstract

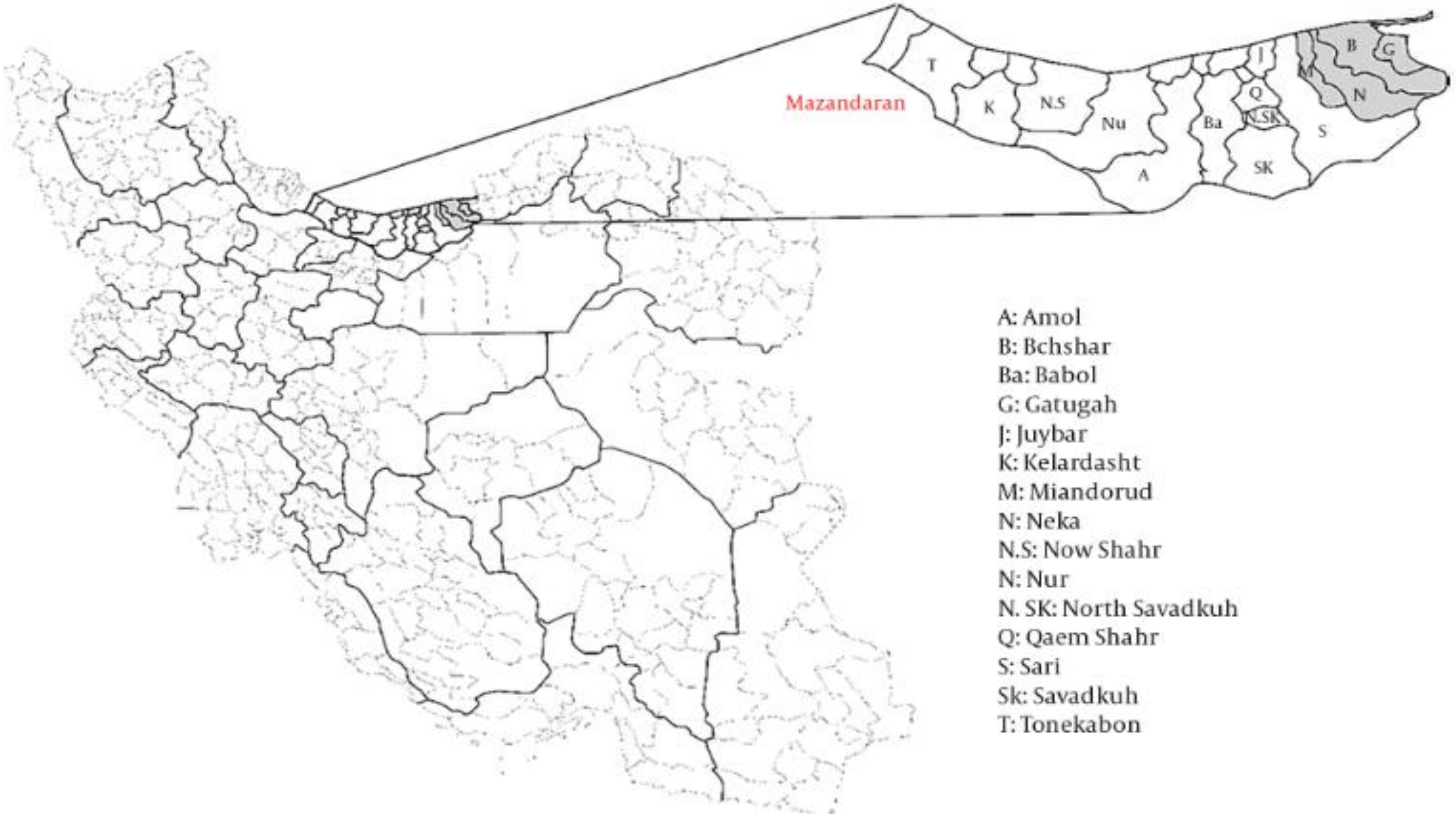
Background: Tick-borne encephalitis virus (TBEV) is a neurotropic tick-borne virus circulating in vast geographical areas of the northern hemisphere. Although the presence of TBEV vector has been documented in north Iran, there is no information about the circulation of TBEV in the country.

Objectives: This study was designed in order to determinate the TBE serologic profile in the Mazandaran Province of Iran.

Methods: In this cross-sectional seroepidemiologic study, from September to November 2018, 448 serum samples collected (by random sampling method) from general population residing in rural areas of Behshar, Galugah, Miandorud and Neka cities were evaluated for presence of anti TBEV IgG antibodies using a commercial Anti-TBE Virus ELISA (IgG) kit.

Results: Sixteen (3.6%) samples were detected as reactive, in which 43.8% had a history of tick bite or tick squish. The highest seropositivity was observed in farming/animal industry-related jobs (33.3%) or were housewives (33.3%). All reactive cases reported a history of consuming local unpasteurized dairy.

Conclusions: This study provides first evidence on the circulation of TBEV in Northern Iran, where climatic conditions, presence



A: Amol
B: Bchshar
Ba: Babol
G: Gatugah
J: Juybar
K: Kelardasht
M: Miandorud
N: Neka
N.S: Now Shahr
N: Nur
N. SK: North Savadkuh
Q: Qaem Shahr
S: Sari
Sk: Savadkuh
T: Tonekabon

PREVENTION

- Travelers should avoid consuming unpasteurized dairy products
- using insect repellents and protective clothing to prevent tick bites.
- The chemical DEET (diethyltoluamide) is often used in insect repellents
- Wear light-coloured clothes so ticks are easier to spot and brush off

Prevention

- Five commercial inactivated vaccines
- In Europe, vaccines contain one European strain
- In Russia, they contain Far Eastern strains
- In China the vaccine contains a Far Eastern strain
- The virus is propagated in cell culture (e.g. primary embryo fibroblasts), inactivated in formalin or formaldehyde, and the purified antigens are adjuvanted with aluminium hydroxide.
- Naive patients need three to four vaccine injections to be fully immunized and vaccination needs then to be repeated every three to five years

Thank you for your attention!!!