West-Nil-Virus in Deutschland?

Georg Pauli

Robert Koch-Institut

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Familie: Flaviviridae

Genus Flavivirus

Prototyp: YFV

(≈ 60 Spezies)

Genus Hepacivirus

Prototyp: HCV

(2 Spezies)

Genus Pestivirus

Prototyp: BVDV

(5 Spezies)

Familie: Flaviviridae

Genus: Flavivirus

Japan. Enzephalitis-Virus (JEV)

West Nil Virus (WNV)

St. Louis Encephalitis-Virus (SLEV)

Dengue Virus Typ 1–4 (DENV)

Gelbfieber-Virus (YFV)

Frühsommermeningoenzephalitis-Virus (FSMEV)

Eigenschaften der Flaviviren

Viruspartikel

Morphologie:	sphärisch (icosahedral)
umhüllt:	ja
Durchmesser:	45–50 nm

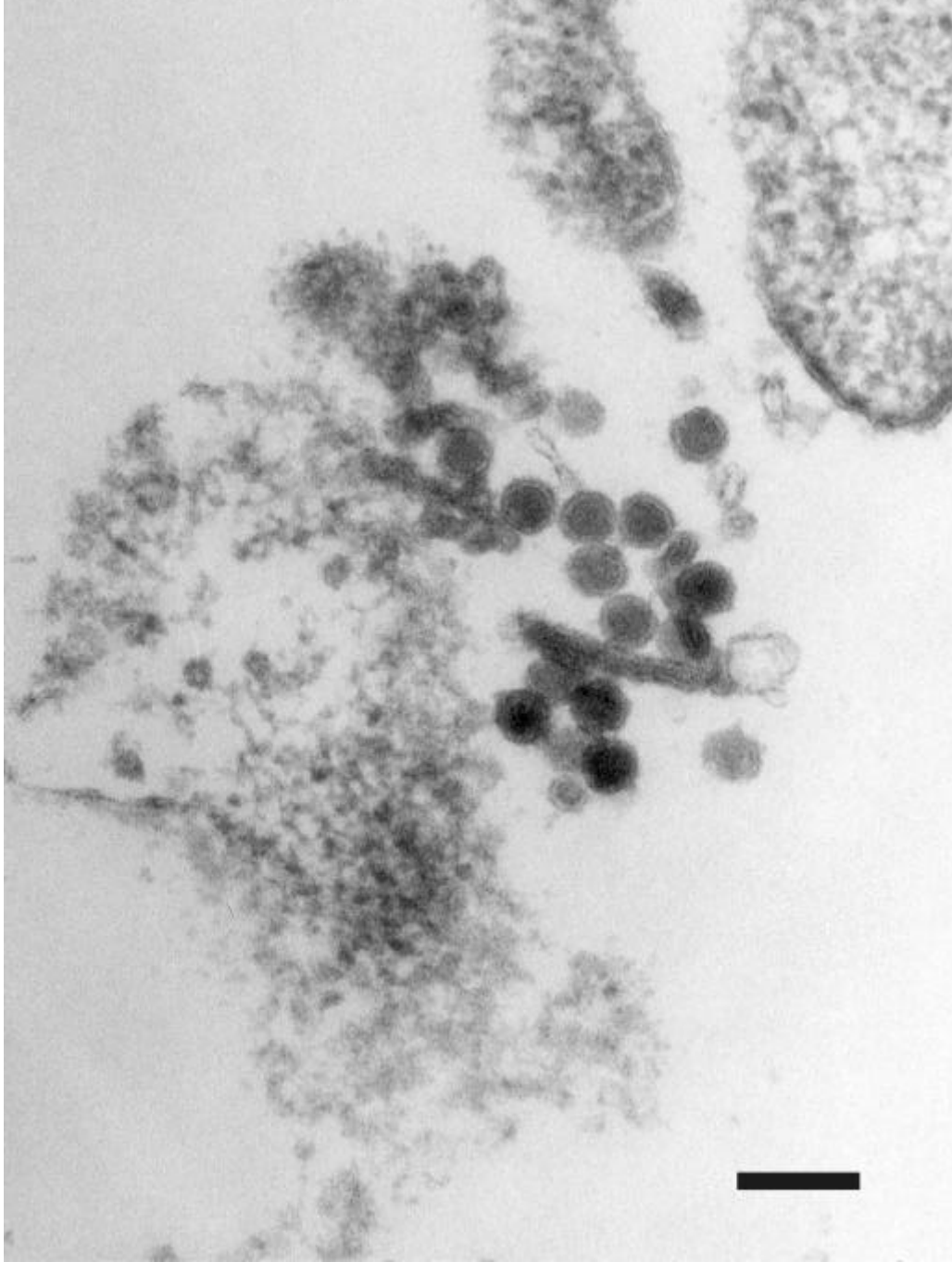
Genom

Nukleinsäure:	einsträngige RNA
Polarität:	positiv-strang
Konfiguration:	linear
Grösse:	11 kb

West Nil Virus

HR Gelderblom, RKI

(the bar equals 100 nm)



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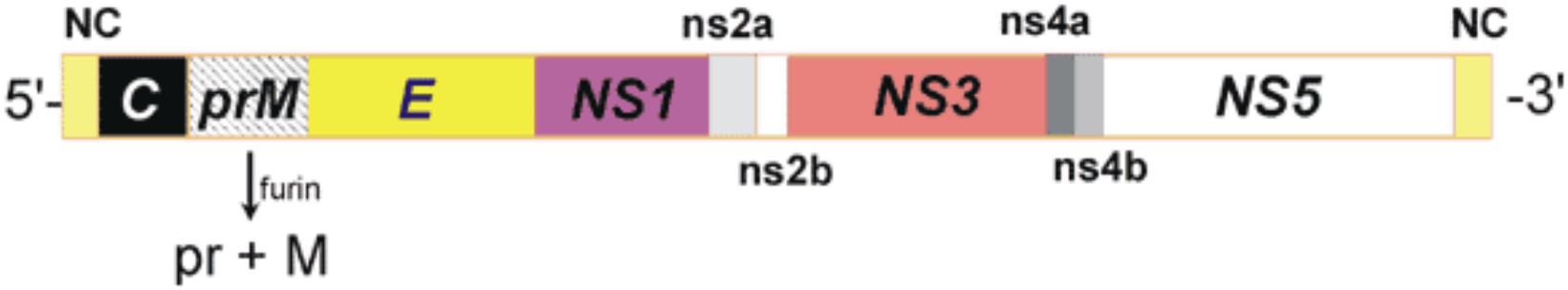
Genomic structure of flaviviruses

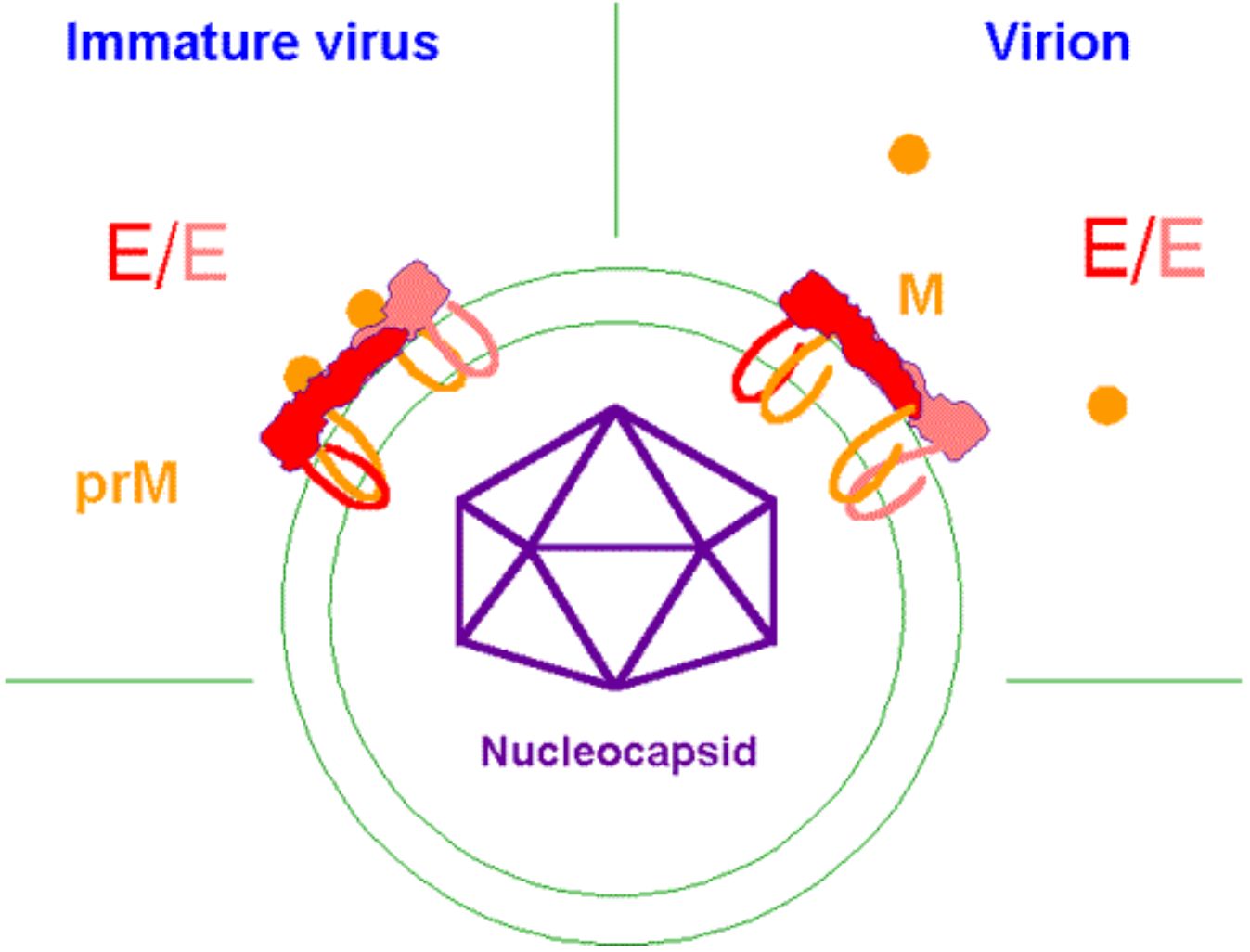


genome length: 11–12 kb

5'- and 3'- ends contain noncoding (NC) regions

10 proteins, 3 structural proteins (C, M, and E), and 7 nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5)

M protein is synthesized as a precursor (prM) protein. The prM protein is processed to pr + M by furin

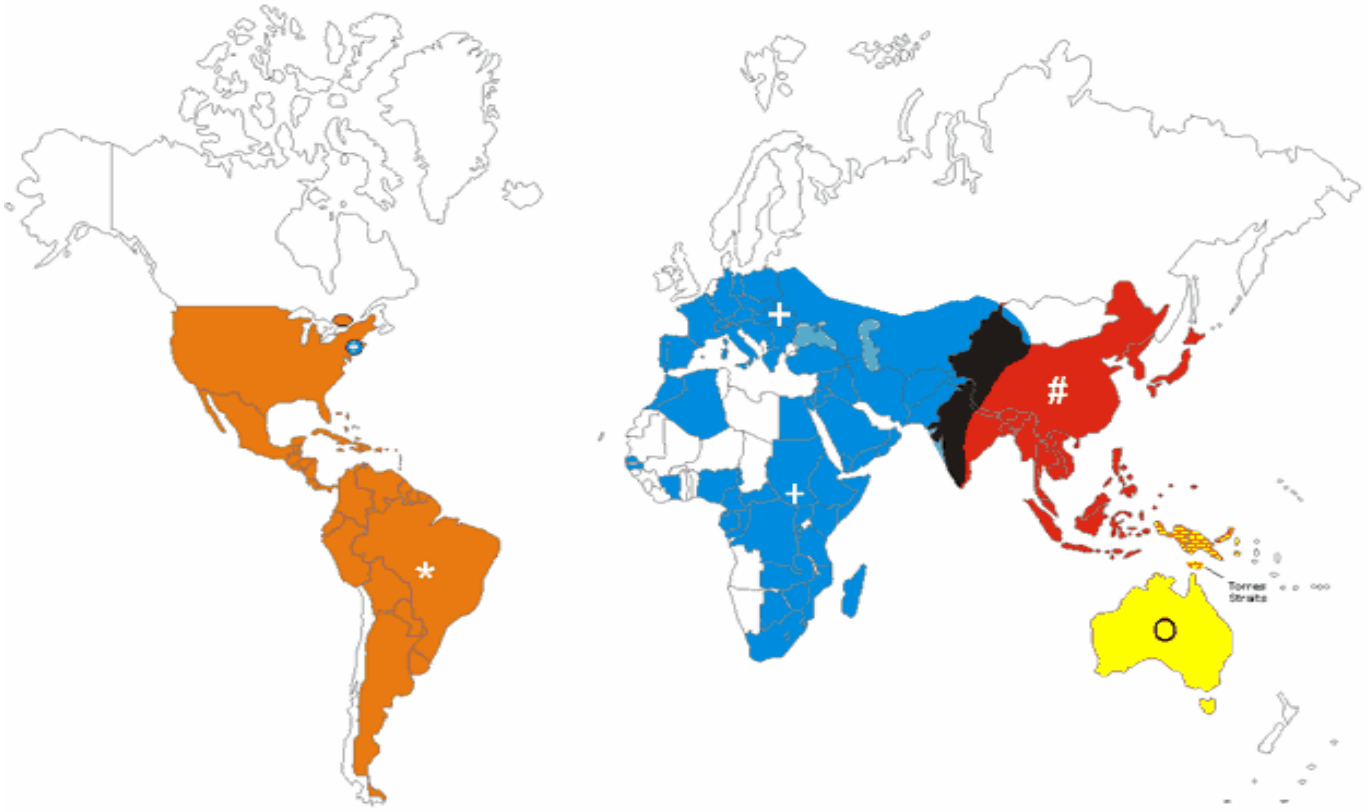


(Source: John Roehrig, Fourth National Conference on West Nile Virus in the United States, New Orleans, Louisiana, February 9-11, 2003)

Japanese Encephalitis Antigenic Complex

Alfuy Virus	Australia
Cacipacore Virus	Brazil
Japanese Encephalitis Virus	Asia
Kokobera Virus	Australia/Papua N. Guinea
Koutango Virus	Africa
Kunjin Virus	Australia
Murray Valley Encephalitis Virus	Australia
St. Louis Encephalitis Virus	North + South America
Rocio Virus	Brazil
Stratford Virus	Australia
Usutu Virus	Africa (Europe)
West Nile Virus	world-wide ?
Yaounde Virus	Africa

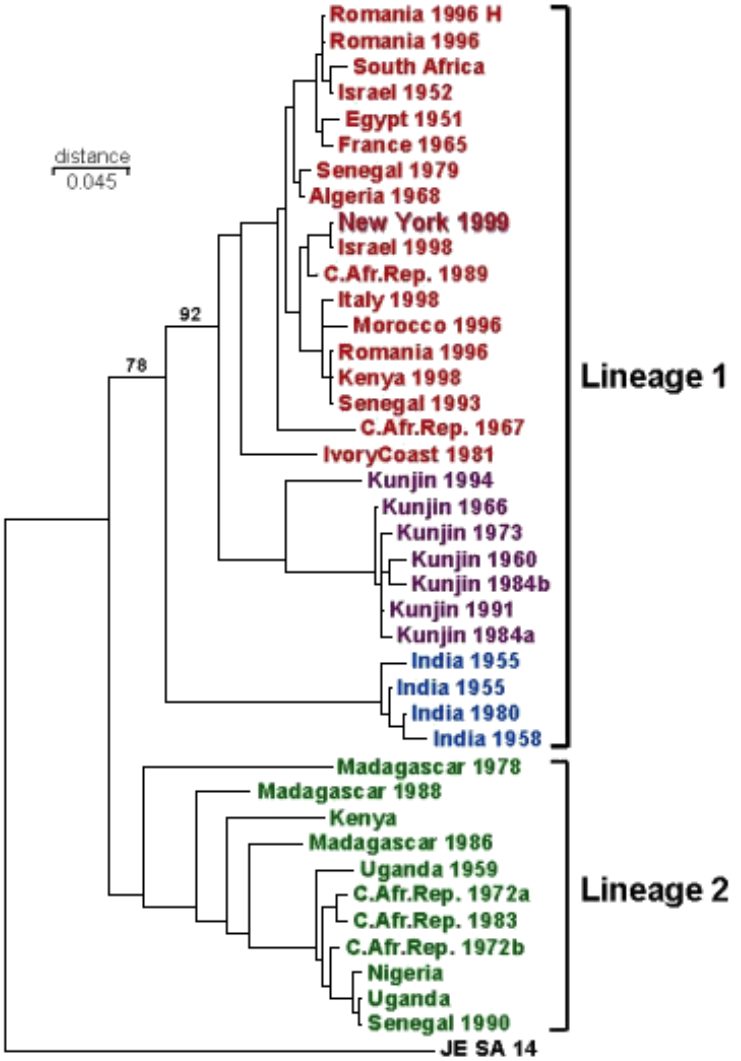
The Geographic Distribution of the Japanese Encephalitis Serocomplex of the Family Flaviridae, 2000.



- St. Louis encephalitis
- * Rocio and St. Louis (Brazil)
- + West Nile virus
- # Japanese encephalitis
- West Nile and Japanese encephalitis
- Japanese and Murray Valley encephalitis
- O Murray Valley and Kunjin

CDC

Phylogenetic Tree Based on Envelope Glycoprotein Sequence Data



MEGA, distance tree, Kimura 2-parameter, neighbor-joining

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West Nil Virus

Linie 1

alle Stämme aus Nordafrika, Europa, Indien, Israel, USA und Kunjin Virus aus Australien

Linie 2

Stämme aus West-, Zentral und Ostafrika und Madagascar

West Nil Virus

- Signifikante Unterschiede zwischen Isolaten aus verschiedenen Regionen, aber auch zwischen Viren einer Region

- Zwei Antigengruppen bei Linie 1:

1. Isolate aus Afrika/Mittlerer Osten (Congo, Egypten, Frankreich, Israel, Pakistan, Uganda) Australien (Kunjin) Russland, Südafrika und USA
2. Isolate aus Indien

West Nil Virus

- Unterschiede in der Infektiosität von WNV-Isolaten für einen Wirt

- Unterschiede zwischen Stämmen in der Pathogenität für erwachsene Mäuse

- Änderungen des Virus im Bezug auf Morbidität und Mortalität für Vögel?

Klinische Eigenschaften - Milde Infektion

- Mehrzahl der Infektionen verläuft mild oder inapparent (= 99%)

- ≈ 20% fieberhafte Erkrankung mit plötzlichem Beginn (West Nil Fieber)

Übelkeit	Kopfschmerzen
Anorexie	Muskelschmerzen
Erbrechen	Ausschlag
Lymphadenopathie	Augenschmerzen

- Inkubationszeit 3 bis 14 Tage
- Symptomdauer 3 bis 6 Tage

Klinische Eigenschaften - Schwere Infektion(1)

≈ 1 in 150 Infektionen schwere neurologische Erkrankung

- Risikofaktor: hohes Alter
- Enzephalitis häufiger als Meningitis

Symptome:

Fieber, Beteiligung des Gastrointestinaltrakts, Schwäche, Wesensänderungen,
(Ausschlag Nacken, Rumpf, Arme, Beine)

Klinische Eigenschaften - Schwere Infektion(2)

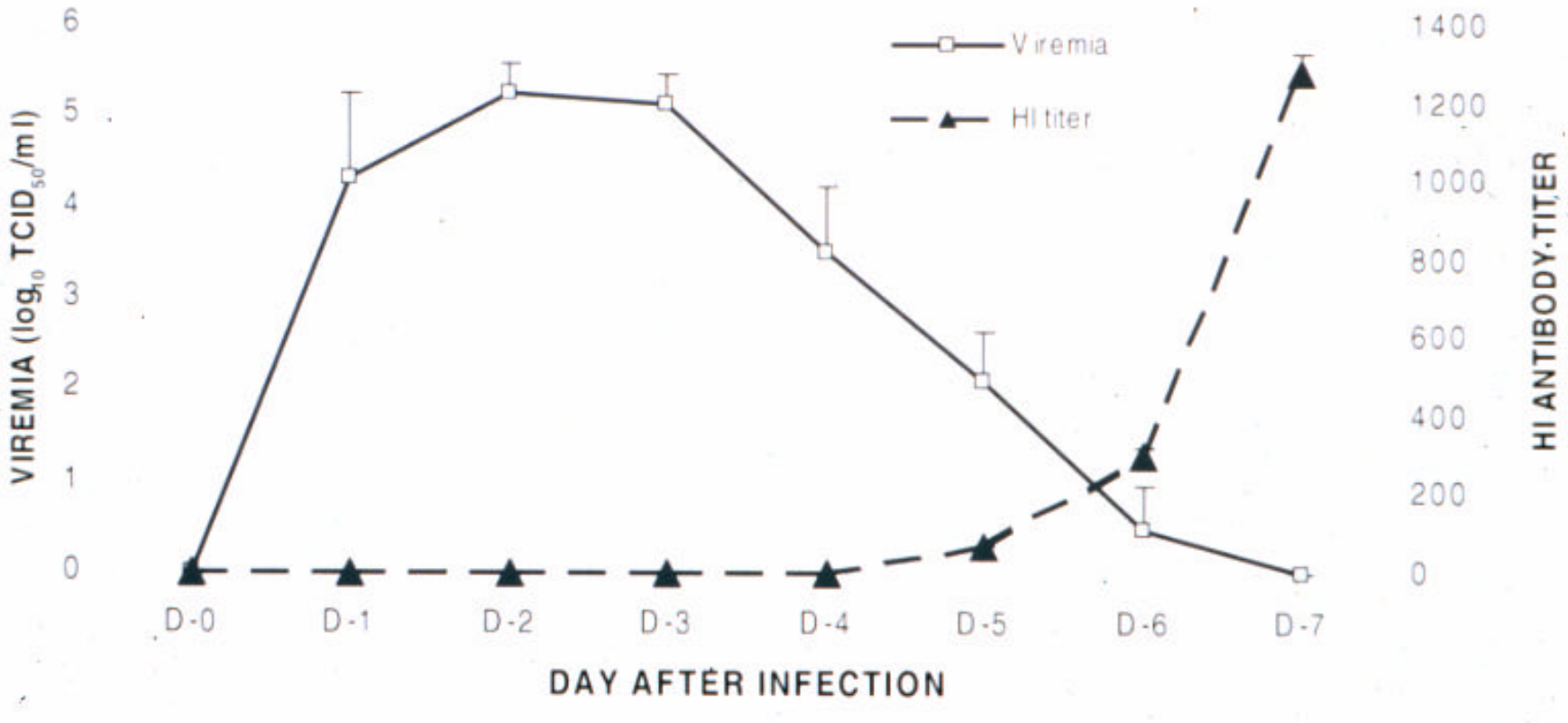
Muskelschwäche und schlaffe Lähmungen

Neurologische Symptome:

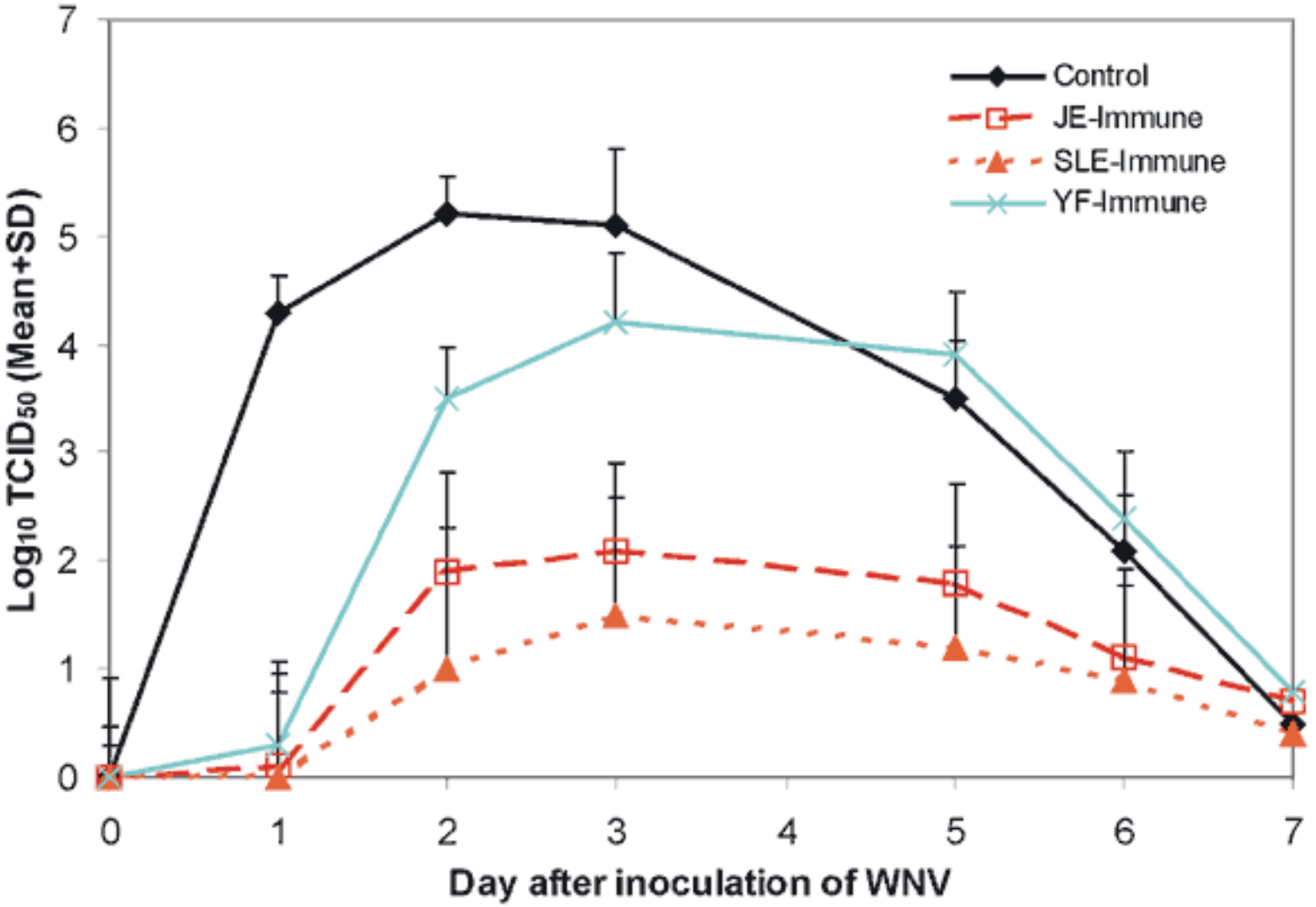
- Ataxie und extrapyramidale Symptome
- Optikusneuritis
- Beteiligung der cranialen Nerven
- Polyradiculitis
- Myelitis

(Myocarditis, Pancreatitis, fulminante Hepatitis)

Viremia and HI Antibodies

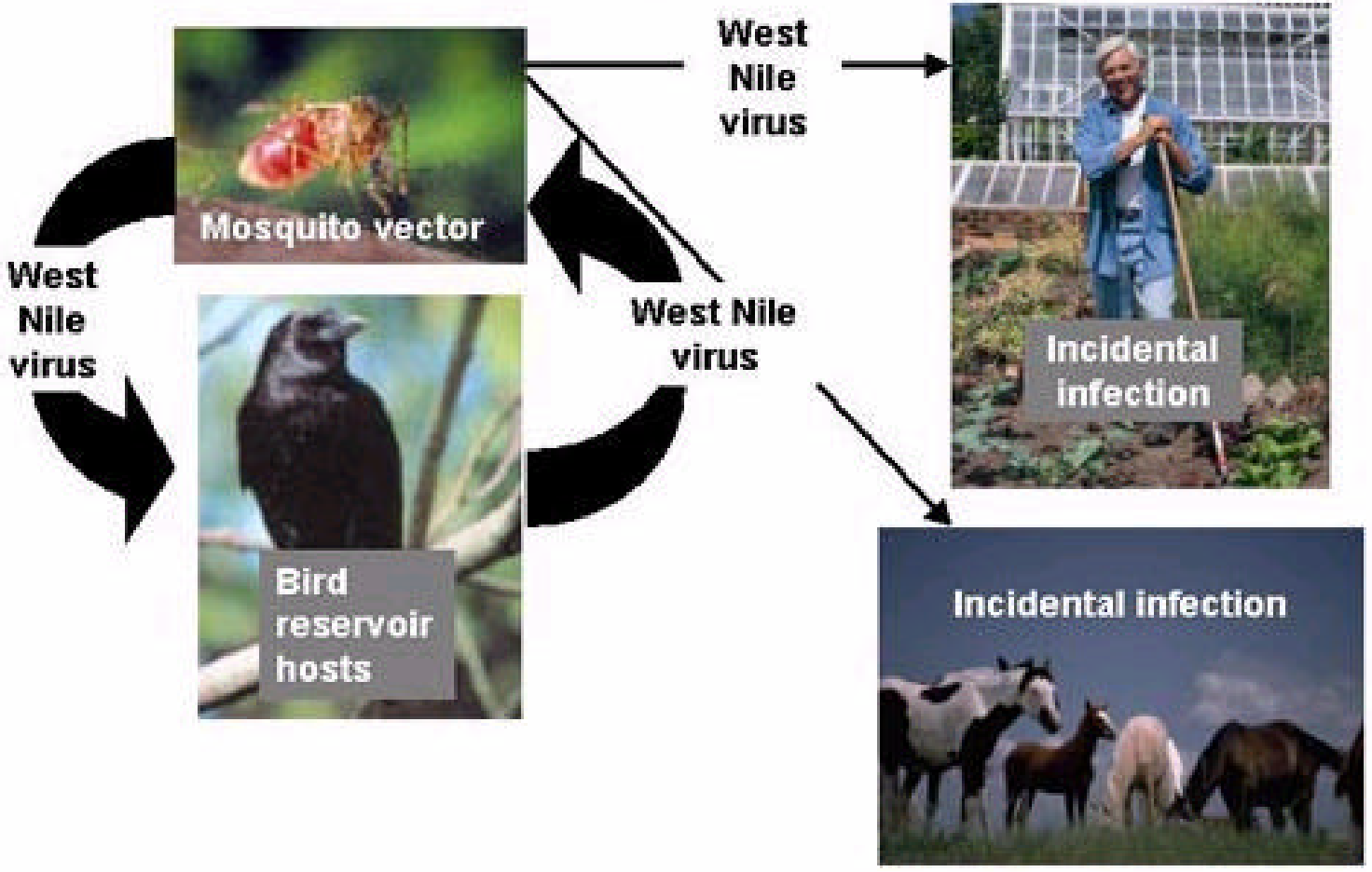


Robert B. Tesh: Cross Immunity: West Nile vs. St. Louis Encephalitis in Areas of Overlap

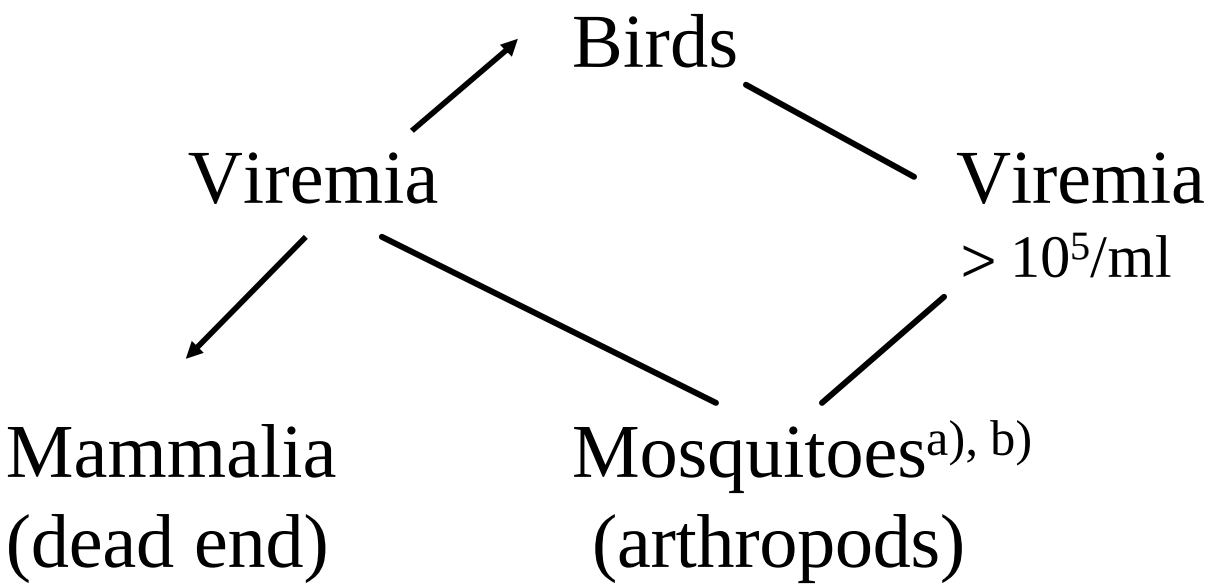


Robert B. Tesh: Cross Immunity: West Nile vs. St. Louis Encephalitis in Areas of Overlap

West Nile Virus Transmission Cycle

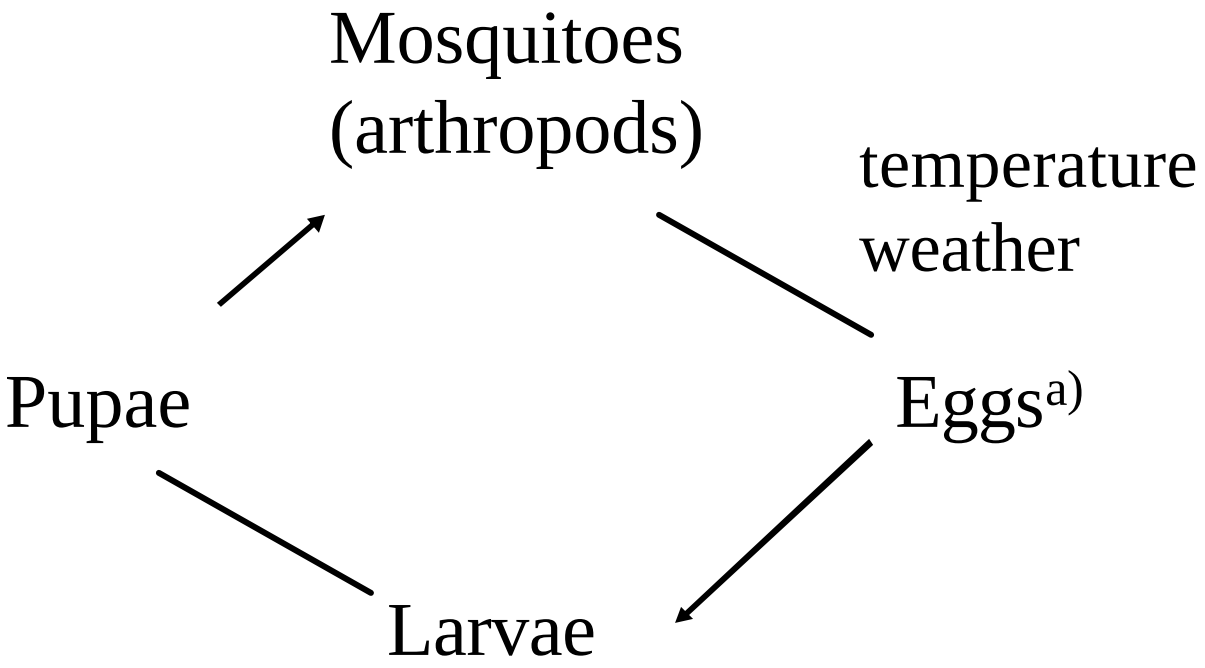


West Nile Virus Transmission Cycle



a) **bird–bird-feeding mosquitoes**
Culex pipiens pipiens*, *Culex modestus*, *Mimoyia richardii
b) **bird–mammalia-feeding (humans),** ***Culex pipiens molestus***

West Nile Virus Transmission Cycle



[a)] ≈ 1% transmission of flavivirus

West Nile Virus in Arthropods (1)

Mosquitoes

Culex spec. (> 18)

Coquillettidia spec. (3)

Mansonia uniformis

Aedes spec. (11)

Anopheles spec. (> 6)

Mimomyia spec.

Aedeomyia africana

Hubalek and Halouzka, EID 5: 643-650, 1999

West Nile Virus in Arthropods (2)

Soft ticks

Argas hermanni

Ornithodoros capensis

Hard ticks

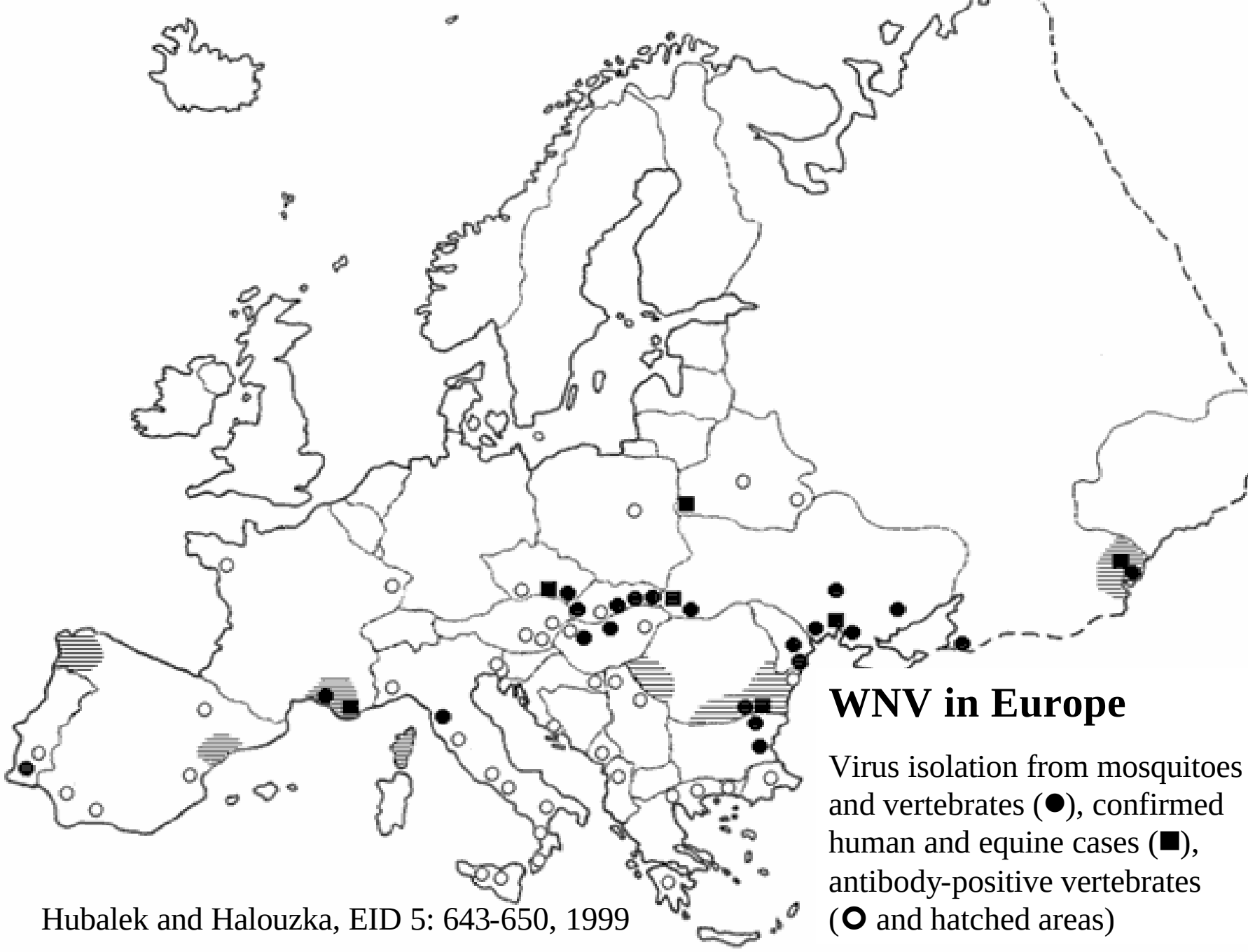
Hyalomma marginatum detritum

Rhipicephalus turanicus muhsamae

Amblyomma variegatum

Dermacentor marginatus

Hubalek and Halouzka, EID 5: 643-650, 1999



WNV in Europe

Virus isolation from mosquitoes and vertebrates (●), confirmed human and equine cases (■), antibody-positive vertebrates (O and hatched areas)

Hubalek and Halouzka, EID 5: 643-650, 1999

West Nile Virus in Europe (1960–1998)

Human cases	Year(s)	% seropositive
France (10)	1962	19
	1975–80	5
	1965	30–50
Romania (453)	1996	17
(94)	1997	
Belarus	1997	1
Ukraine	1970s	
(38)	1985	
Russia (> 10)	1963–68	7–31
Volgograd (> 900)	1999	

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Hubalek and Halouzka, EID 5: 643-650, 1999

West Nile Virus in Europe (human)

Country	Year(s)	% seropositive
Portugal	1967–70	3
Spain	1960/79	17; 8–30
Italy	1967–69	5–23
Albania	1958	2
Bulgaria	1960–70	3
Hungary	1970s	4–6
Slowakia	1970–1973	1–4
Austria	1964–1977	1–6
Moldavia	1970s	3

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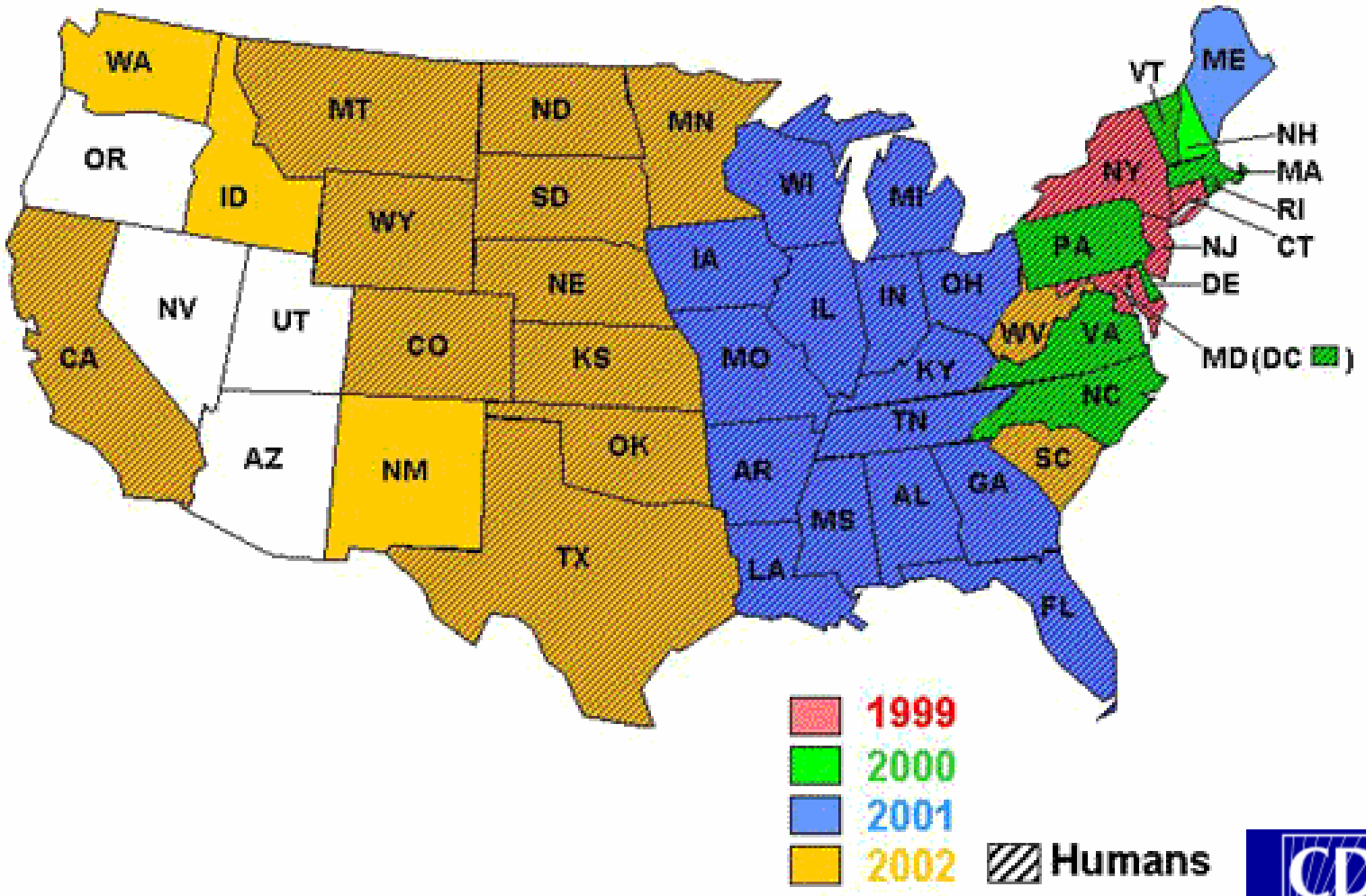
Hubalek and Halouzka, EID 5: 643-650, 1999

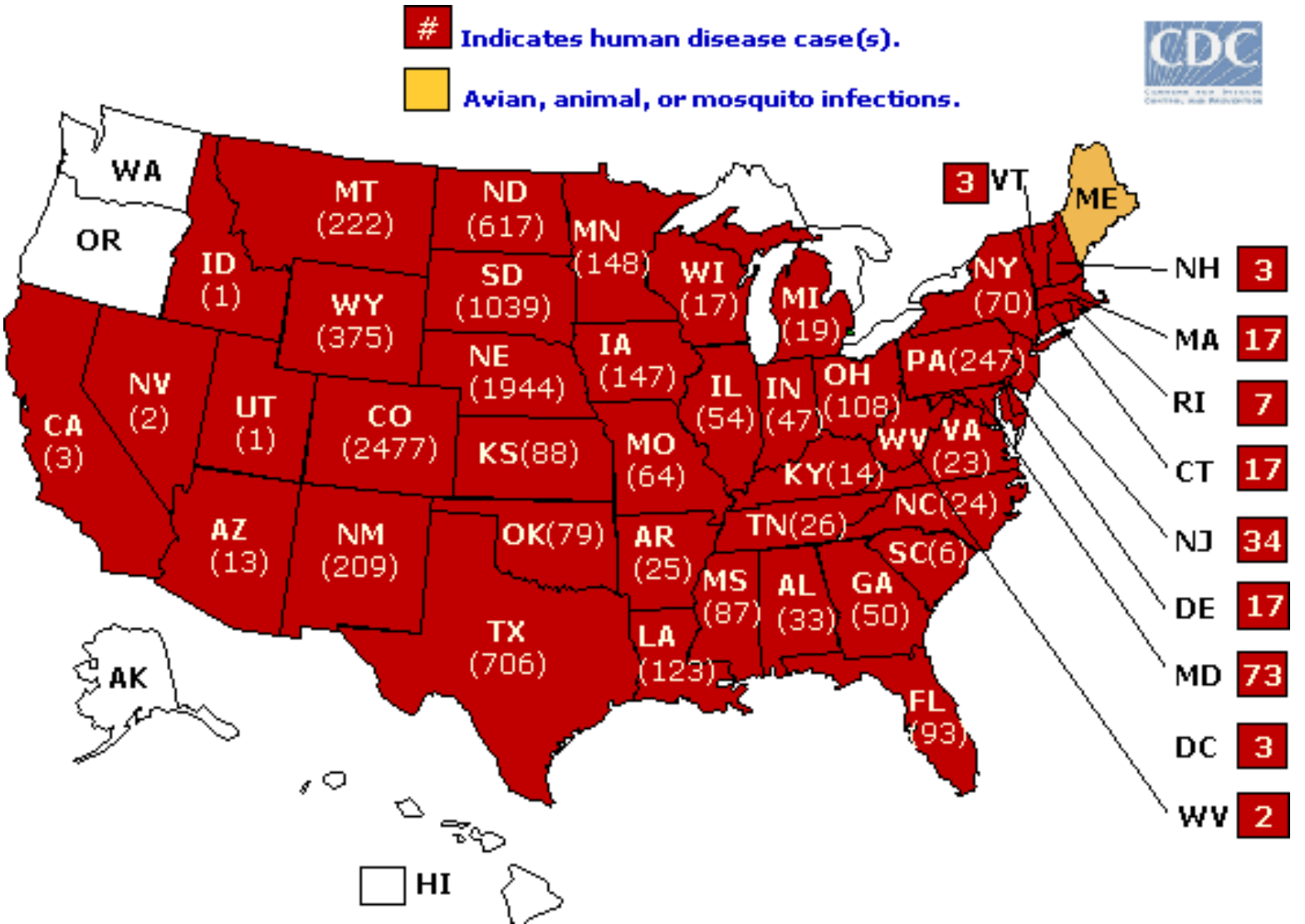
Robert B. Tesh: Cross Immunity: West Nile vs. St. Louis Encephalitis in Areas of Overlap

138 bird species have been reported
to CDC's West Nile Virus
Avian Mortality Database
from 1999 to November 2003

Spread of WNV by state, 1999–2002.
WNV activity in the U.S. in Birds, Horses, Mosquitoes, Animals, or Humans



Spread of WNV by state, as of March 10, 2004.
WNV activity in the U.S. in 2003
in Birds, Horses, Mosquitoes, Animals, or Humans



WNV disease in humans in the US

	2000	2001	2002	2003 (March 10, 2004)
WNV infections meningoencephalitis (2768/30%) and WN fever (6446/69%)	**21**	**66**	**4,156**	**9,377**
Deaths	**2**	**9**	**284**	**244**

In all reported human cases, the median age of infected persons was 55 years (range: 1 month--99 years); for persons with WNME, the median age was 59 years (range: 1 month--99 years); and for persons with WNF, the median age was 48 years (range: 1--93 years).

WNV disease in humans in the US in 2003 (I), as of March 10, 2004

State	Cases 2002	Deaths 2002	Cases 2003	Deaths 2003
Alabama	49	3	33	3
Arizona			13	1
Arkansas	43	3	25	
California	1		3	
Colorado	14		2477	45
Connecticut	17		17	2
Delaware	1		17	
District of Columbia	34	1	3	6
Florida	28	2	93	
Georgia	44	7	50	4
Illinois	884	64	54	1
Indiana	293	11	47	4
Iowa	54	2	147	6
Kansas	22		88	4
Kentucky	75	5	14	1

WNV disease in humans in the US in 2003 (II), as of March 10, 2004

State	Cases 2002	Deaths 2002	Cases 2003	Deaths 2003
Louisiana	329	25	123	8
Maryland	36	7	73	7
Massachusetts	23	3	17	1
Michigan	614	51	19	2
Minnesota	48		148	4
Mississippi	192	12	87	1
Missouri	168	7	64	7
Montana	2		222	4
Nebraska	152	7	1944	29
Nevada			2	
New Hampshire			3	
New Jersey	24		34	3
New Mexico			209	4
New York	82	5	70	11
North Carolina	2		24	2
North Dakota	17	2	617	7

WNV disease in humans in the US in 2003 (III), as of March 10, 2004

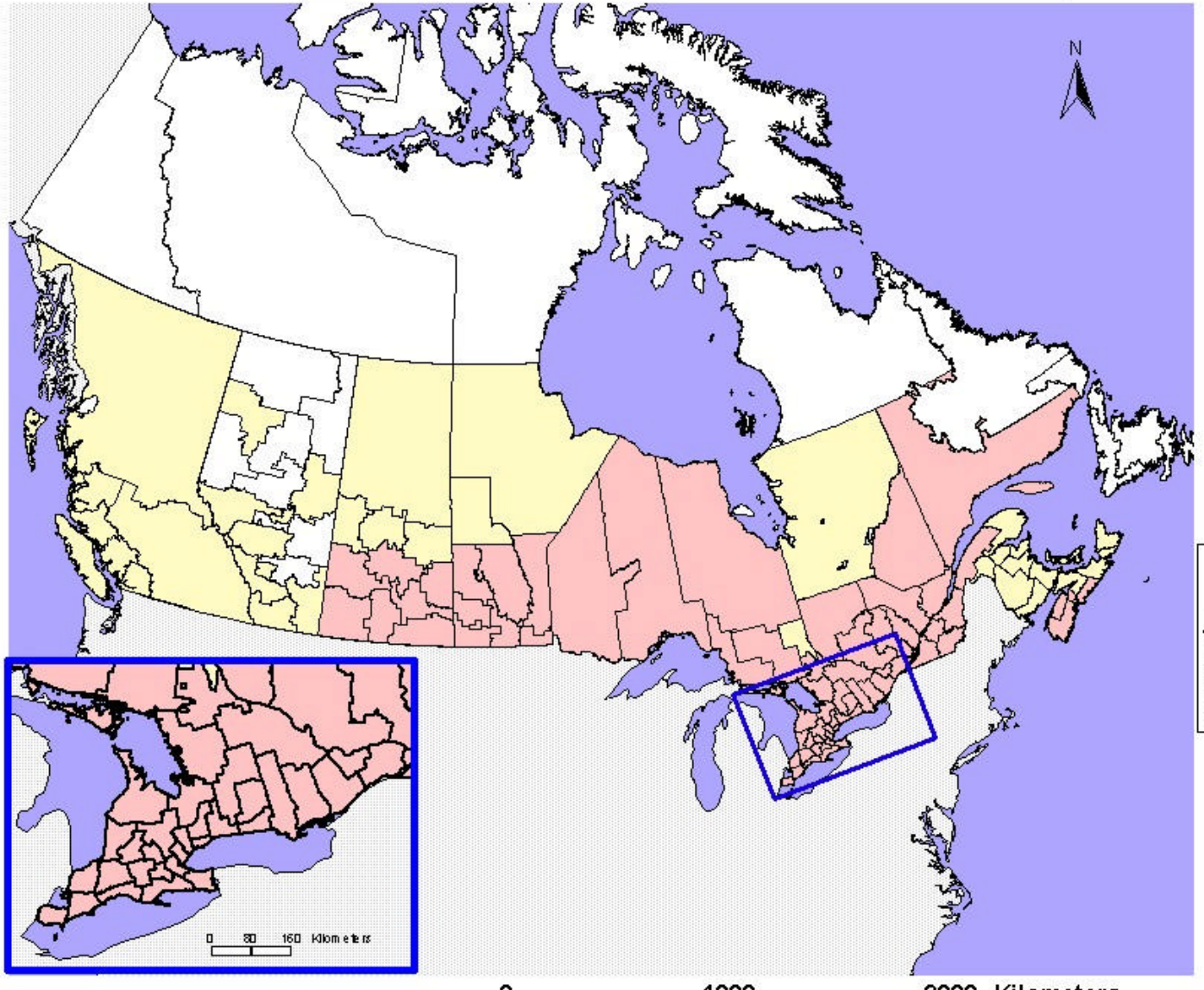
State	Cases 2002	Deaths 2002	Cases 2003	Deaths 2003
Ohio	441	31	108	8
Oklahoma	21	2	79	8
Pennsylvania	62	7	247	1
Rhode Island	1		7	
South Carolina	1		6	
South Dakota	37		1039	14
Tennessee	56	7	26	1
Texas	202	13	706	35
Utah			1	
Vermont	1		3	1
Virginia	29	2	23	
West Virginia	3	2	2	
Wisconsin	52	3	17	
Wyoming	2		375	9
Totals	4156	284	9377	244

6446 as West Nile Fever (69%), 2768 as meningitis/encephalitis (30%), 163 unspecified (2%)

Health Canada Santé Canada

Dead Birds Submitted for West Nile Virus Diagnosis by Health Region in Canada as of December 18, 2002

Canada



See the regional maps for more detailed information

Legend

Birds Submitted for diagnosis (by Health Region)

- NO
- YES
- POSITIVE

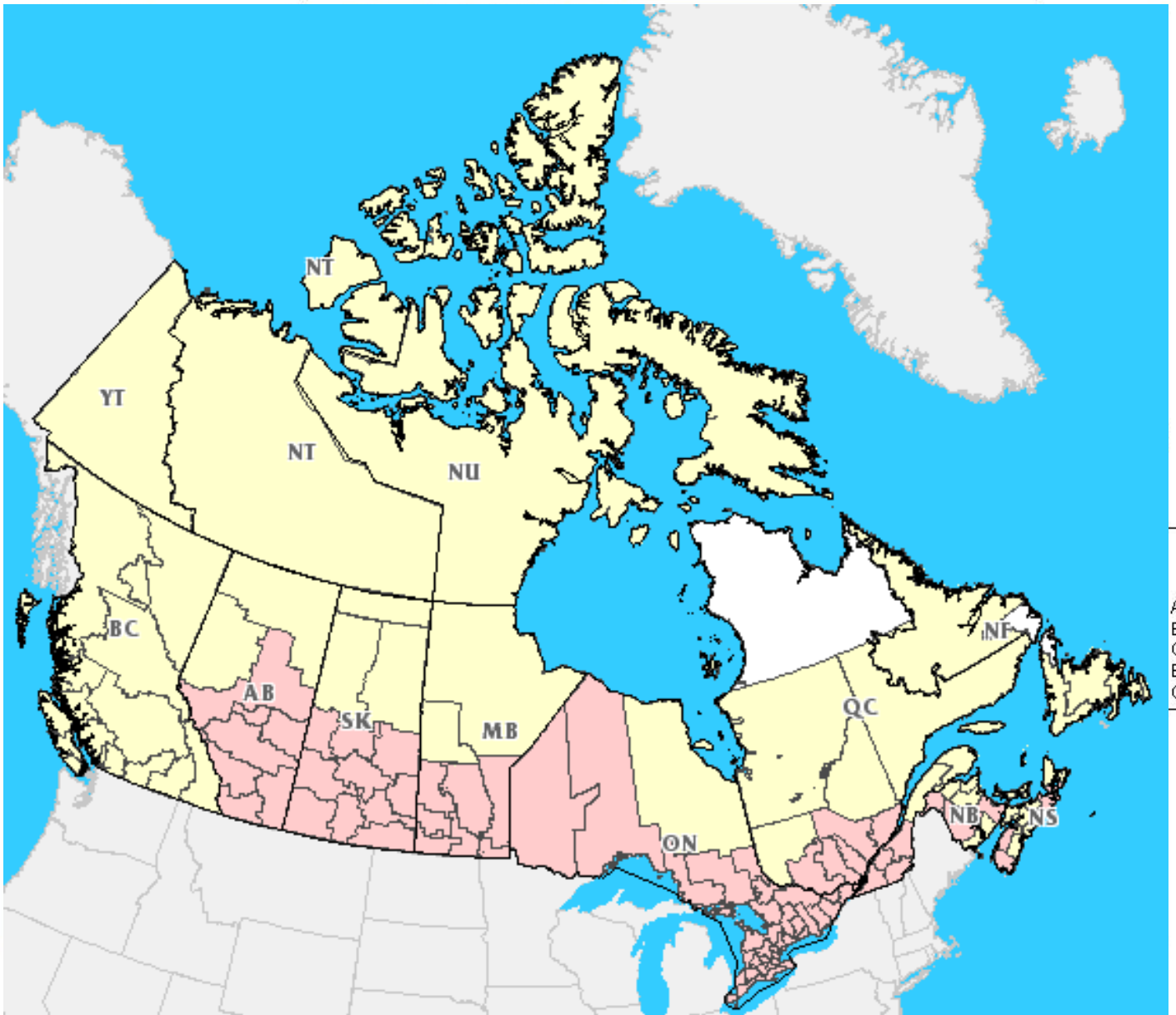
Numerical Summary of Birds Submitted to Health Canada for Diagnosis

Birds	# Positive/ # Submitted
American Crow	488/2677
Blue Jay	26/433
Common Raven	5/200
Black-billed Magpie	20/154
Other Birds	24/194

Health Canada Santé Canada

Dead Birds Submitted for West Nile Virus Diagnosis by Health Region Canada as of November 21, 2003

Canada



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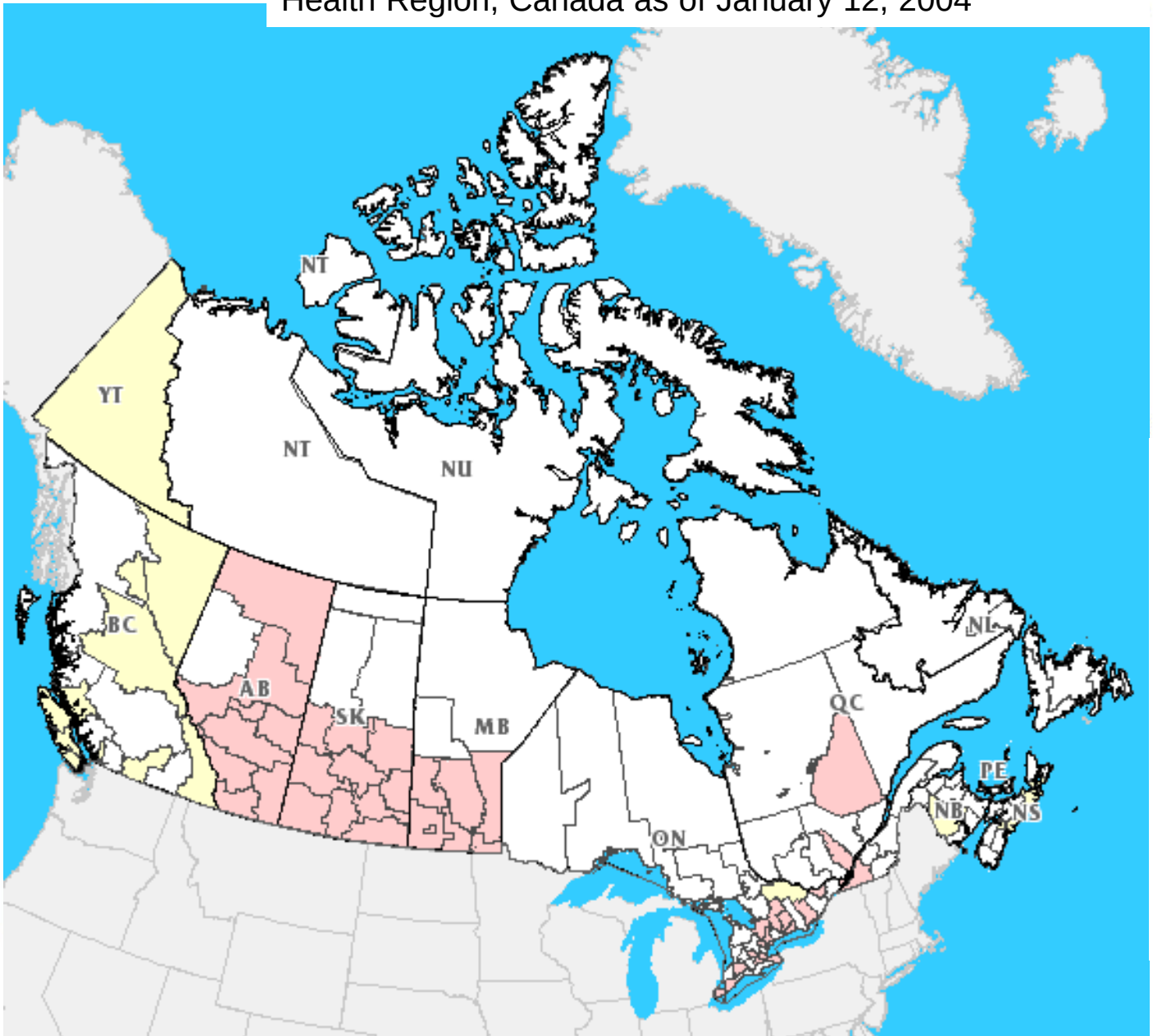
Health Canada Santé Canada

Human Results: WNV Neurological Syndromes, WNV Fever and WNF Asymptomatic Infection Diagnosis by Health Region, Canada as of January 12, 2004

Canada



See the regional maps for more detailed information

Legend

Human results

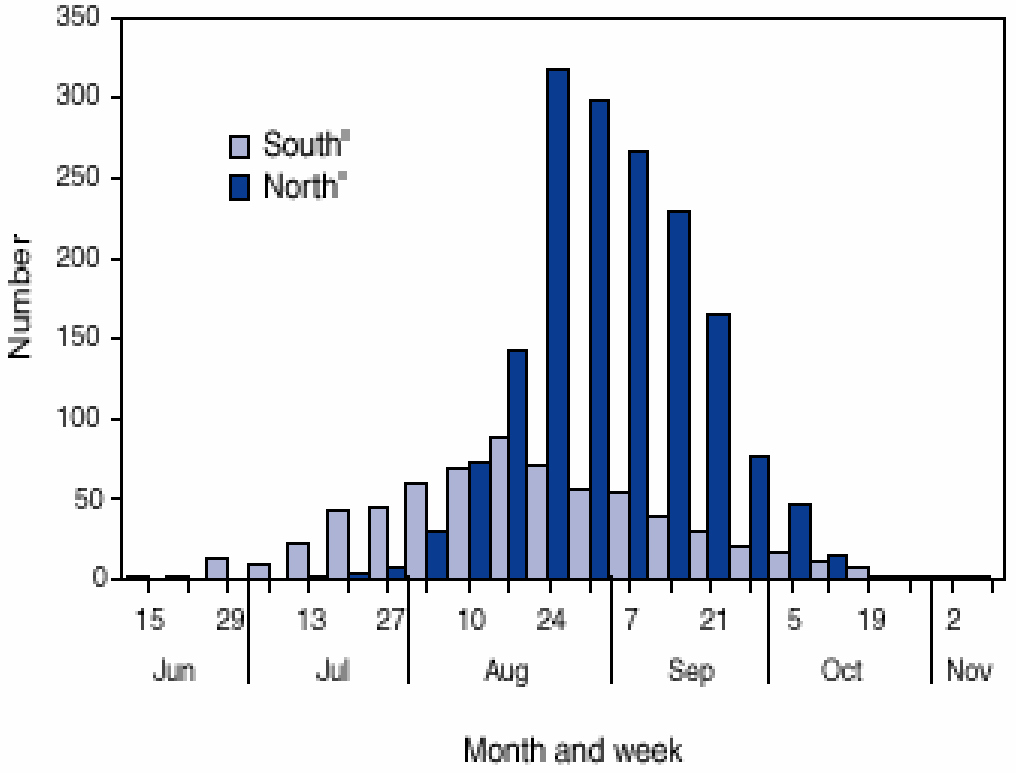
- NO
- TRAVEL-RELATED
- POSITIVE

Human Results by Province [1]

Province/Territory	Total Clinical Cases[2]	WN virus Neurological Syndromes (WNNS)[3]		WN virus Fever (WNF)[4]		WN virus Asymptomatic Infection (WNAI)[5]		Total Death
		Probable	Confirmed	Probable	Confirmed	Probable	Confirmed	
Nova Scotia (NS)	2[6]	0	1[6]	0	1[6]	0	0	0
New Brunswick (NB)	1[6]	0	0	0	1[6]	0	0	0
Quebec (QC)	17	1	13	0	3	0	0	0
Ontario[10] (ON)	89[7]	-	-	-	-	-	-	2[8]
Manitoba (MB)	141	28	7	78	27	0	1	2
Saskatchewan (SK)	792	0	53	0	729	0	10	6[9]
Alberta[11] (AB)	272	0	48	0	221	0	3	0
British Columbia (BC)	20[6]	3[6]	6[6]	3[6]	8[6]	0	0	0
Yukon Territory (YT)	1[6]	0	0	0	1[6]	0	0	0
Canada - Total	**1335**	**32**	**128**	**81**	**991**	**0**	**14**	**10**

WNV Infectionen bei Menschen und Tieren korreliren mit der Mückendichte und deren Aktivität (Arthropoden?)

FIGURE 2. Number of human West Nile meningoencephalitis cases, by location and by week and month of illness onset — United States, June–November 2002*

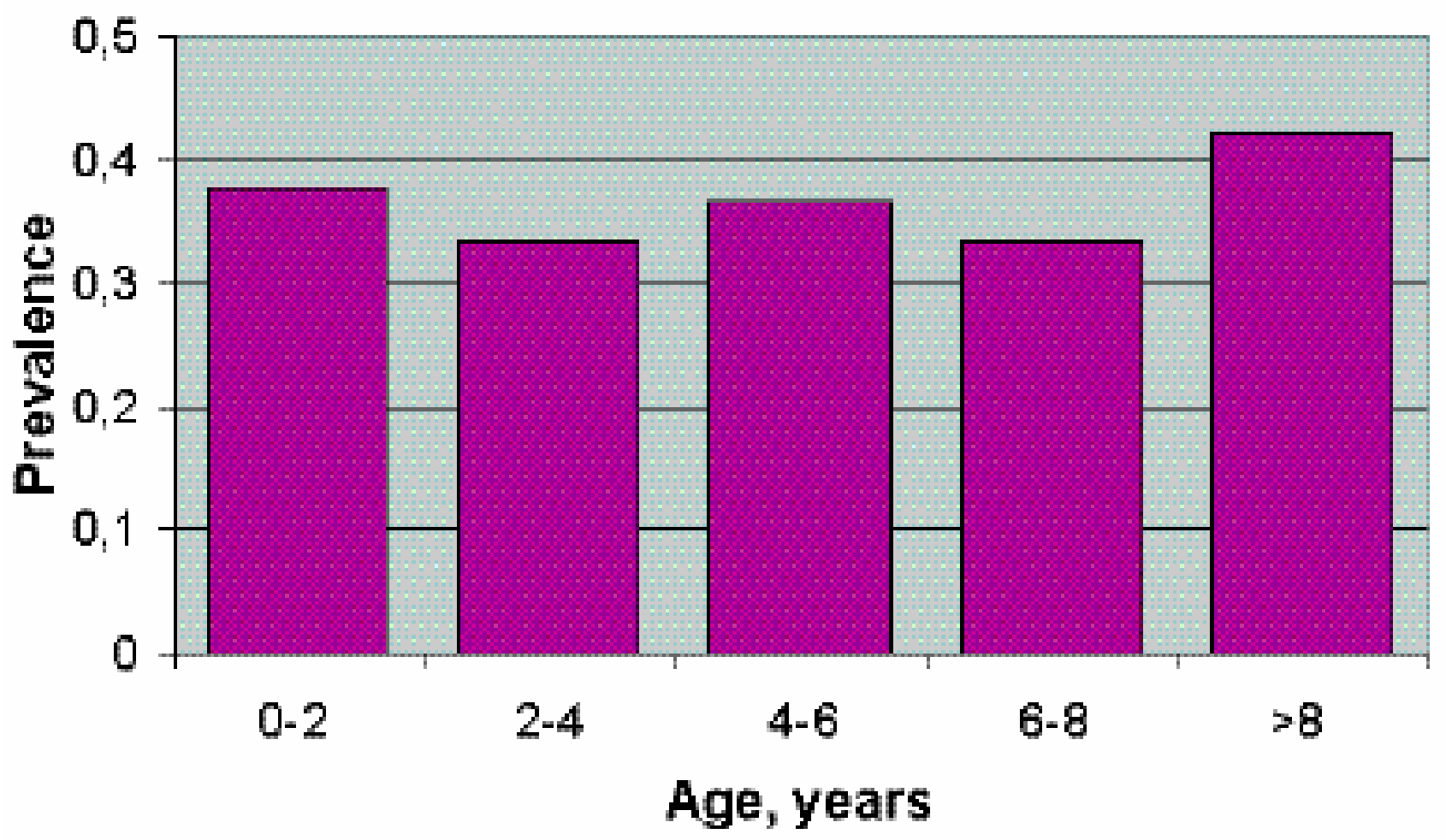


* N=2,354 as of November 30.

† *East South Central region:* Alabama, Kentucky, Mississippi, and Tennessee; *South Atlantic region:* Delaware, the District of Columbia, Florida, Georgia, Maryland, North Carolina, South Carolina, Virginia, and West Virginia; *West South Central region:* Arkansas, Louisiana, Oklahoma, and Texas; and *Pacific region:* California.

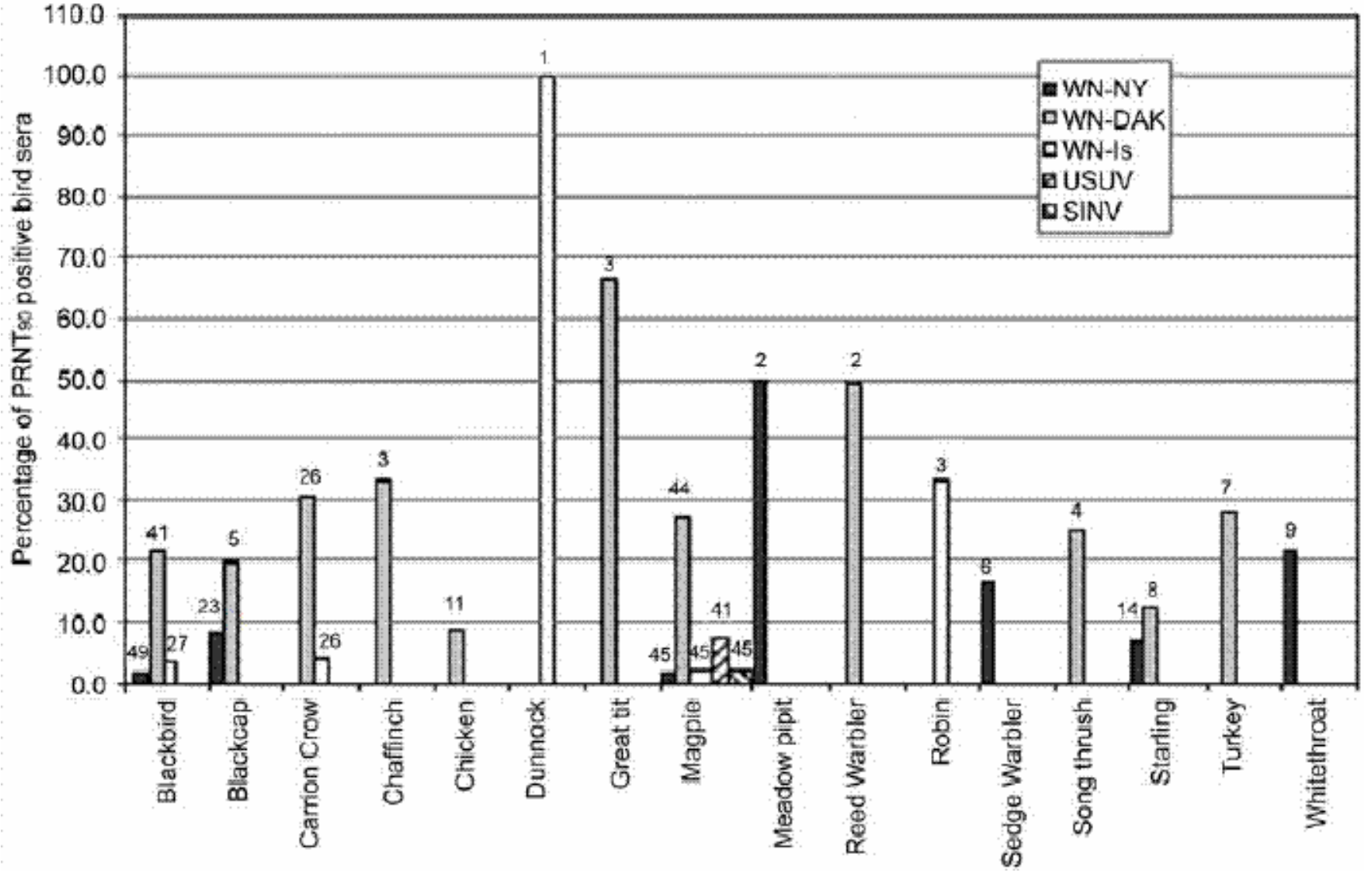
§ *East North Central region:* Illinois, Indiana, Michigan, Ohio, and Wisconsin; *Mid-Atlantic region:* New Jersey, New York, and Pennsylvania; *Mountain region:* Colorado, Montana, and Wyoming; *New England region:* Connecticut, Massachusetts, and Rhode Island; *West North Central region:* Iowa, Kansas, Minnesota, Missouri, Nebraska, and South Dakota.

WNV age-specific prevalence (n=282) in horses, Tuscany Region, 1998–1999



Source: Gian Luca Autorino et al., EID 8: 1372-1378 (2002)

WNV antibody-positive birds in UK



Alan Buckley et al., Journal of General Virology (2003), 84 (10): 2807-2817

West Nil Virus Infectionen in Deutschland

Virus und Antikörper in jungen Störchen aus Deutschland in Israel gefunden

Antikörper in erwachsenen Störchen in Deutschland (Malkinson and Banet; Kooperation mit Prof. Kaleta, Gießen)

Von 1986 bis 1995	0/48
Von 1997 bis 2000	14/98 (14.3%)

West-Nil-Virus in Deutschland

Kooperationsprojekt:

Robert Koch-Institut (RKI)

Paul-Ehrlich-Institut (PEI)

Bernhard-Nocht-Institut (BNI)

Finanzierung durch BMGS

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West-Nil-Virus in Deutschland

Untersuchungskollektive:

- Blutspender (PEI)
- Zugvögel (RKI)
- Pferde mit ZNS-Störungen (RKI)
- Menschen mit ZNS-Störungen unklarer Genese (RKI)

West-Nil-Virus in Deutschland

Untersuchungsmethoden

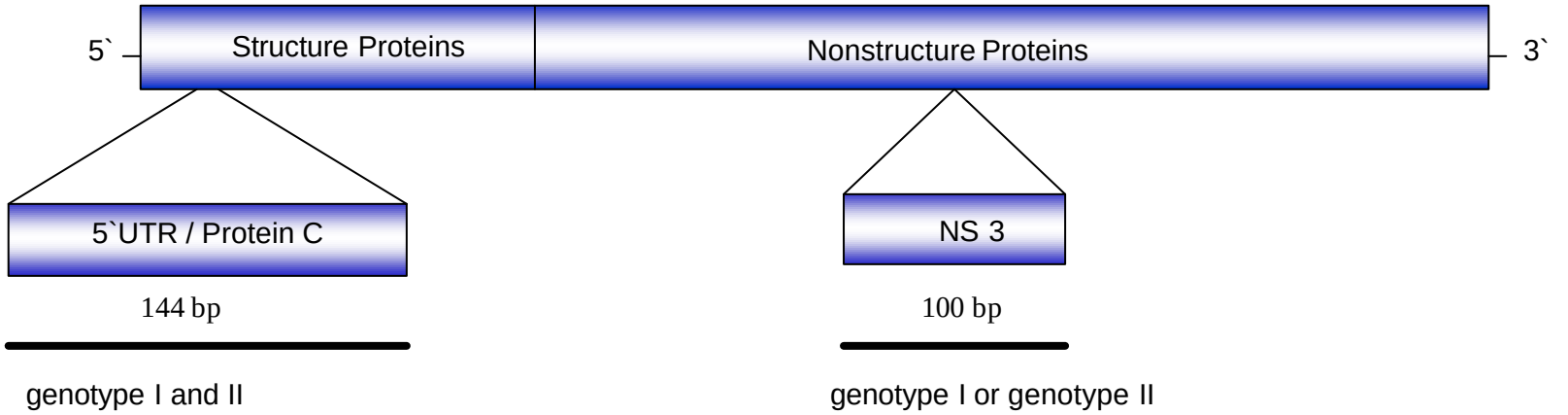
Inzidenz:

Virusnachweis: Polymerasekettenreaktion (PCR)
Differenzierung von Isolaten
Anzucht

Prävalenz:

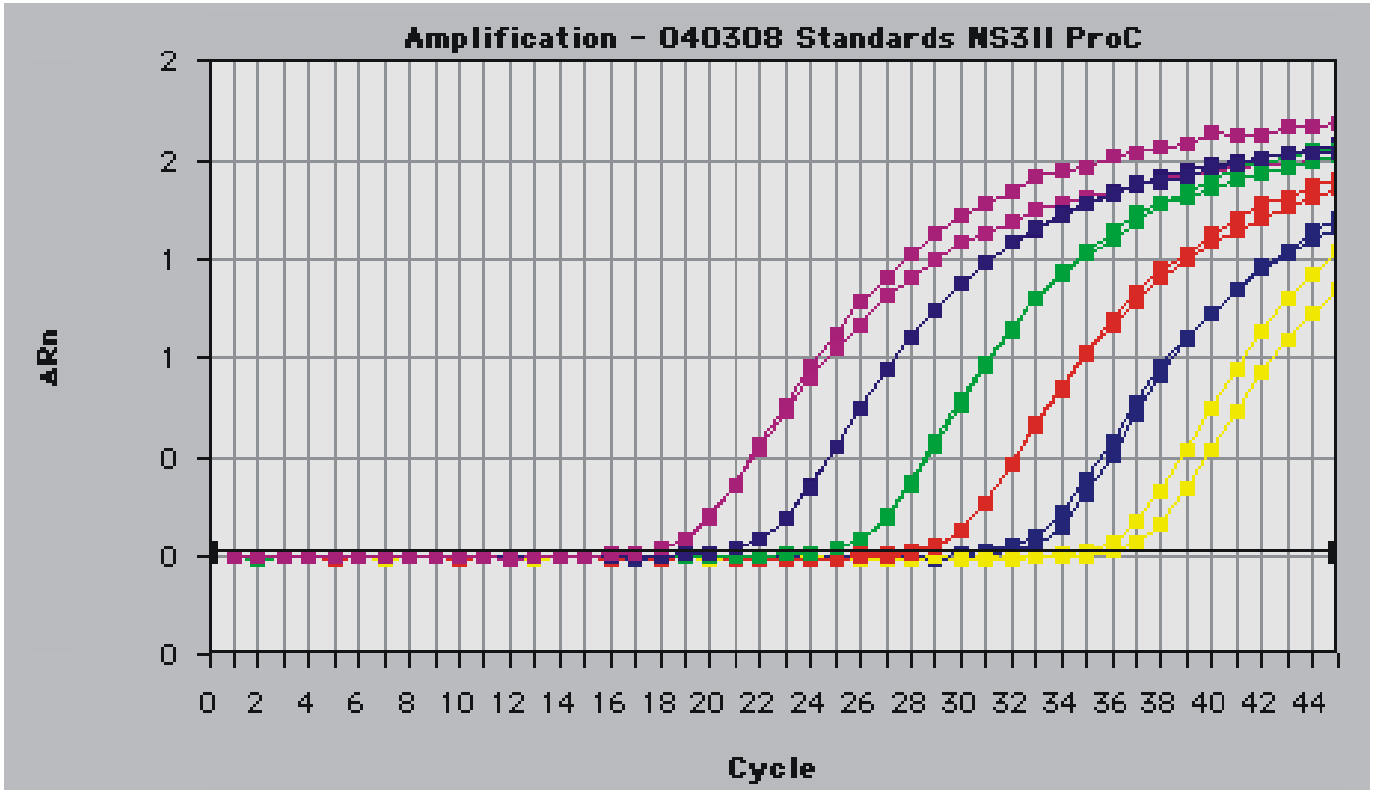
Antikörpernachweis: Virusneutralisationstest (NT)
Immunfluoreszenztest
ELISA

West-Nil-Virus in Deutschland



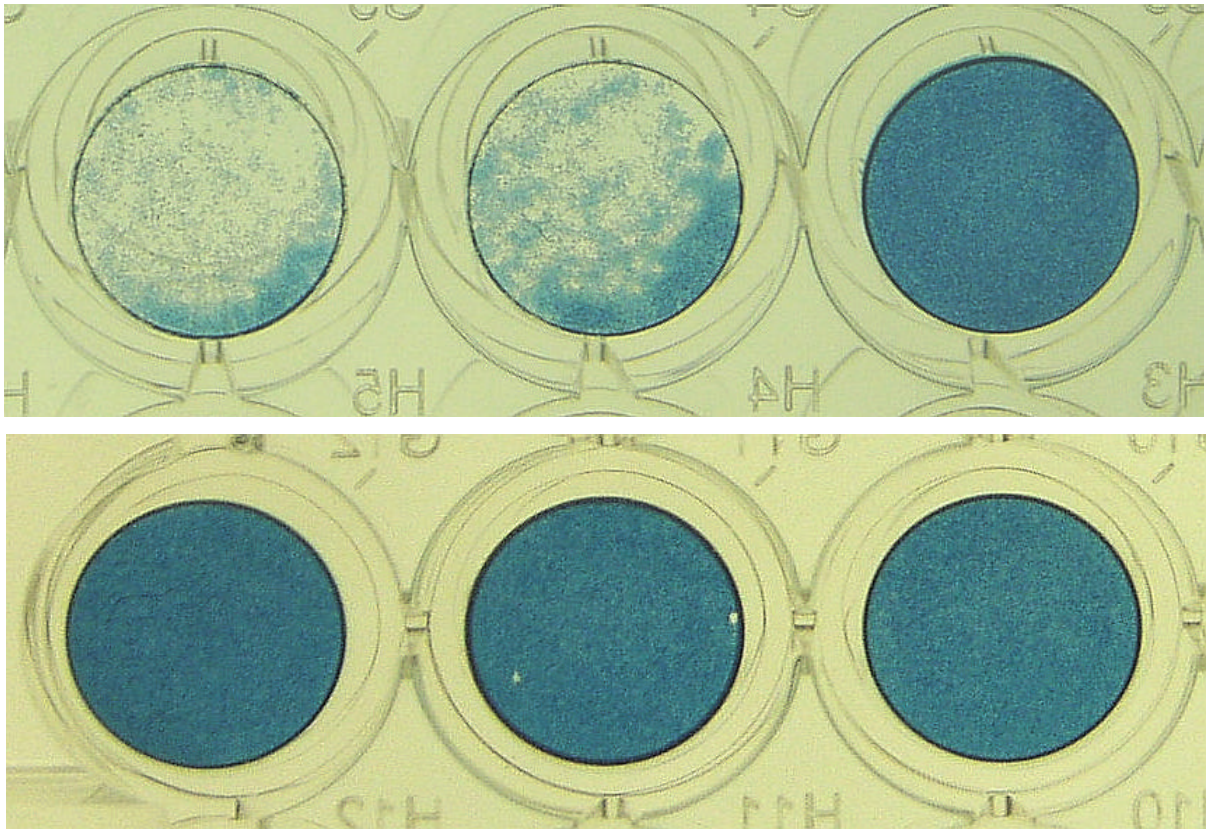
Schematische Darstellung des WNV-Genoms. Angegeben sind die Positionen der etablierten RT-TaqMan PCR-Teste.

West-Nil-Virus in Deutschland



TaqMan-PCR: Repräsentative Amplifikationskurve des 5‘ UTR/Protein C-Tests, wobei die Sensitivität des real-time-Tests angegeben ist (1 x 10^6 – 10 Kopien/µl)

West-Nil-Virus in Deutschland



WNV-Neutralisationstest; obere Reihe: Storchenprobe ohne WNV-spezifische Antikörper; untere Reihe: Storchenprobe mit WNV-spezifischen Antikörpern;

(von links nach rechts: Storchenplasma [2 mal], Plasmakontrolle ohne Virus)

West-Nil-Virus in Deutschland

Weißstörche (Jungvögel) bei der Blutabnahme

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West-Nil-Virus in Deutschland

Vorläufige Ergebnisse des Zugvogelkollektivs

Virusnachweis PCR:

414 Weißstörche (Nestlinge) negativ

Antikörpernachweis (NT):

127 Weißstörche (Nestlinge) 19% positiv

Unterschiede in der Epidemiologie von WNV in der „Alten Welt“ und in den Amerikas

„Old World“

- verschidene WNV sind endemisch in Afrika, Asien, Ozeanien/Australien und Europe
- „natürlich Immunität“ der (Zug)Vögel (und Vertebraten)

Nord- und Südamerika

- ≈ 1999 Einschleppung eines (hochpathogenen) WNV
- keine Immunität in der Vogelpopulation
- Ausbreitung von WNV durch Zugvögel in einer „naïven“ Vogelpopulation durch den Vektor Arthropode/Mücke

Epizootic West Nile Virus in the United States, 1999-2002

- Alpaca
- Horses
- Big Brown Bat
- Little Brown Bat
- Cat
- Dog
- Grey Squirrel
- Llama
- Alligator
- Wolf
- Fox Squirrel
- Sheep
- Eastern Chipmunk
- Rocky Mountain Goat
- Striped Skunk
- Reindeer
- Domestic Rabbit
- Harbor Seal

http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-apr-2001.pdf

HI antibody responses in Egyptian children following recent WN virus infection

Age (yrs.)	Antigen		
	WNV	SLEV	JEV
2	1:640	1:80	1:80
3	1:160	1:80	1:40
1 ½	1:640	1:80	1:80
4	1:640	1:320	1:160

Robert B. Tesh: Cross Immunity: West Nile vs. St.Louis Encephalitis in Areas of Overlap
Fourth National Conference on West Nile Virus in the United States
New Orleans, Louisiana, February 9-11, 2003

HI antibody response of persons previously infected with SLE virus after receiving YF vaccination

Subject	Day post vaccination	HI Antibody Titer (reciprocal)					
		YF	SLE	WN	DEN-1	DEN-2	DEN-3
CM	0	0	40	0	0	0	0
	8	20	80	80	10	0	20
	10	40	160	160	20	40	80
	17	80	320	640	10	80	160
	39	80	320	640	20	80	160
SH	0	0	40	20	0	0	0
	8	20	80	80	0	0	20
	10	160	1280	2560	320	160	640
	17	≥1280	5160	10240	1280	1280	1280
	38	≥1280	2560	5120	640	320	1280

0 = < 10

Robert B. Tesh: Cross Immunity: West Nile vs. St. Louis Encephalitis in Areas of Overlap

Robert Koch-Institut:

Sonja Linke

Heinz Ellerbrok

Matthias Niedrig

Kim Hattermann

Anette Teichmann

Inga Nehlmeier

ROBERT KOCH INSTITUT

Kooperationspartner:

Andreas Kaiser
Institut für Zoologie, Universität Mainz

Hafez Mohamed Hafez
Inst. für Geflügelkrankheiten, Freie Univ. Berlin

Kerstin Borchers
Inst. für Virologie, Veterinärmedizin, FU Berlin

Franz J. Conraths
BFAV Wusterhausen

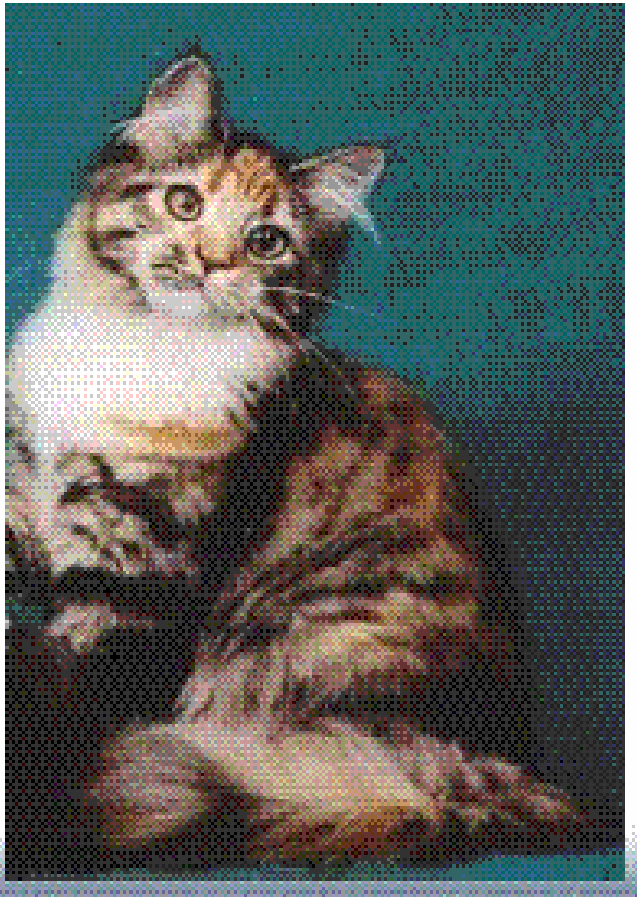
Peter Berthold
MPI für Ornithologie, Radolfzell

Franz Bairlein
Institut für Vogelforschung, Vogelwarte Helgoland

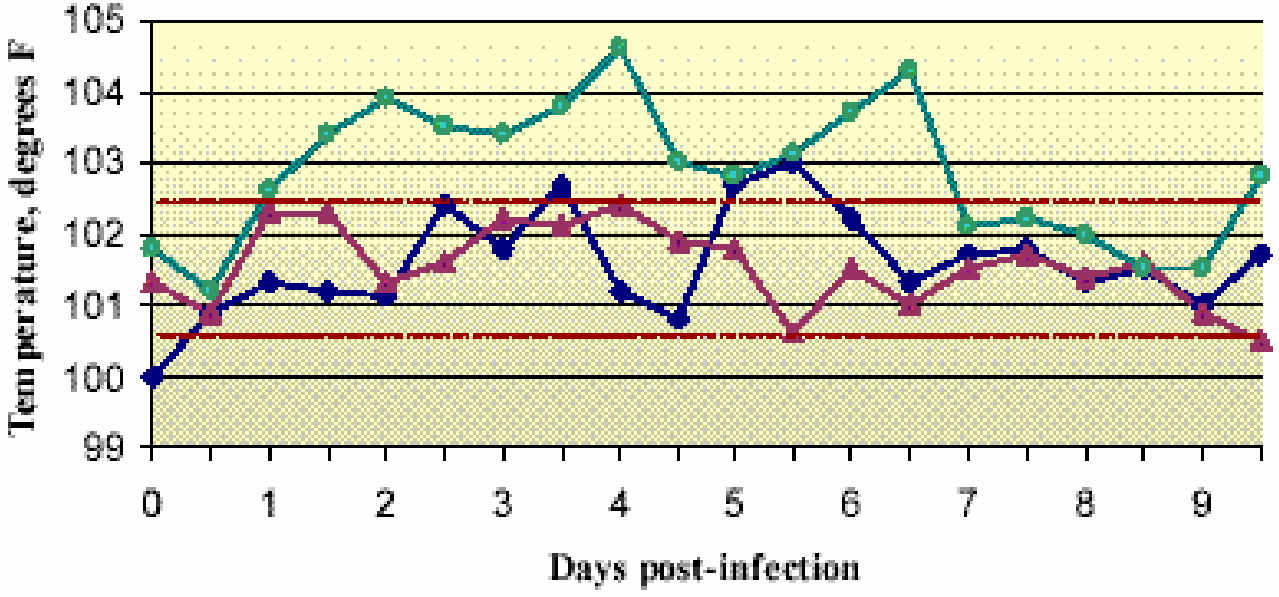
Ulrich Köppen
Beringungszentrale Hiddensee

Hermann Müller/Gerald Fritz Schusser
Med. Tierklinik, Univ. Leipzig

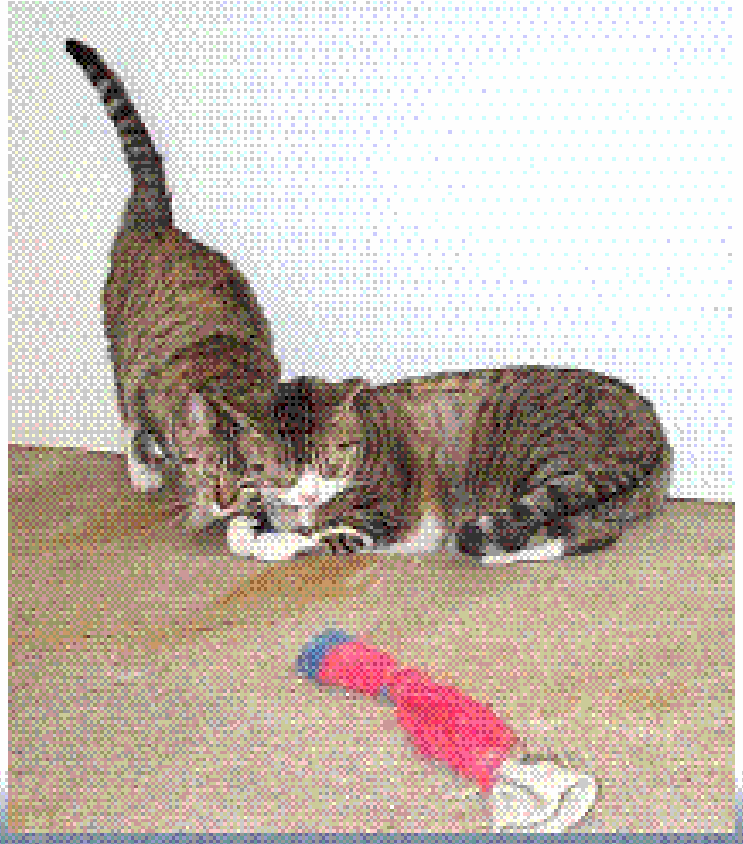
Feline Research



Cats as West Nile virus hosts



Clinical Disease in Feline



- Mild clinical disease, lasting two to three days

Feline Oral Transmission Studies

Oral Transmission

- 2 cats fed a West Nile virus infected mouse, one daily for 3 days
- 2 cats fed a single West Nile virus infected mouse
- Clinical exams, BID serum for virus isolation

Feline Oral Infections

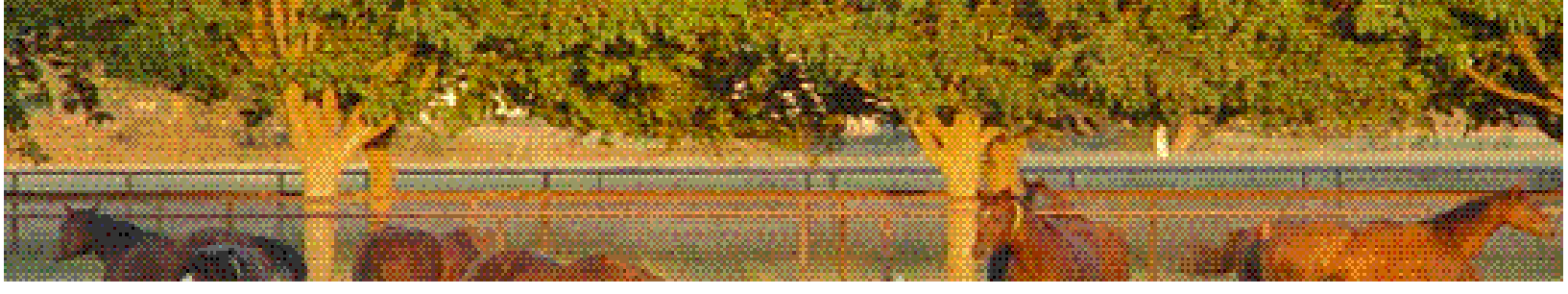
- Cats were readily infected by consuming infected mice (4/4)
- No clinical disease
- Occasional mosquitoes may become infected by feeding on infected cats

Feline Research Results

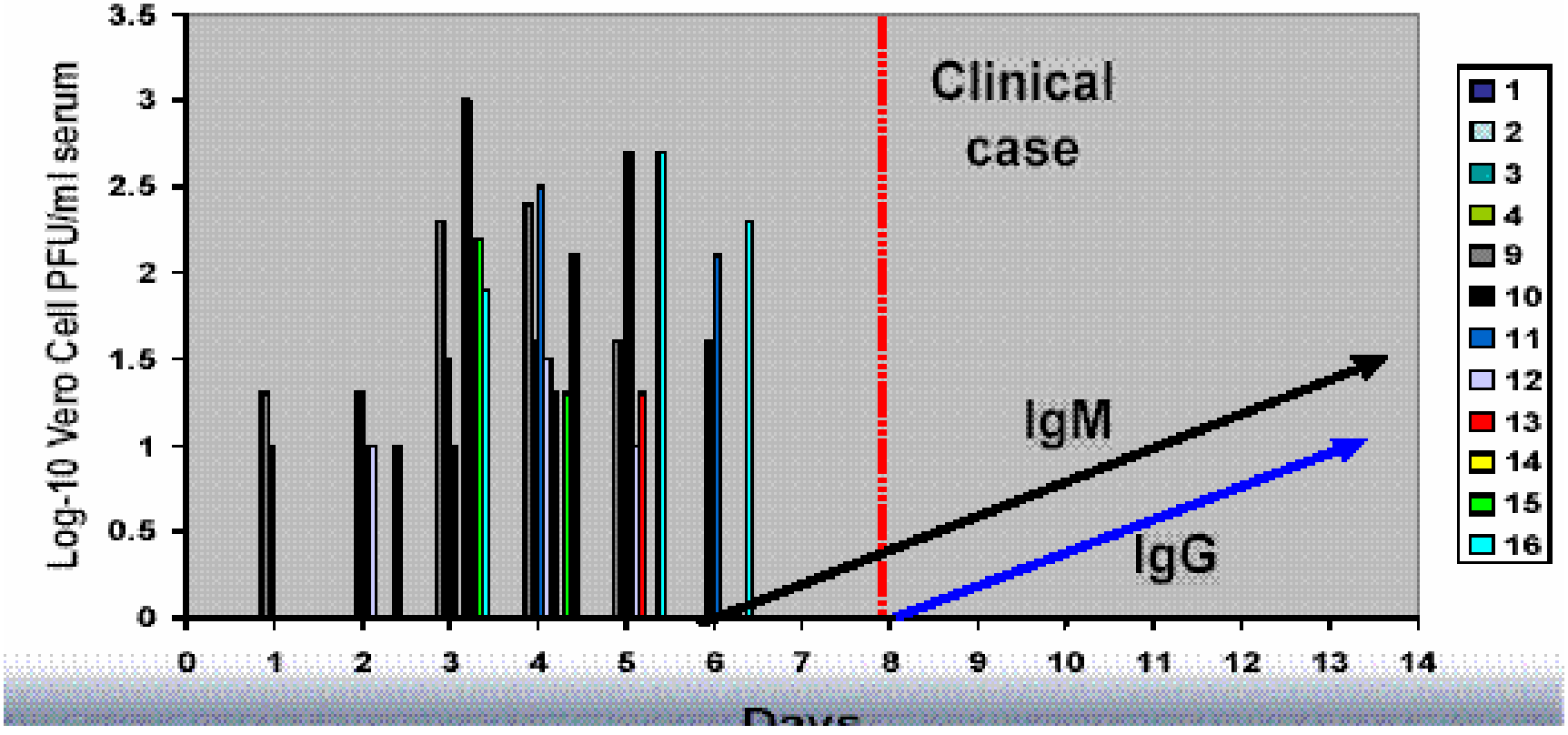
- Virus capable of replicating (12/12)
- Virus not isolated from saliva
- Mild, nonspecific disease
- Inconsistent hematologic disturbances, fever
- Cats develop a level of viremia that may be capable of infecting mosquitoes

Equine Background

- West Nile virus responsible for outbreaks of encephalomyelitis in humans and equids
- Concerns about equine
  - Sentinel host for humans
  - Dead end or amplification host
  - Public Health ramifications
- Incidence in 2002: ~12,000 cases in US
  - Clinical attack rate ~ 0.1 (10%)
  - Roughly 1 in 3 affected animals die or are euthanized

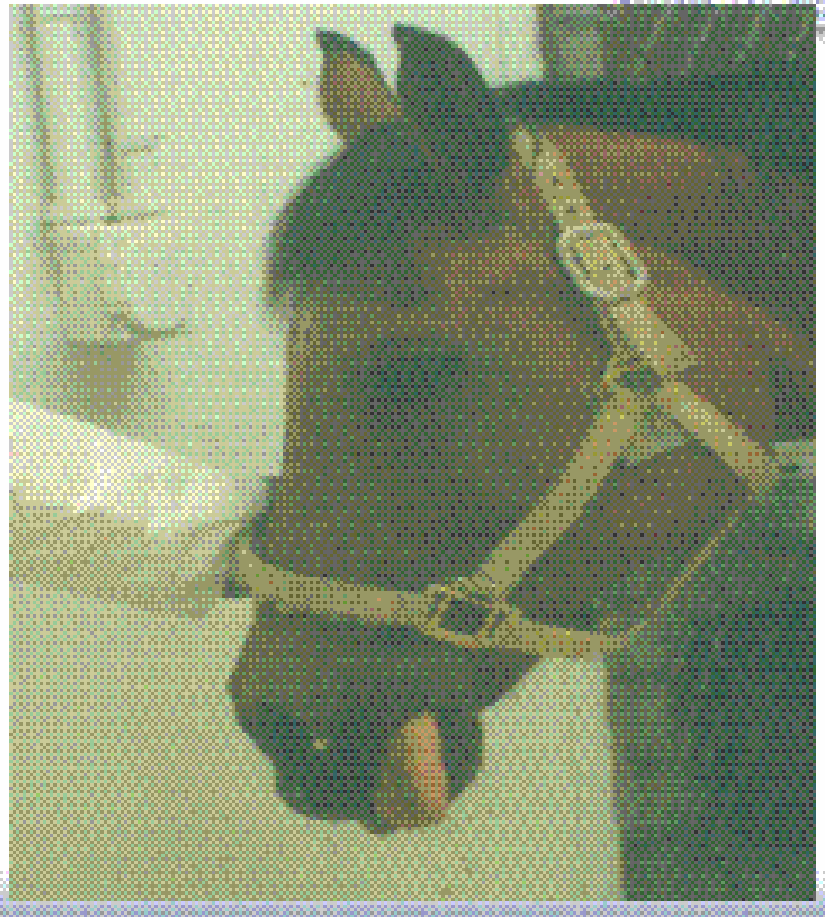


Equine Serology/Viremia Time Line



Equine Clinical Case

- CNS Disease
  - Ataxia
  - Circling
  - Hind limb weakness
  - Proprioceptive deficits
  - Lip droop/paralysis



Horse 11, Tissue Virus (Log10 PFU/gram)

Medulla	6.8	Cervical cord	5.0
Cerebellum	5.0	Thoracic cord	4.0
Frontal cortex	5.2	Lumbar cord	4.3
Occip cortex	4.3	Radial n.	neg
Hippocampus	3.3	Spleen	neg
		Liver	neg

Equine Conclusions

- Viremias
  - Highest viremia
    - Day-3 post infection
    - 3 Log-10 Vero cell PFU/ml serum
  - One clinical case
    - Apparent to inapparent = 1:11
    - Virus titers in brain and spinal cord, day-9
      - Log $10^{4.0}$ to $10^{6.8}$ PFU/gram
- None of the virgin mosquitoes became infected

Equine Conclusions

- Equines infected with WNV develop viremias of low magnitude and short duration
- Infected horses are unlikely to serve as amplifying hosts for WNV in nature
- Care should be taken on postmortem exam
- Clinical attack rate is roughly 10% in experimental and field studies
- Clinical signs usually characteristic of encephalomyelitis

Canine Research

Experimental Infection of Canine with WNV

- Is WNV readily transmitted to dogs by feeding of infected mosquitoes?
- What is the duration and magnitude of viremia and antibody response
- Are dogs likely to serve as amplifying hosts?

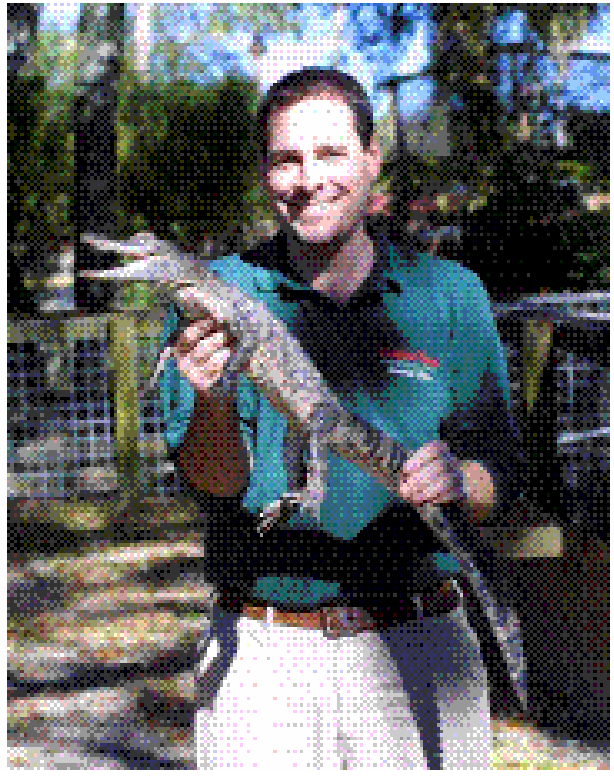
Experimental Infection of Canine with WNV

- There was no evidence of clinical disease
- Mild leukopenia
- Virus capable of replicating (4/4)
- Virus was not isolated from saliva
- Dogs are not likely to be amplifying hosts

Alligators

- Epizootic characterized by neurologic disease which occurred at a 9,000-head alligator farm in Florida.

- Approximately 300 alligators (*Alligator mississippiensis*) died during this outbreak



Alligators

- Of the tissues sampled, liver had the highest viral loads (maximum $10^{8.9}\log_{10}$ pfu/0.5cm^3)
- Brain and spinal cord had the lowest viral loads (maximum $10^{6.6}\log_{10}$ pfu/0.5cm^3) each
- Viral loads in plasma ranged from $10^{3.6}$ to $10^{6.5}$ $\log_{10}$ pfu/mL

Effect of route of administration of a DNA West Nile virus vaccine on the protection of fish crows from challenge with virulent West Nile virus

Treatment[a,b]	No. tested	% seropositive[c]	% viremic	Peak viremia[d]	% survival
Room control	10	0	0	<1.7 (0.0)	100
IM	9	56	67	2.9[b] (0.4)	100
Oral	8	0	88	5.2[c] (0.8)	50
Placebo	10	0	100	4.3[c] (0.3)	50

[a]IM, intramuscularly. Crows were inoculated IM with 0.5 mg of the DNA vaccine. Oral, crows were given 0.5 mg of the DNA vaccine orally. Placebo, crows were inoculated IM with 0.5 mg of nonspecific DNA and given 0.5 mg of nonspecific DNA orally.

[b]Room controls were placebo inoculated and then challenged with diluent.

[c]Percentage of crows whose serum produced $\geq$80% neutralization at 1:20 dilution.

[d]Logarithm$_{10}$ mean peak viremia in crows bled every third day after challenge (S.E.). No virus was detected in any of the room control birds and a value of 1.7 was assigned to birds from which no virus was detected for calculation of mean and S.E. Means followed by the same letter are not significantly different at a = 0.05 by student t test.

Viremia levels in fish crows that survived or died after challenge with virulent West Nile virus

	Survived (peak)		Died	
Treatment[a]	No.	Viremia[b]	No.	Peak viremia[b]
IM	9	2.9 (0.4)	0	n/a
Oral	4	3.6 (0.7)	4	6.9 (1.0)
Placebo	5	3.8 (0.4)	5	4.8 (0.4)

[a]IM, intramuscularly. Crows were inoculated IM with 0.5 mg of the DNA vaccine. Oral, crows were given 0.5 mg of the DNA vaccine orally. Placebo, crows were inoculated IM with 0.5 mg of nonspecific DNA and given 0.5 mg of nonspecific DNA orally.

[b]Logarithm mean peak viremia in crows bled every third day after challenge (S.E.). A value of 1.7 was assigned to birds from which no virus was detected for calculation of mean and S.E.