EXOTIC VECTOR-BORNE VIRAL ZOONOSES - A THREAT FOR DISEASES-FREE COUNTRIES?

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Many viruses have co-evolved with animals and people on a specified geographic area over long period of time.

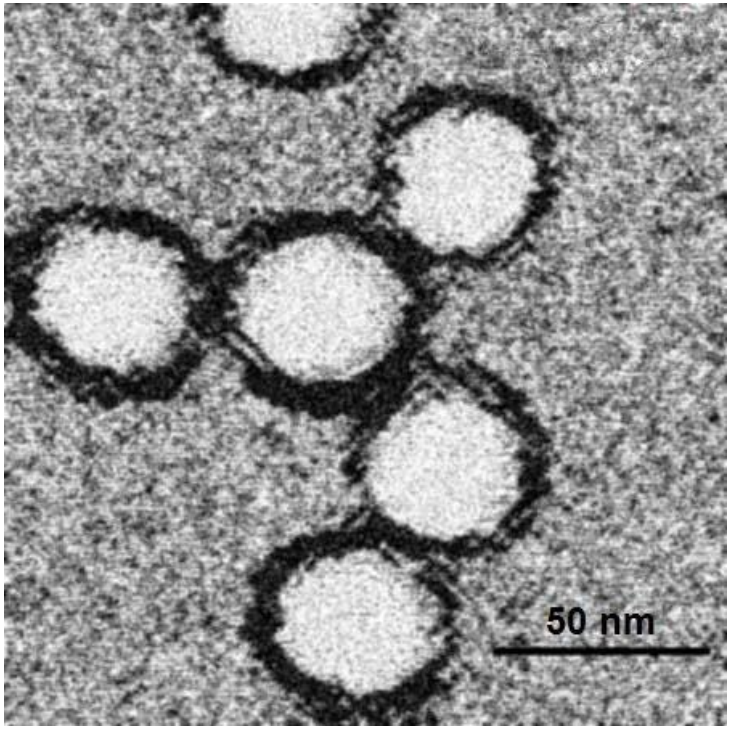
Latterly, many of them have entered new territory, due to movement of viremic hosts (both intentionally- e.g., trade and naturally – e.g., migration), movement of virus-carrying vectors, changes in the environment, climate, and agriculture.

Certain viruses pose threat to health and life of humans or animals.

West Nile Virus, Crimean- Congo Hemorrhagic Fever Virus and Rift Valley Fever Virus infections belong to the one of the most dangerous exotic vector-borne viral diseases.

The goal of the study was the assessment of the epidemiological situation of WNV, CCHFV and RVFV infections in Poland.

WEST NILE VIRUS (WNV)



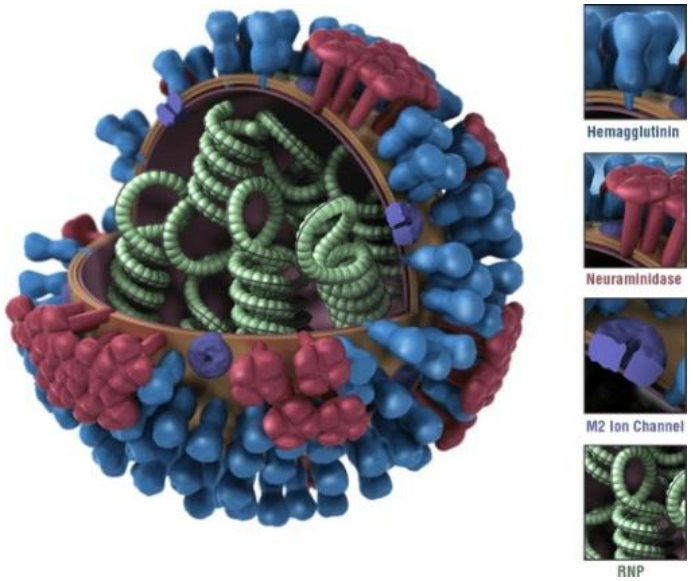
Virus classification and morphology

West Nile virus (WNV) - a mosquito-borne zoonotic arbovirus

Order: ***Unassigned***
Family: ***Flaviviridae***
Genus: ***Flavivirus***
Species: ***West Nile virus***

First isolation- West Nile district of Uganda (1937) – blood a woman suffering from a mild febrile illness

enveloped, 45 nm in diameter,
linear single-stranded RNA+ ((+)ssRNA)



source:http://imgbucket.com/pages/w/west-nile-virus-structure-and-shape/

Vector and animal hosts

Mosquitoes of the genus *Culex* (*Cx. Pipiens)* - the principal vectors of WNV.

In mosquito populations - vertical transmission (adults to eggs).

Source:http://www.medicinenet.com/west_nile_encephalitis/article.htm

Vector and animal hosts

Birds - the reservoir hosts of WNV.

In Europe, Africa, Middle East and Asia, mortality in birds associated with WNV infection - rare.

In the Americas - highly pathogenic for birds.

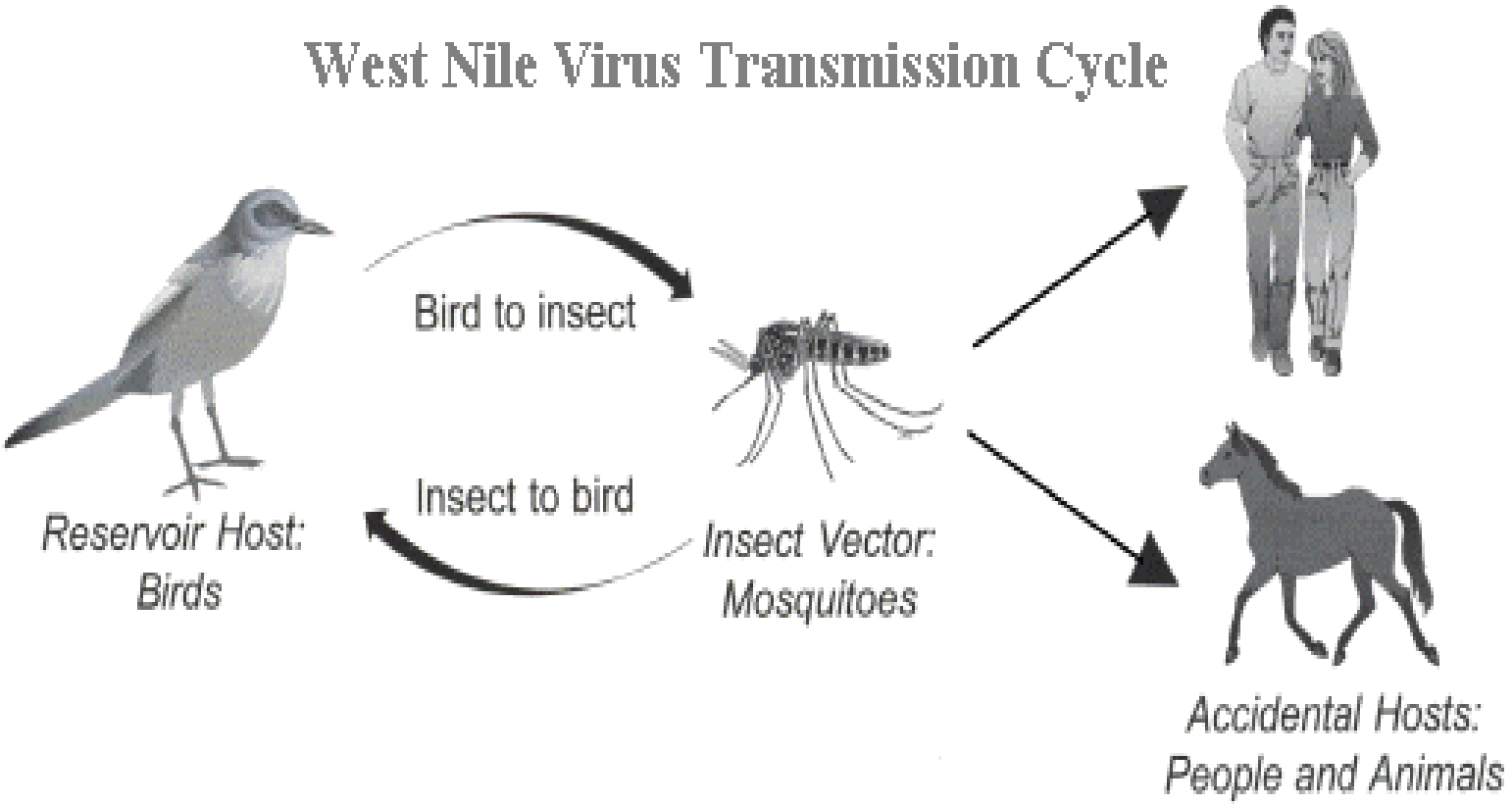
Members of the crow family (*Corvidae*) are particularly susceptible, but virus has been detected in dead and dying birds of more than 250 species.

Vector and animal hosts

Horses (and humans) are “dead-end” hosts - while they become infected, they do not spread the infection.

Symptomatic infections in horses are rare and generally mild, but can cause neurologic disease, including fatal encephalomyelitis.

Transsmision cycle



Source: http://www.bcmvcd.com/viruses.html

Clinical symptoms in people

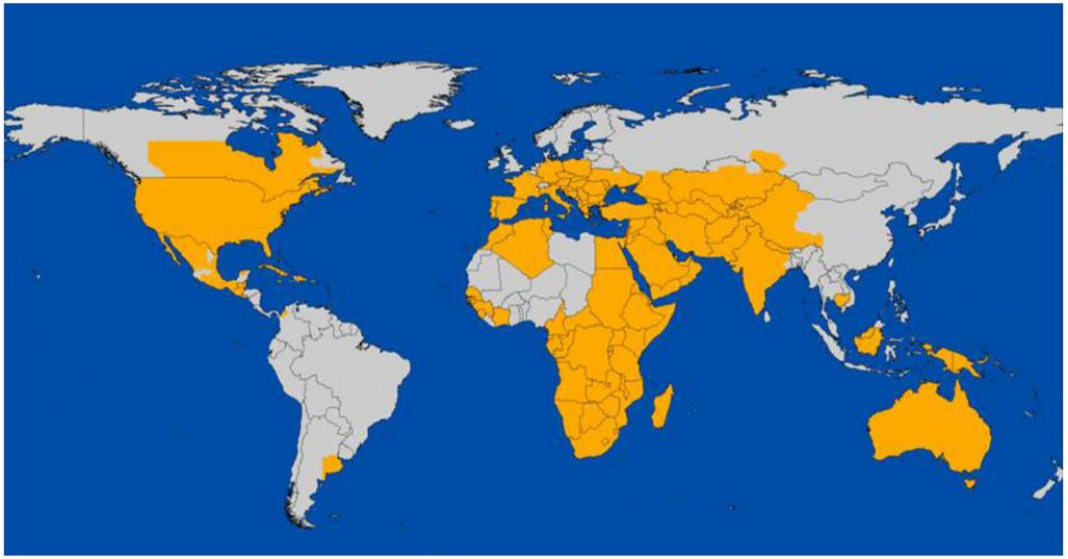
Most have no signs or symptoms

Mild infection signs and symptoms (about 20% of people) : fever, headache, body aches, fatigue, back pain (occasionally: skin rash, swollen lymph glands, eye pain)

Serious infection signs and symptoms (less than 1% of infected people) - a serious neurological infection (encephalitis, meningoencephalitis, meningitis, West Nile poliomyelitis); high fever, severe headache , stiff neck , disorientation or confusion, stupor or coma, tremors or muscle jerking , lack of coordination, convulsions , pain, partial paralysis or sudden muscle weakness

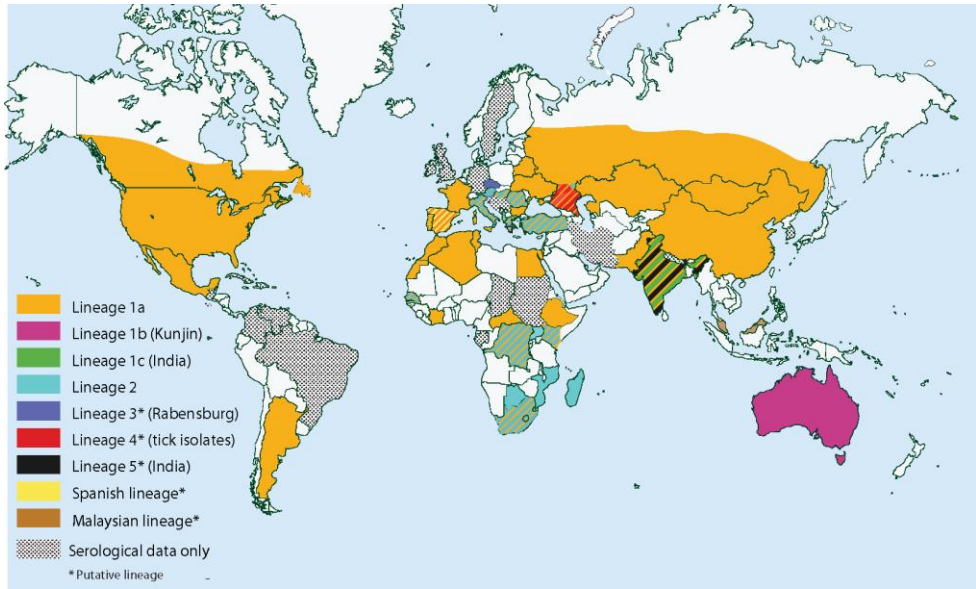
Signs and symptoms of West Nile fever usually last a few days, but signs and symptoms of encephalitis or meningitis can linger for weeks, and certain neurological effects, such as muscle weakness, may be permanent.

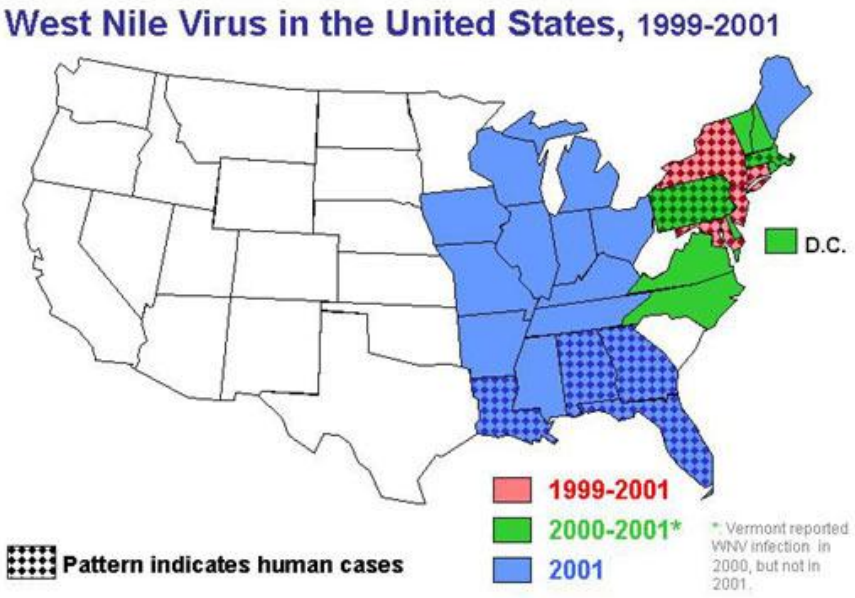
Global distribution of West Nile virus

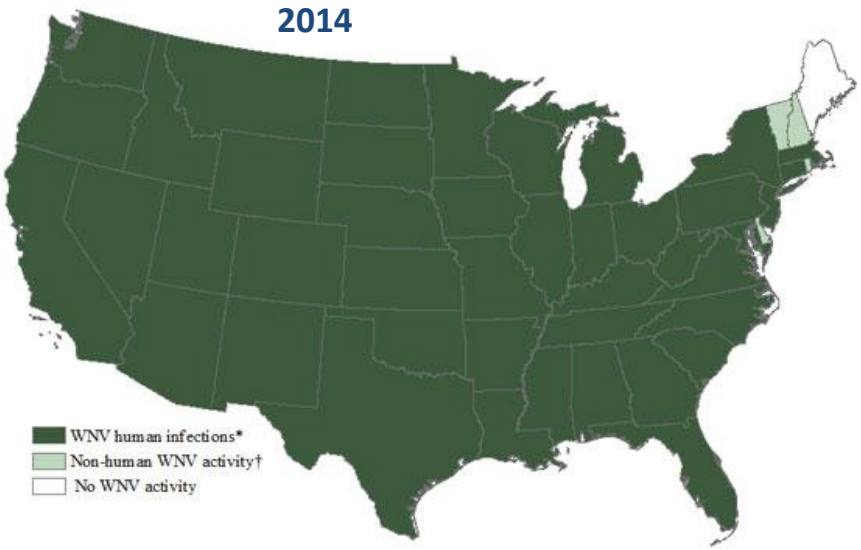


the US Centers for Disease Control and Prevention, 2006

Vector-Virus Interactions and Transmission Dynamics of West Nile Virus. Ciota and Kramer, 2013





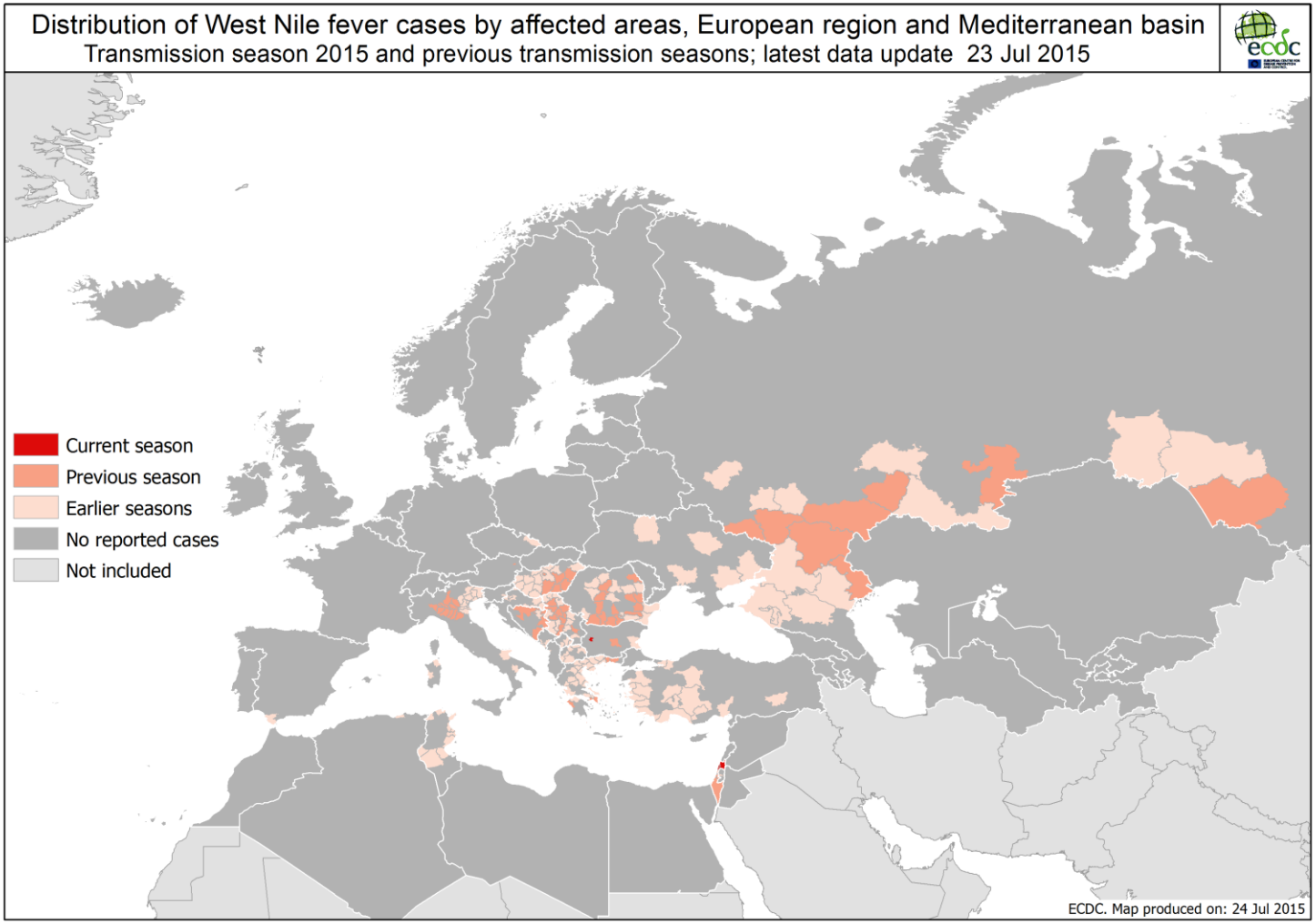


http://www.microbiologybook.org/mhunt/arbo.htm

The first case in the Western Hemisphere: New York City in 1999;

over the next five years, the virus spread across the continental United States,

north into Canada, and southward into the Caribbean islands and Latin America



Source: the ECDC (European Centre for Disease Prevention and Control)

WNV IN POLAND

In the nineties of the last century, Juricova et al. confirmed WNV antibodies in 12.1% of house sparrows (*Passer domesticus)* and in 2.8% of Eurasian tree sparrows (*Passer montanus)* in Campinas forest area in Poland.

In the next few years, WNV antibodies were found in three storks, one crow (*Corvus corone cornix),* and one mute swan (*Cygnus olor)* on the other part of Poland.

In 2008 antibodies were found in 2 horses (0.65 %) (Bażanów et al.).

In 2015 In Poland, WNV antibodies were detected in one (0.26%) out of 378 horses, in 63 (13.29%) out of 474 wild birds and in 14 (33.33%) out of 42 human patients displayed neurological symptoms (Niporczuk et al.).

Materials – birds (tissue samples)

Organs of 30 birds (brain, liver, lungs, heart spleen, trachea, kidneys) –

dead birds from Treatment And Rehabilitation Centre of Wild Animals of Wrocław University of Environmental and Life Sciences in Złotówek (OLiRDZ UP) and dead birds found in a field:

white-tailed Eagles (*Haliaeetus albicilla)* -9, common buzzard (*Buteo buteo)* – 4, goshawk (*Accipiter gentilis)* -1, peregrine falcon (*Falco peregrinus*)-1, capercaillie (*Tetrao urogallus*) -4, mute swans (*Cygnus olor*) -4, saker falcon (*Falco cherrug*) -1, crossbreed peregrine falcon/gyr falcon (*Falco peregrinus/Falco rusticolus*) -1, European herring gul (*Larus argentatus*) -1, mallard (*Anas platyrhynchos*) -1

Materials –birds (serum samples)

Serum samples from 14 birds:

1.healthy birds entrapped in live traps – 10 goshawks, 1 common buzzard

- capercaillie reintroduction programme

- health status of goshawk evaluation programme

2. sick birds, treated in OLiRDZ UP - 3 white-tailed Eagles, 1 common buzzard

Materials – horses (serum samples)

Serum samples from 411 horses from different farms (both small and stables) located throughout Poland.

Horses: different age, sex, breed (particularly Arabian, Thoroughbred, Hucul pony)

Methods

Virus isolation and identification (birds)

Soon after preparation, the material was inoculated into:

1. Cell lines: RK-13 (rabbit kidney), Vero (green monkey kidney), chicken fibroblast cell culture (primary cell culture);

Cell cultures were examined daily for up to 4 days for the development of viral cytopathic effects (CPE), using an inverted microscope. In the absence of visible CPE, up to 4 subsequent passages were done.

Methods

Virus isolation and identification (birds)

2. Embryonated chicken eggs (ECE) (inoculation on chorioallantoic membrane, into chorio-allantoic sac, intracerebellary);

ECE were opened after 4-5 days and examined for the changes in embryo and membranes. In the absence of visible changes, up to 4 subsequent passages were done.

Supernatant from cell cultures and allantoic liquid : hemagglutination test, RT-PCR

Serological methods
(birds and horses)

All sera were tested by:

-a microneutralisation procedure on Vero cells.

Serum samples with a virus neutralisation titer of ≥1:4 were considered positive;

-reverse ELISA;

- **IFA test (Euroimmun, Groß-Grönau,Germany).**

Results

Virus isolation:

WNV - negative

Isolated: herpesvirus, paramyxovirus (Newcastle disease virus), orthomyxovirus (flu virus); negative – circovirus.

Results

Serological investigations:

Birds:

6 (42.85%) serum samples - positive (4 healthy goshawks, 2 sick white-tailed Eagles)

Titers: 1:10 -1; 1:20 – 1; 1:80 – 1; 1:160 – 2; 1:1280 - 1

Horses:

83 (20.2%) serum samples – positive (different age, sex, breed ; 56 percent of horses, according to their travel history, never left the country).

Titers: 1:10 – 10; 1:20 - 19, 1:40 – 46; 1:80 – 1, 1:320 – 1; 1:640 – 2; ≥1:1280 - 4

Discussion and conclusion

BIRDS

- **Antibiodies against WNV have been found in birds in Poland since over 15 years, but so far, the rate of seropositive birds was relatively low. The high percentage in our research can suggest that the virus is already present in Poland.**

- **According Komar et al. blue jays, American crows, and house sparrows are some of the most susceptible birds to West Nile Virus infection. Our investigations involved goshawks and white-tailed Eagles - potentially less sensitive species, yet the rate of seropositive birds was high.**

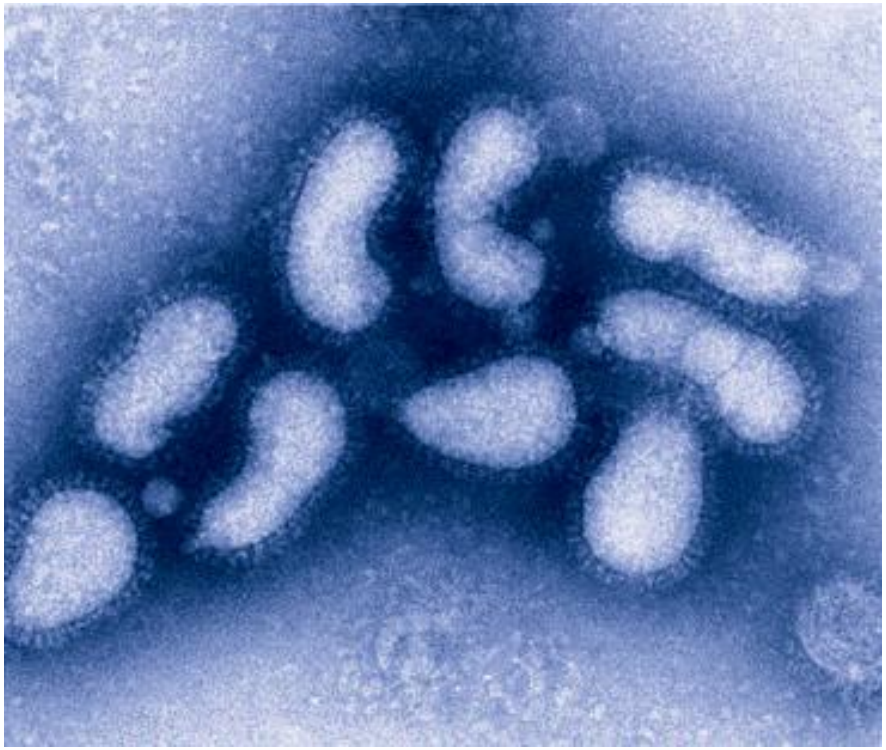
- **Admittedly positive wild birds could have contact with virus outside the country, but in the case of tested raptors species, adults do not migrate (the highest titers were found in 3-4 years old birds) .**

Discussion and conclusion

HORSES

- **We obtained a surprisingly high percentage of seropositive horses in comparison to earlier research (2008) - 2 horses -0.65% and research from Puławy PIW (2015) - 1 horse - 0.26%.**
- **In our research 20.2 percent of horses were serospositive (56% horses never left the country), it confirm the thesis that the virus is present in our ecosystem.**

CRIMEAN–CONGO HEMORRHAGIC FEVER VIRUS (CCHFV)



source: http://www.topnews.

Virus classification and morphology

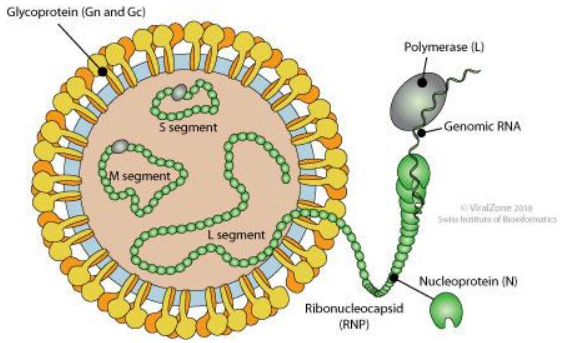
CCHF- a widespread tick-borne viral disease that is endemic in Africa, the Balkans, the Middle East and Asia.

Order: ***Unassigned***

Family: ***Bunyaviridae***

Genus: ***Nairovirus***

Species: ***Crimean-Congo hemorrhagic fever virus***



http://education.expasy.org/images/Bunyaviridae_virion.jpg

- first recognision - the Crimean peninsula in the mid-1940s; first isolation- from a patient in Kisangani, Democratic Republic of Congo, in 1956

-80–120 nm, pleomorphic, enveloped, the genome:

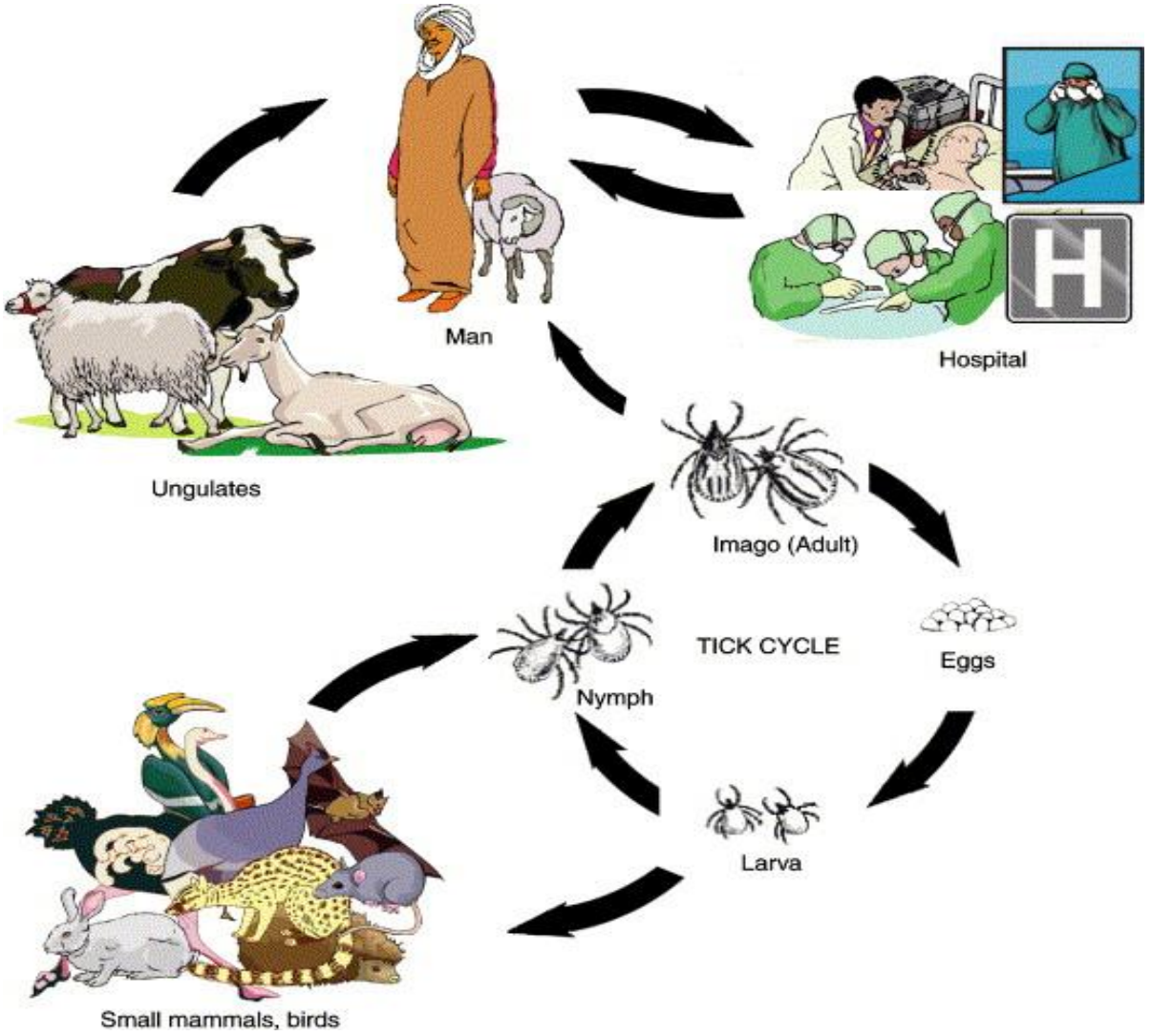
three copies of negative-strand RNA (ssRNA-) segments.

Vectors

-ticks : argasids and ixoids (especially the genus Hyalomma, Rhipicephalus and Dermacentor).

source: http://www.afrivip.org/sites/default/files/Ticks-importance/hyalomma.html

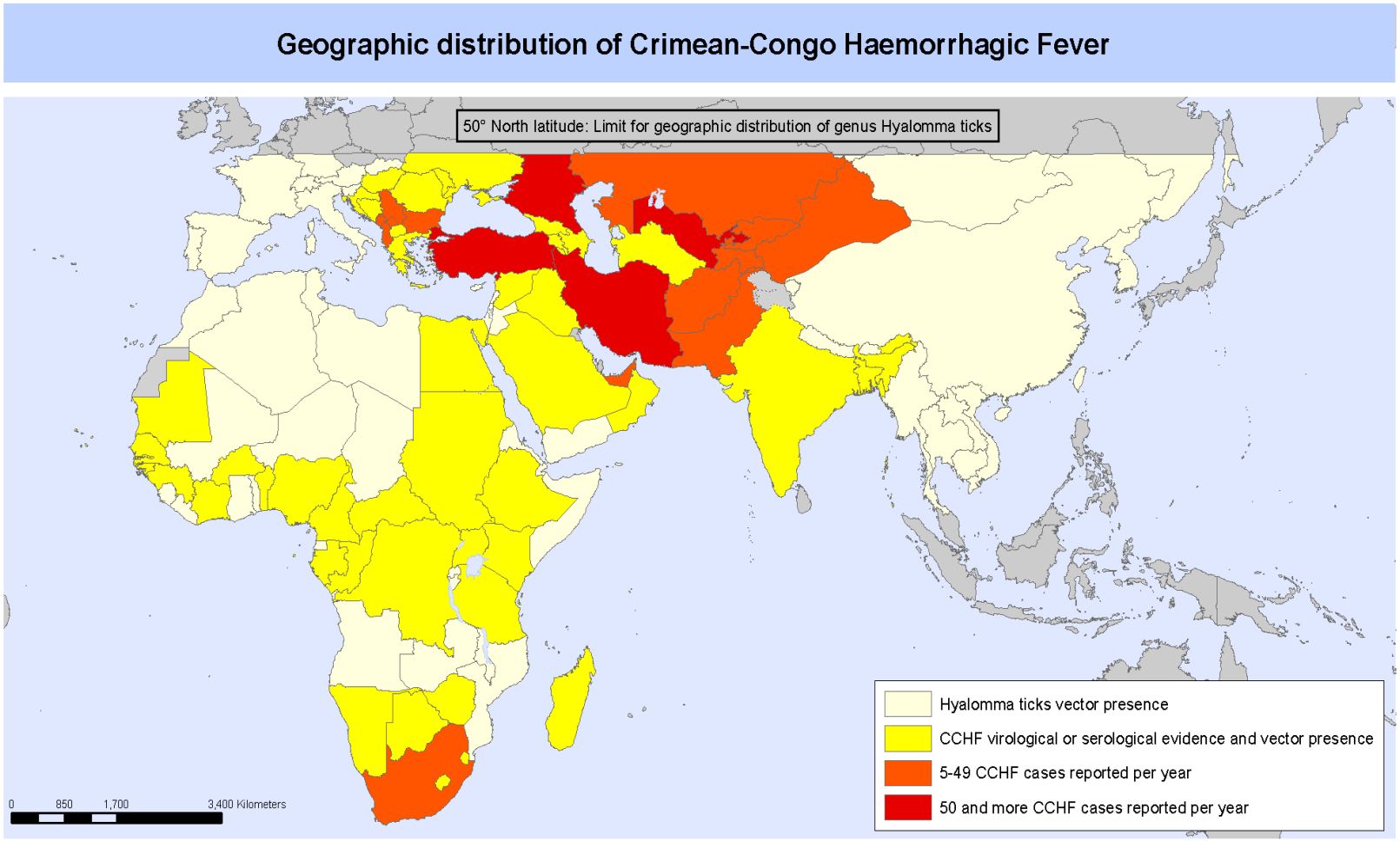
Transsmision cycle



source:http://www.infectionlandscapes.org/2012/10/crimeancongo-hemorrhagic-fever.html

50° north latitude set limit for the geographic distribution of *Hyalomma spp*. ticks, thus CCHFV infections appear or can appear in areas underlying this latitude.

Poland - not considered as a country at risk, but southern parts of Poland reaches 49° north latitude, thereupon at least this territory should be under control



Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

World Health Organization

Clinical symptoms

Humans : a severe hemorrhagic disease.

The most common clinical signs*:* fever, nausea, headache, myalgia, diarrhea, petechial rash, and bleeding. Case fatality rate - 5-80%.

Animals, in particular small and large mammals (e.g. hedgehogs, hares, foxes, sheep and cattle) develop viremia (for up to 2 weeks), but do not show clinical signs .

Materials

- **592 bovine serum samples.**

The tested animals derived from a small farms located mainly in south- east part of the country (Subcarpathian Voivodenship that borders Ukraine and Slovakia, Lesser Poland Voivodeship and Świętokrzyskie Voivodenship - Holy Cross Province).

The animals had a direct access to the pasture.

Blood samples were collected at routine screening of tuberculosis and bovine leukemia.

Methods

-Indirect immunofluorescence assay

-Enzyme-linked immunosorbent assay (ELISA)

Results

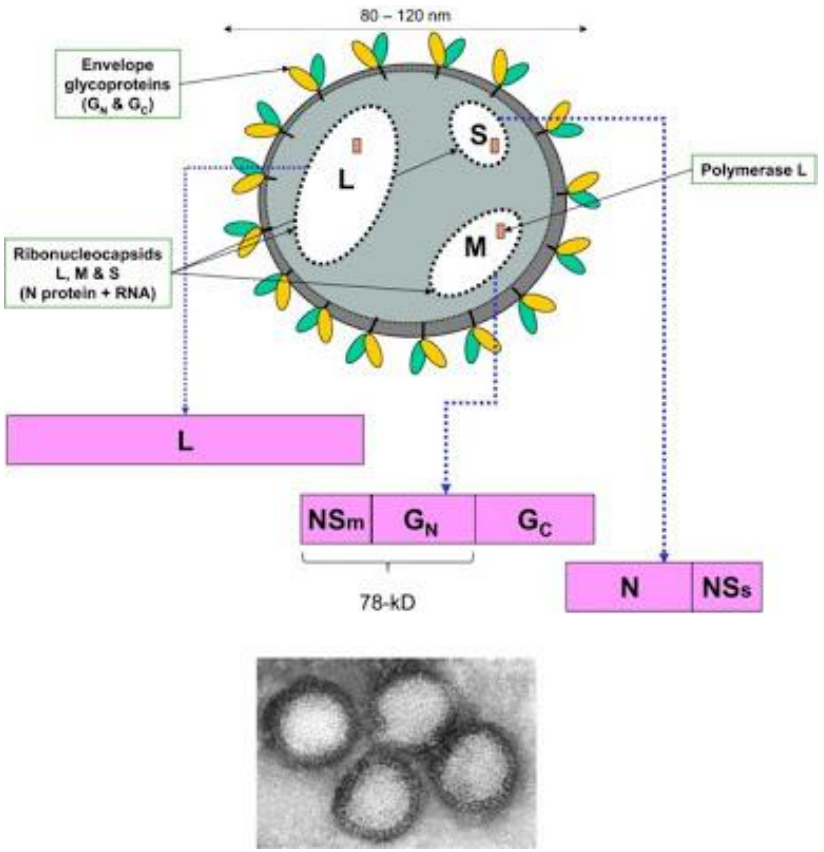
All sera - negative

Discussion and conclusion

The geographic range of the CCHFV is the most extensive among the tickborne viruses, which pose a health threat to the people, and the second (after dengue virus) most widespread of all medically important arboviruses.

Given the fact that there are many unknown factors about this disease and about dynamics of spread of CCHFV and, on the other hand, the geographic location of Poland as a country that borders countries at risk, it seems justifiable the control of CCHFV infection on this territory.

RIFT VALLEY FEVER VIRUS



RVFV structure (Published in: Vet. Res. 2010. 41(6): 61.)

Virus classification and morphology

Rift Valley fever (RVF) - an arthropod-borne zoonotic disease responsible for widespread outbreaks in both humans and ruminants.

Order: ***Unassigned***
Family: ***Bunyaviridae***
Genus: ***Phlebovirus***
Species: ***Rift Valley fever virus***

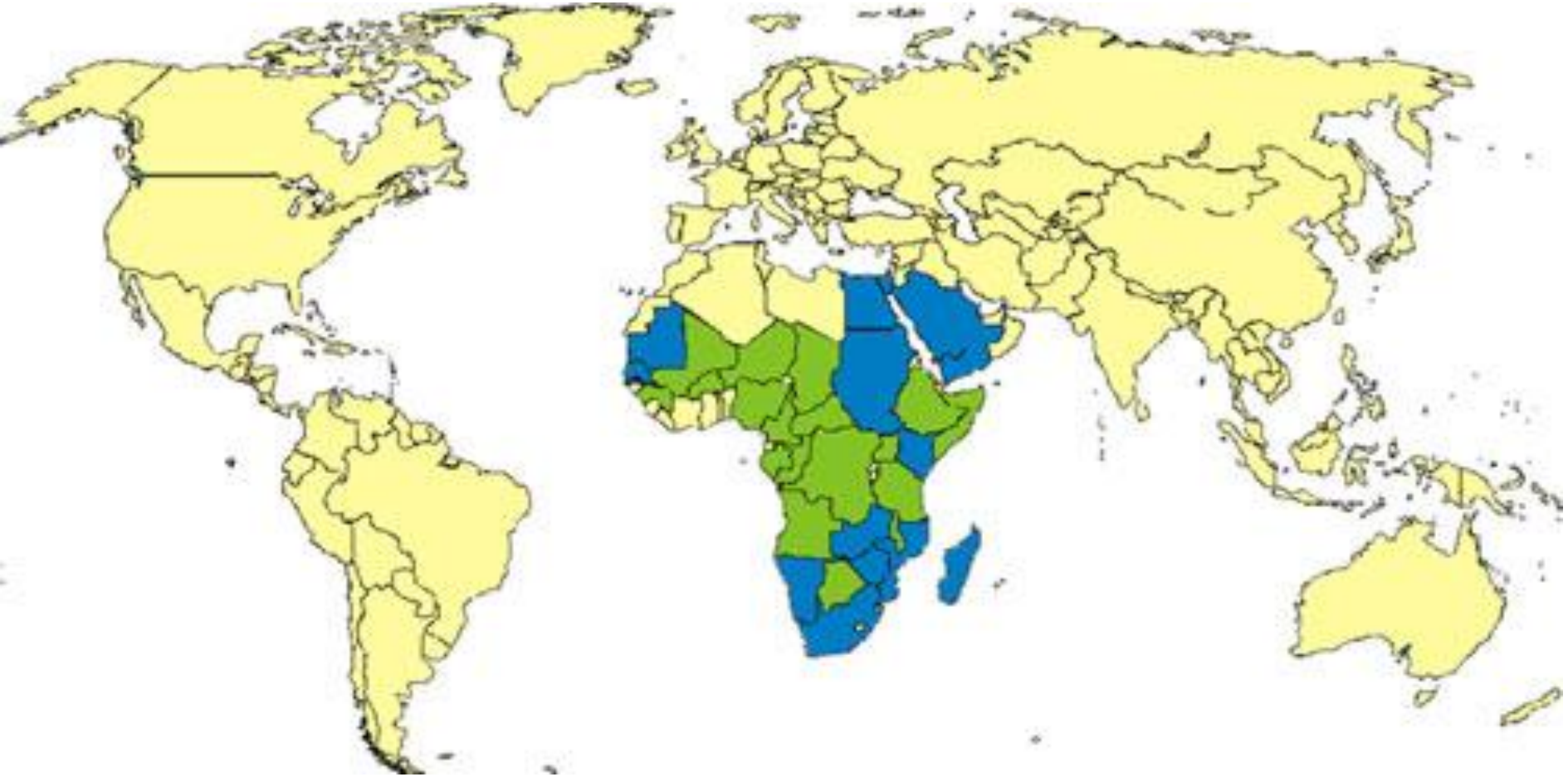
-spherical shaped, 80-120 nm, enveloped, a negative-sense single-stranded RNA (ssRNA -) 3 segments

- first identified in Kenya in 1930, the geographical range - the African continent.

-over the past 50 years - in over 30 countries (parts of western Africa, Egypt, Madagascar, the Comoros, the Arabian Peninsula (2000).

Vectors

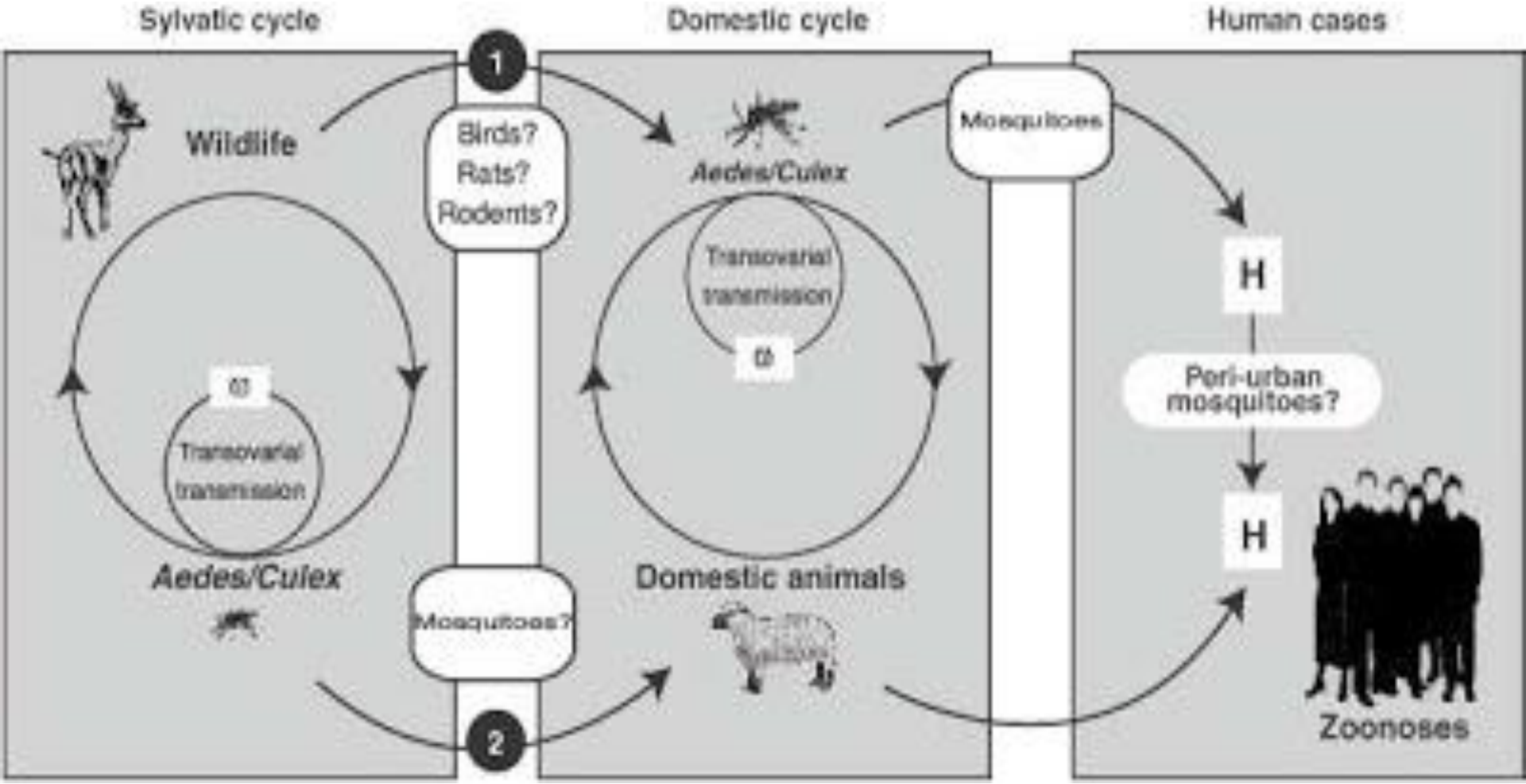
- a range of mosquito vector species as well as other arthropods, many of which are currently present in North America and Europe



•Countries with endemic disease and substantial outbreaks of RVF (blue)
•Countries known to have some cases, periodic isolation of virus, or serologic evidence of RVF (green)

source: CDC - http://en.wikipedia.org/wiki/Image:Rift_valley_fever_distribution.jpg

Transsmision cycle



source: http://www.infectionlandscapes.org/2012/10/rift-valley-fever.html

RVFV – THE RISK FACTOR

- is considered to have high colonization capacity;
- has been identified as a potential emergent risk in other nations, both as a natural exotic pathogen and an intentionally introduced biological weapon.
- classified as:

- a category A priority pathogen- by the National Institute of Allergy and Infectious Diseases—indicating the potential to cause social disruption and requiring public health preparedness;
- a high-consequence pathogen- by the World Organization for Animal Health;
- the third most dangerous animal threat- by the United States Department of Agriculture Animal and Plant Health Inspection Service after avian influenza and foot-and-mouth disease.

Clinical symptoms

In ruminants: epizootics are characterized by mass abortions and high mortality resulting in high economic burden.

In humans: most human cases - relatively mild, severe complications - in a small proportion of people (hemorrhagic fever, blindness and residual neurological deficits). The total case fatality rate - less than 1%.

Material and methods

Material: 592 bovine serum samples used in CCHFV investigations.

Method: Enzyme-linked immunosorbent assay (ELISA).

Results

All sera - negative

Discussion and conclusion

The risk of importing RVFV into the US or EU is generally assumed to be low, but not excluded because of movement of viremic hosts, movement of virus-carrying vectors and intentional entry (bioterrorism).

Admittedly the negative result was expected, but it was worth to check it out.

EXOTIC VECTOR-BORNE VIRAL ZOONOSES - A THREAT FOR DISEASES-FREE COUNTRIES? – GENERAL CONCLUSION

The probability that exotic arboviruses may spread to other continents is high, hence a monitoring of exotic vector-borne viral zoonoses is necessary nowadays.

It is important to capture the moment when virus appears in new area, then there are possibility to prepare and implement the prophylaxis.

THANK YOU