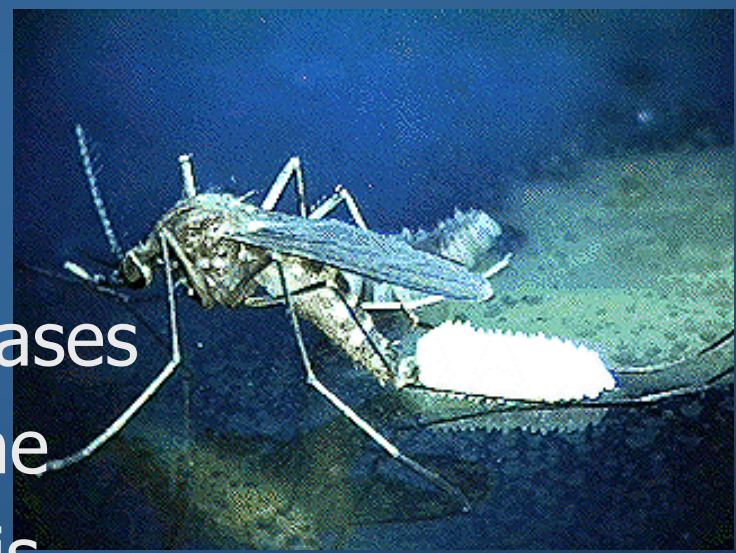
Overwintering and vertical transmission



William K. Reisen
Arbovirus Field Station
Center for Vectorborne Diseases
School of Veterinary Medicine
University of California, Davis

Talk Content

- Evidence for overwintering
  - Virus genetics
  - Infections during winter
- Possible mechanisms
  - Continued transmission
  - Avian chronic infections
  - Mosquito vertical transmission

Phylogenetic Relationships Among West Nile Virus Strains Collected in New York, 1999-2002

Strain	Year	Location	Source	Accession #
Eg101	1951	Egypt	Human	Af260968
RO97-50	1996	Romania	Cx. pipiens	Af260969
NY99	1999	New York	Equine	Af260967
NY00a (3000017)	2000	Staten Island, NY	Cx. pipiens	Af346309
NY00b (3100365)	2000	Staten Island, NY	Cx. pipiens	Af346310
NY01a (32010157)	2001	Suffolk CO, NY	Cx. pipiens/restuans	n/a
NY01b (01-1398)	2001	Suffolk CO, NY	American Crow	n/a
NY02a (0055)	2002	Schenectady, NY	Squirrel	n/a
NY02b (3557)	2002	Rockland CO, NY	American Crow	n/a
NY02c (2640)	2002	Niagara CO, NY	American Crow	n/a
NY02d (2395)	2002	Niagara CO, NY	American Crow	n/a
NY02e (2684)	2002	Clinton CO, NY	American Crow	n/a

Ebel, GD, Jan-02

Phylogenetic Relationships Among West Nile Virus Strains Collected in New York, 1999-2002

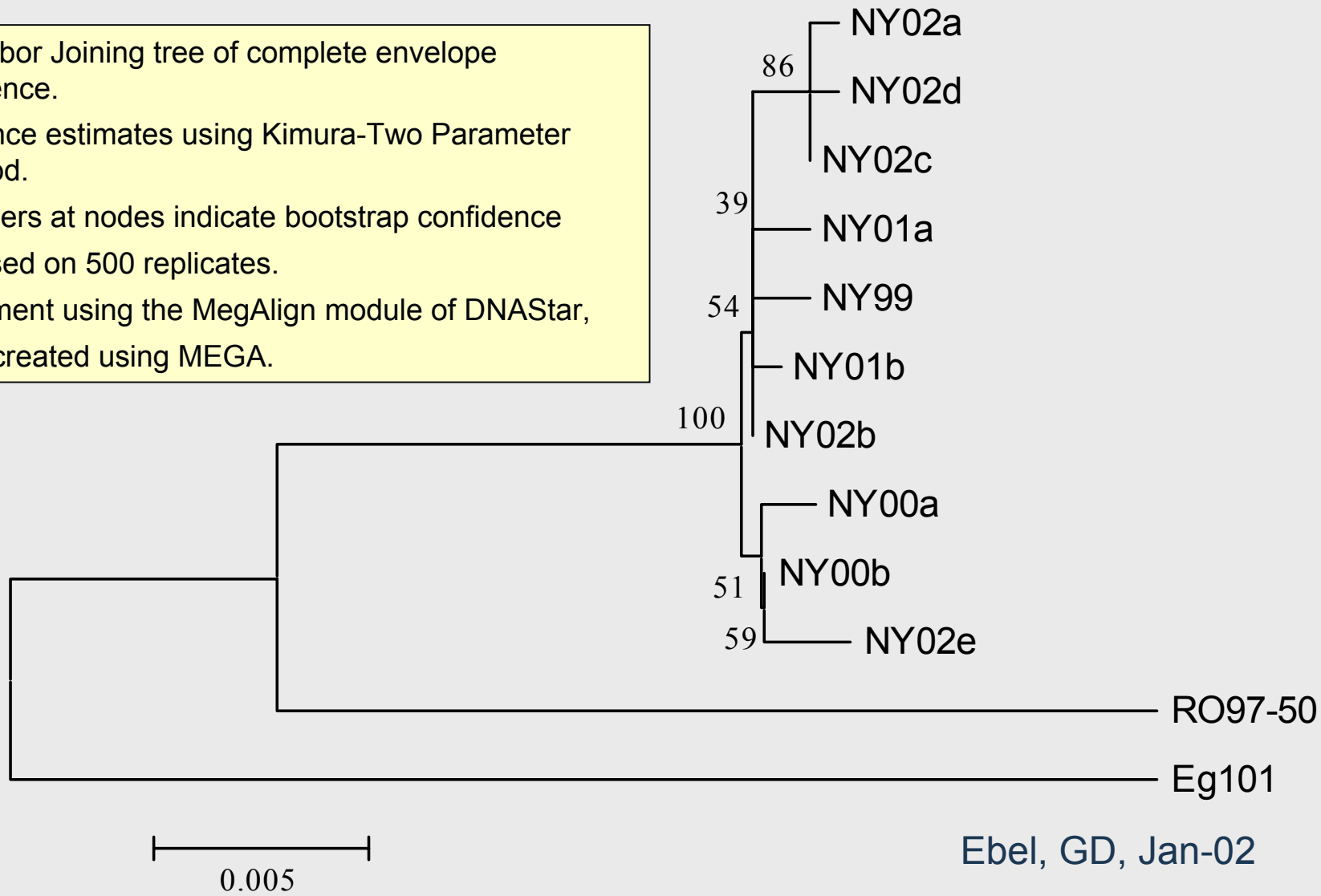
Neighbor Joining tree of complete envelope sequence.

Distance estimates using Kimura-Two Parameter method.

Numbers at nodes indicate bootstrap confidence based on 500 replicates.

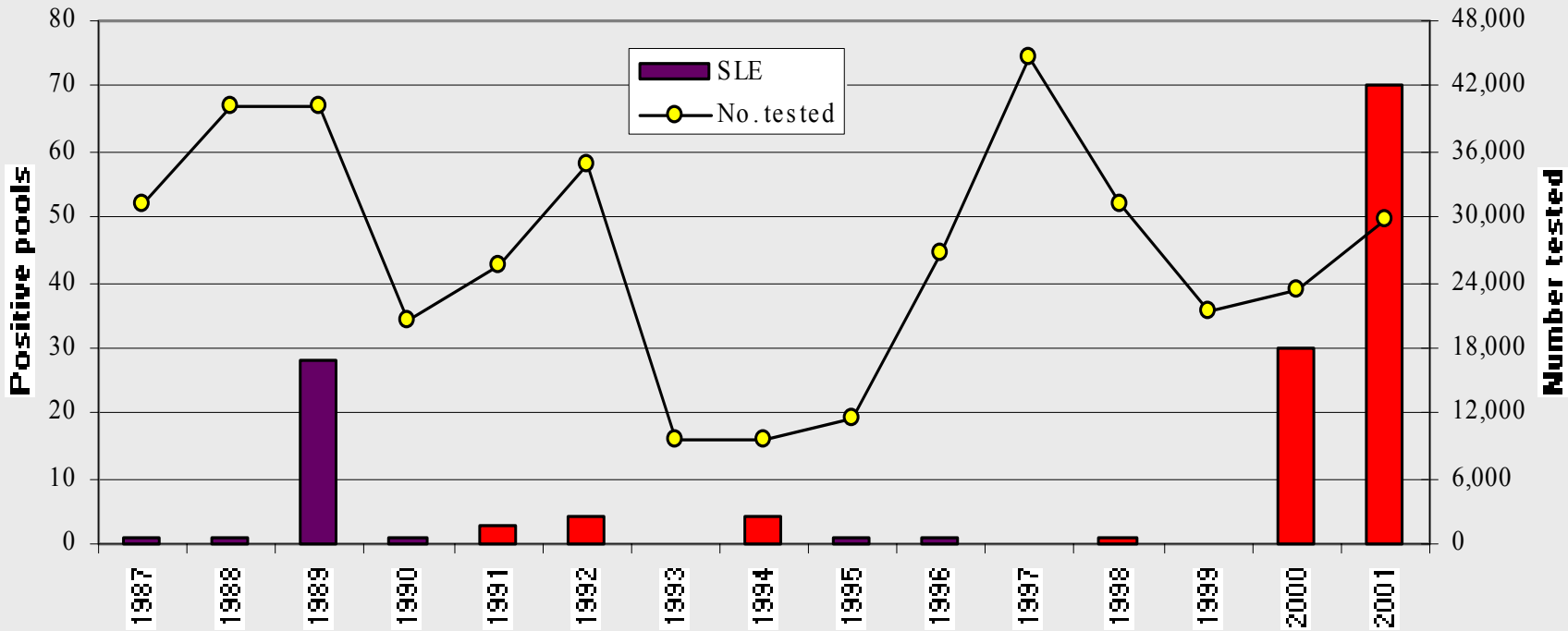
Alignment using the MegAlign module of DNAStar,

Tree created using MEGA.



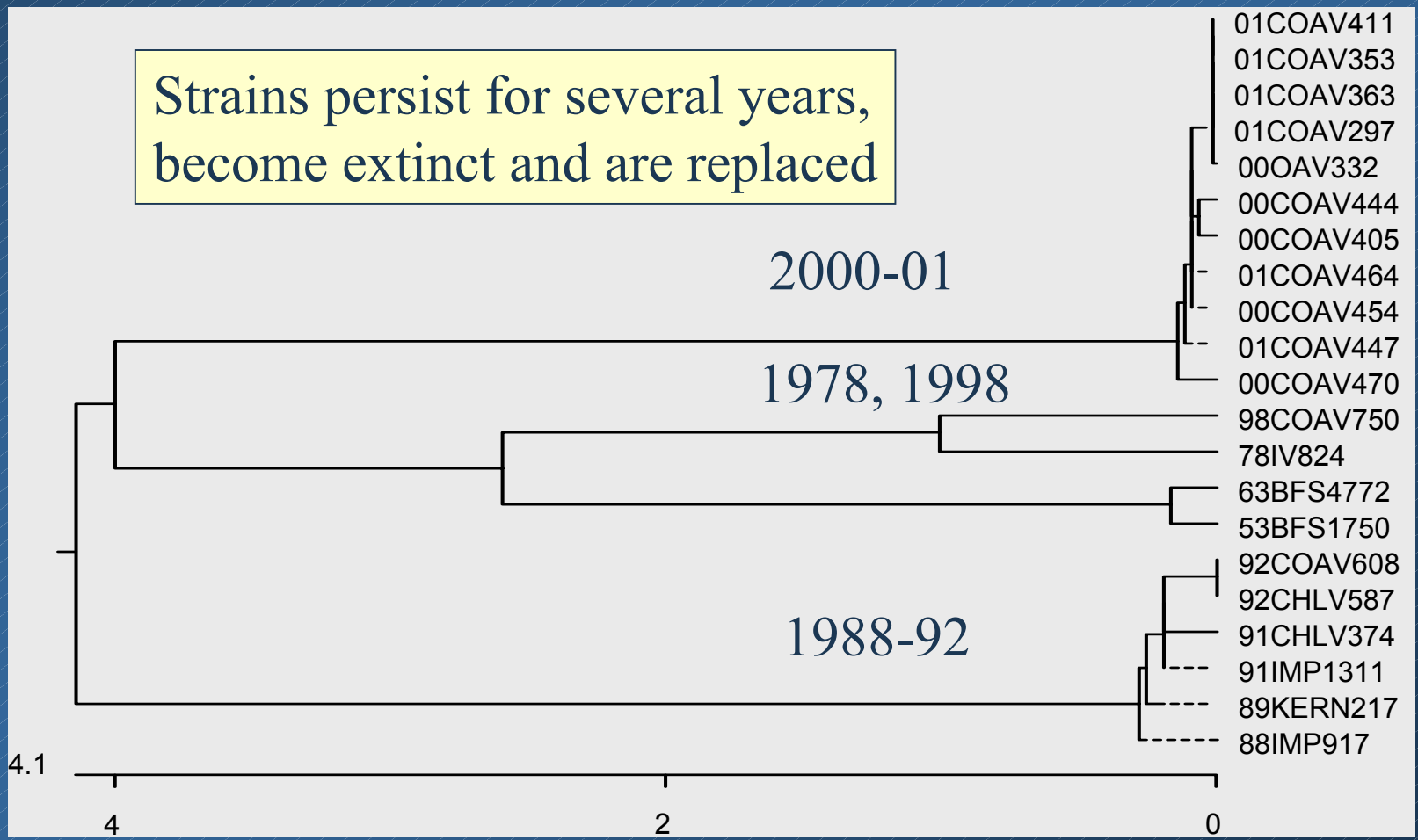
Ebel, GD, Jan-02

Number of mosquitoes tested and viruses isolated, Coachella Valley, 1987-2001



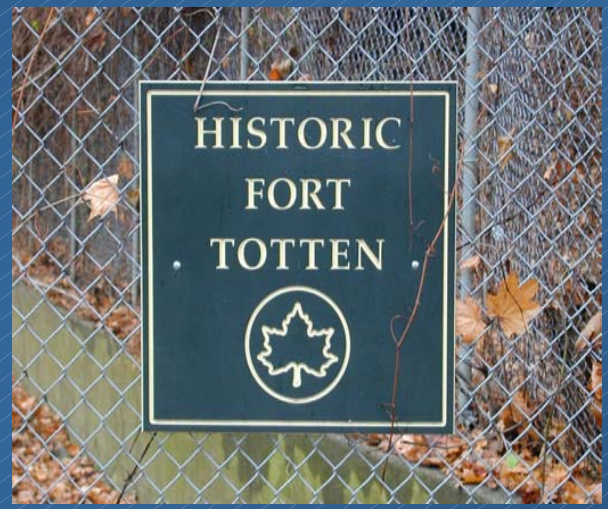
Selected SLE isolates from years in red were sequenced

Differences among strains of SLE isolated from Coachella and Imperial Valleys



Content

- Evidence for overwintering
  - Virus genetics
  - Infections during winter
- Possible mechanisms
  - Continued transmission
  - Avian chronic infections
  - Mosquito vertical transmission



Slide from T. Andreadis

Winter *Culex* infections in New York, 2000

- 3 of 91 pools [2,383 mosquitoes] of *Culex* mosquitoes from NYC positive for WN RNA by TaqMan; 1 infectious virus isolate made in Vero cell culture; other isolation attempts were negative

Note: pools frozen immediately

Nasci et al. 2001. EID 7(4):742

Overwintering Culex pipiens *collections, Ft. Totten, Queens, NY, 2001**

	Date collected	
	Dec 13	Mar 12
No. collected	3,968	1,829
Days held at 22^{0}C	5	3
Pool size	10	10
Vero cell culture	Negative	Negative
Taq-Man assay (mosquito pools)	Negative	Negative

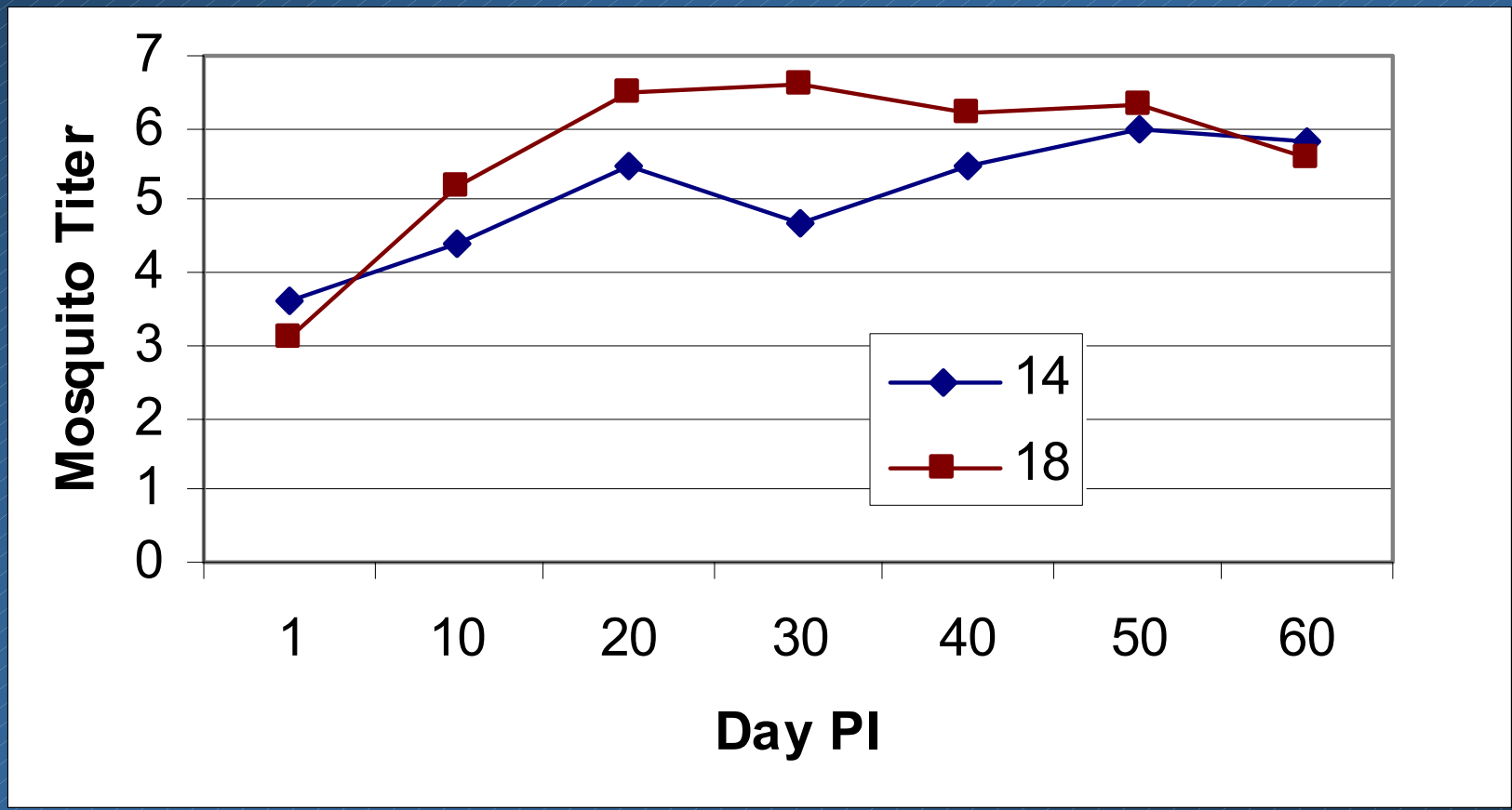
*T. G. Andreadis, CT Agr Exp Stn & V. Kulasekera, NYC DPH

Effect of holding temperature on WN replication in Cx. p. pipiens

Holding temperature (days at)			No.	Infected	Dissem.
18C	10C	26C	tested	(%)	(%)
7	28	0	24	0	0
7	42	0	26	0	0
7	41	1	16	31	0
7	39	3	13	69	0
7	37	5	13	69	0
7	35	7	13	54	0
7	21	7	24	96	4
7	35	14	12	100	42
0	0	14	23	96	48
per os, Crow isolate from NY					

Turell et al. JME 38: 462

Replication of WN virus in Cx. univittatus at 2 temperatures [per os, H442 SA strain]



Cornel et al. 1993. JME 30: 449

Culex pipiens *infection with SLE: similar findings with a different explanation*

- Bailey et al. 1978. Science 199: 1346 reported isolation of SLE from *Cx. pipiens* collected from old forts in Maryland during winter. Females held 7-21 days and then some fed. 2/62 blood fed and 0/53 non-blood fed pools positive by mouse brain inoculation.
- Bailey et al. 1982. AJTMH 31: 1054 reported that *Cx. pipiens* females in diapause that blood fed experimentally survived winter equally well as unfed females and implied that small meals taken during fall were the source of previous SLE infections.

Content

- Evidence for overwintering
  - Virus genetics
  - Infections during winter
- Possible mechanisms
  - Continued transmission
  - Avian chronic infections
  - Mosquito vertical transmission

WN horse cases reported during winter

State	County	Collection Dates
LA	Marion	20020103
LA	Cameron	20020116
FL	Polk	20020207
FL	Dade	20020323
FL	Duval	20020411
FL	Calcasieu	20020415

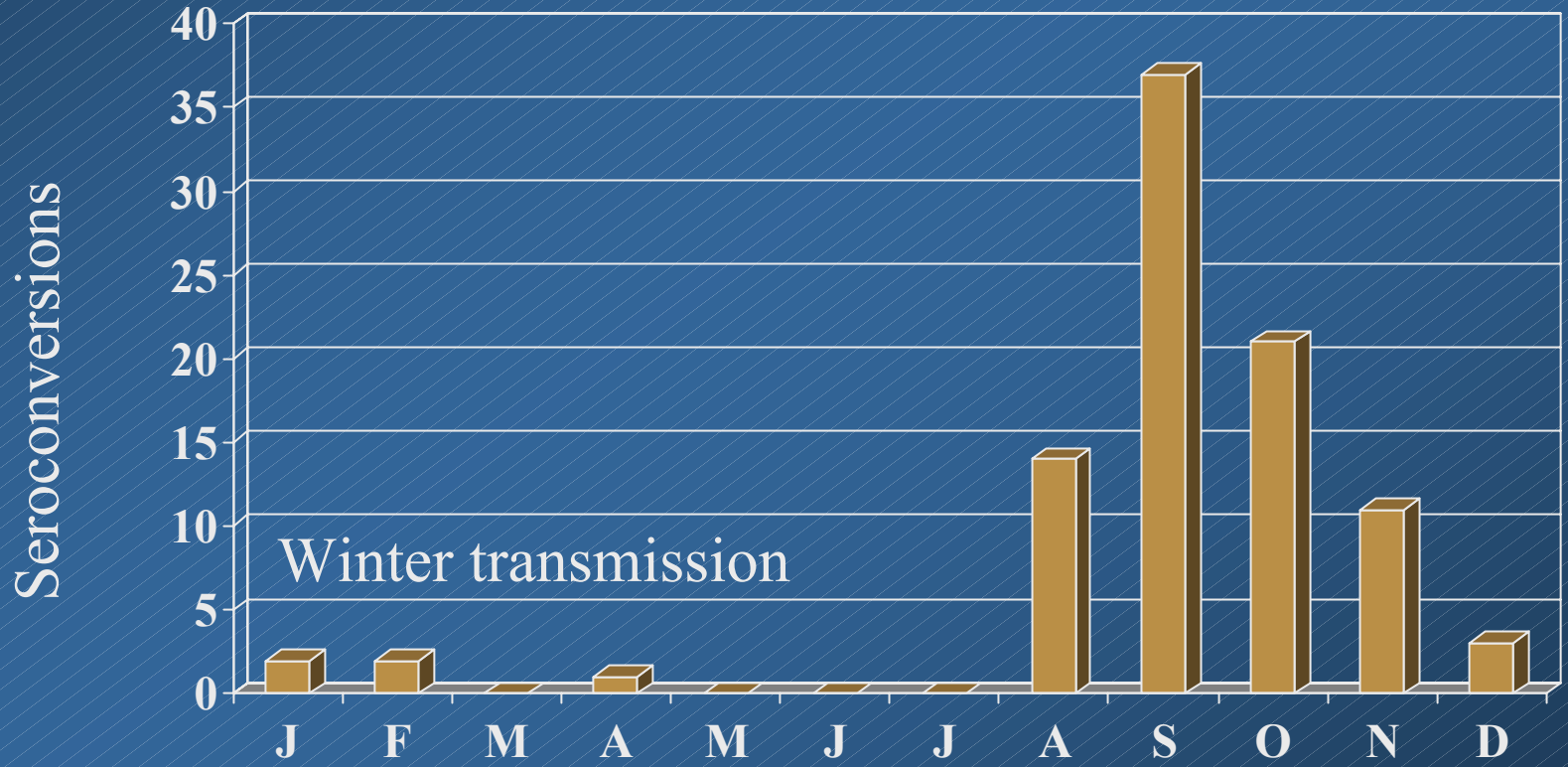
Data From CDC ArbNet

WN isolations from birds during winter: continued transmission?

state	County	Common_Name	date_collected
NY	West Chester	Red-tailed hawk	20000219
FL	Liberty	Other Species	20020110
FL	Alachua	Swallow-tailed Kite	20020129
LA	East Baton Rouge	Northern Cardinal	20020213
LA	East Feliciana	Blue Jay	20020219
FL	Palm Beach	Other Species	20020320

Data from AM Kipp. CDC ArboNet query

Chicken seroconversions to SLE, Sarasota, FL, 1978-88



Drawn from Day et al. 1991. JME 28: 19

Content

- Evidence for overwintering
  - Virus genetics
  - Infections during winter
- Possible mechanisms
  - Continued transmission
  - Avian chronic infections
  - Mosquito vertical transmission

Chronic avian infections, virus isolation

	WEE			SLE**
	Tested	Pos*	%	Tested
Brewer's blackbird	23	1	4.3	1
California quail	18	0	0.0	35
Chinese spotted dove	2	0	0.0	0
Cowbird	99	2	2.0	29
House finch	69	1	1.4	30
House sparrow	122	5	4.1	41
Mourning dove	39	0	0.0	57
Red-winged blackbird	53	3	5.7	19
Savannah sparrow	4	0	0.0	0
Tricolored blackbird	97	3	3.1	128
White-crowned sparrow	0	0	0.0	29
Yellow headed blackbird	8	0	0.0	0
	534	15	2.8	369
*infected >13 d post inoculation				
** All negative				

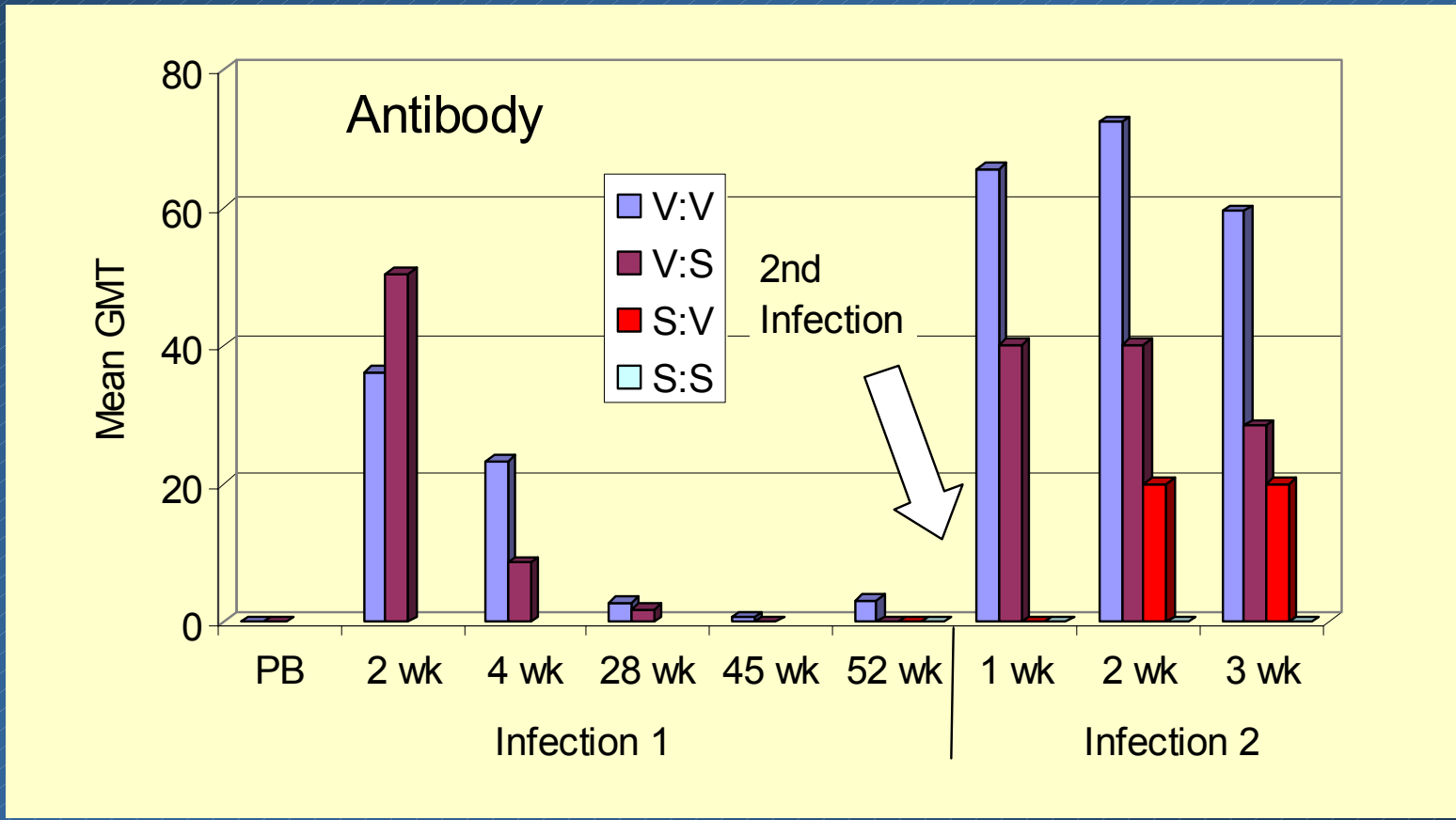
Reeves et al. 1958
Proc.Soc.Exp.Bio.Med.
97: 733

Chronic avian infections, RT-PCR

	WEE				SLE		
Species	Tested	Pos	%		Tested	Pos	%
Brewer's sparrow	9	1	11.1		6	0	0.0
House finch	14	1	7.1		15	2	13.3
Mourning dove	8	3	37.5		8	0	0.0
Orange-crowned warbler	0				4	1	25.0
Song sparrow	7	0	0.0		6	1	16.7
Western scrub jay	5	0	0.0		5	2	40.0
White-crowned sparrow	13	1	7.7		9	3	33.3
21 species	102	0	0.0		96	0	0.0
Totals	158	6	3.8		149	9	6.0
Pos >6 wks post infection							

[Reisen et al. unpublished]

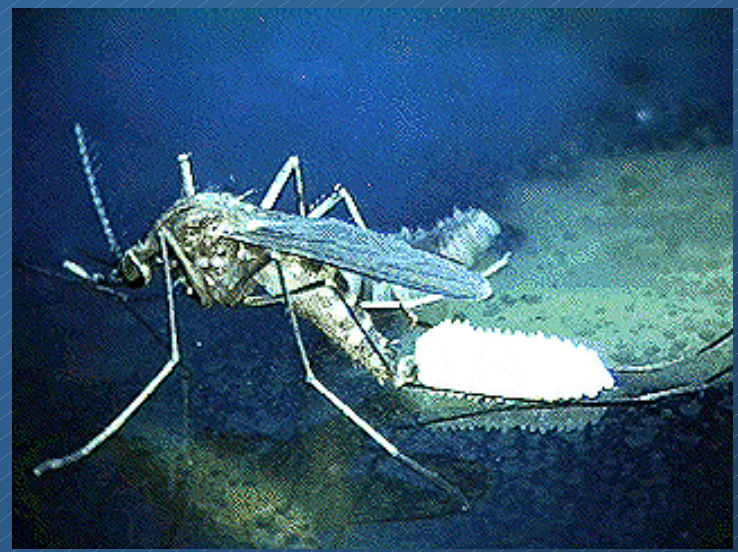
Antibody response to second infection may prevent relapse of chronic infection with SLE



Reisen et al. JME [in press]

Content

- Evidence for overwintering
  - Virus genetics
  - Infections during winter
- Possible mechanisms
  - Continued transmission
  - Avian chronic infections
  - Mosquito vertical transmission



Vertical transmission of WN by Pakistan mosquitoes

Species	F1 tested	Pos pools /total	MFIR/1,000
Ae. albopictus	646	5/14	7.7
Ae. aegypti	3,482	23/71	6.6
Cx. tritaeniorhynchus	1,585	3/34	1.9

[per os infection, Egypt 101 strain]

Baqar et. al. 1993. AJTMH 48: 757

Vertical transmission of West Nile virus by NY and HI mosquitoes

Species	Rearing Temp	Male	Female	Total	MFIR /1,000
Cx. p. pipiens	18	722(2)	695(0)	1,417(2)	1.4
[New York]	26	911(3)	962(1)	1,873 (4)	2.1
Ae. albopictus	20	1,531(0)	1,444(0)	3,975(0)	0
[OAHU]	26	5,173(0)	5,295(0)	10,468(0)	0
[intrathoracic inoculation, Crow 397-99 from NY]					

Turell et al. 2002. JME 39: 640

Vertical transmission of West Nile by California mosquitoes

Species	Egg batch (egg rafts)	No. pos. male pools/ tested	No. pos. female pools/ tested	Total pos pools/tested	MFIR/1,000
Cx. p. pipiens	1 (30)	0/90	0/86	0/176	0
	2 (9)	0/13	0/8	0/21	0
Cx. p. quinq	1 (9)	2/15	0/13	2/28	3
Cx. tarsalis (Yolo Co.)	1 (16)	10/44	5/42	15/86	6.9
Cx. tarsalis (Coachella)	1 (31)	0/50	0/52	0/102	0

[intrathoracic inoculation, NY Strain of WN]

Goddard et al. unpubl.

Vertical transmission: natural occurrence in Africa

- Miller et al. 2000. First field evidence for natural vertical transmission of West Nile virus in *Culex univittatus* complex mosquitoes from Rift Valley Province, Kenya. AJTMH 62: 240.

Isolation made from a male mosquito verifying vertical and transtadial transmission. Strain similar to an isolate from Romania.

Importance of vertical transmission

- Overwintering mechanism
- Enhance amplification rate
- If linked to autogeny, could allow passage between generations without blood feeding.

QUESTIONS????

- Will WN persist?
- At what level?
- By what mechanism?
- Role of vertical transmission in summer amplification?