

**Molecular Detection of Canine Heartworm
(*Dirofilaria immitis*)
in Mosquitoes by PCR (Polymerase Chain Reaction)
in San Joaquin County, 2010-2011**

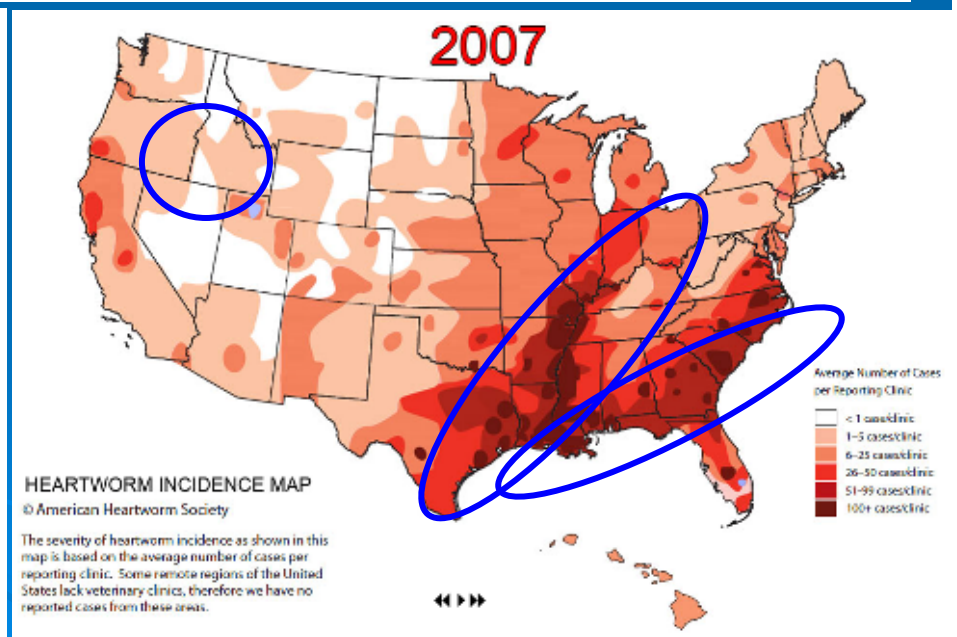
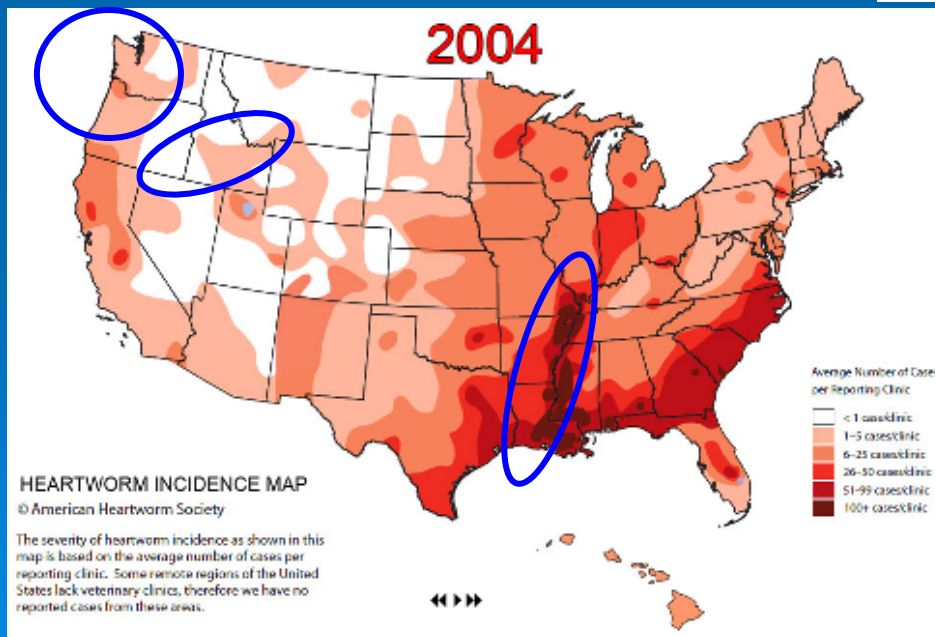
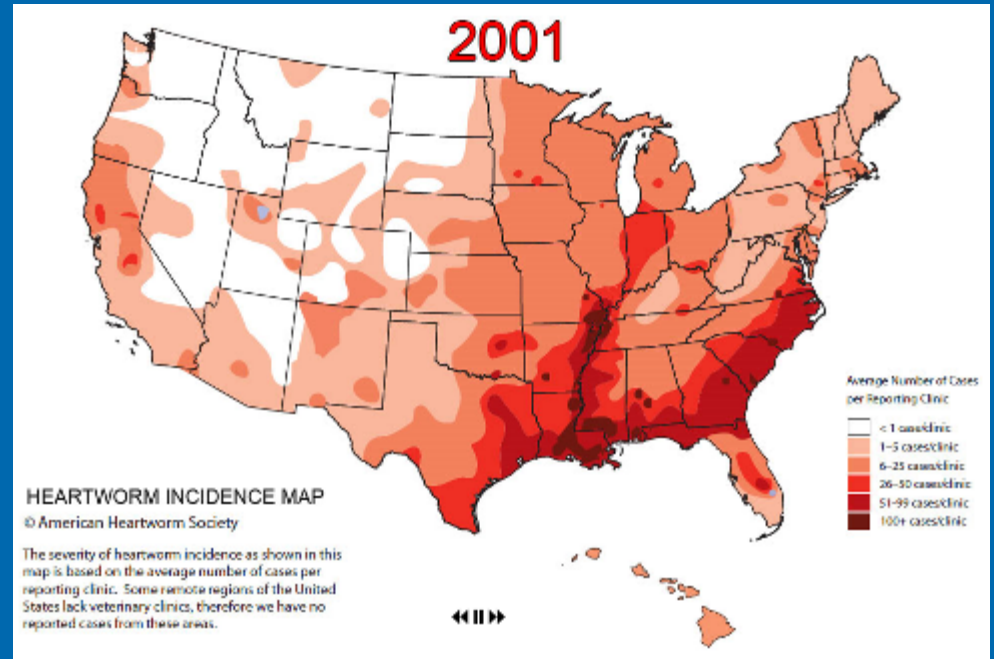
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March 2011



Dog heartworm cases on the rise across United States



San Joaquin County Survey

Year	2004	2005	2006	2007	2008	2009	2010	2011
Cases	1		4	25	29	11	14	6

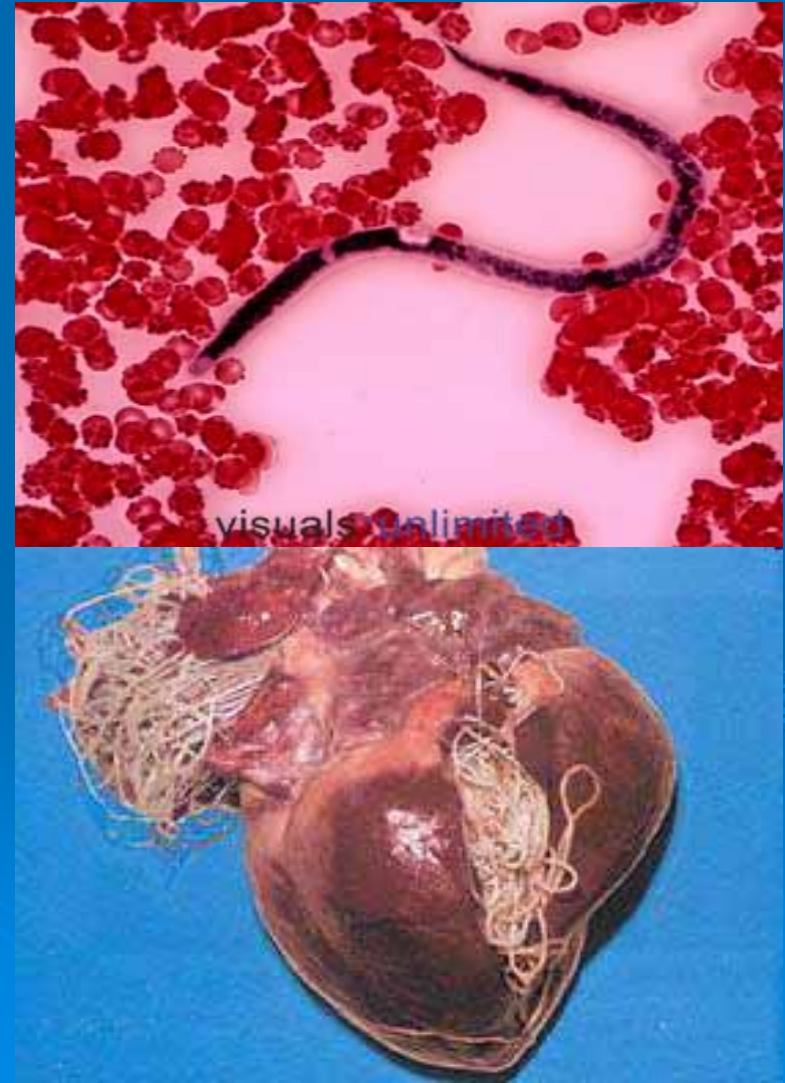
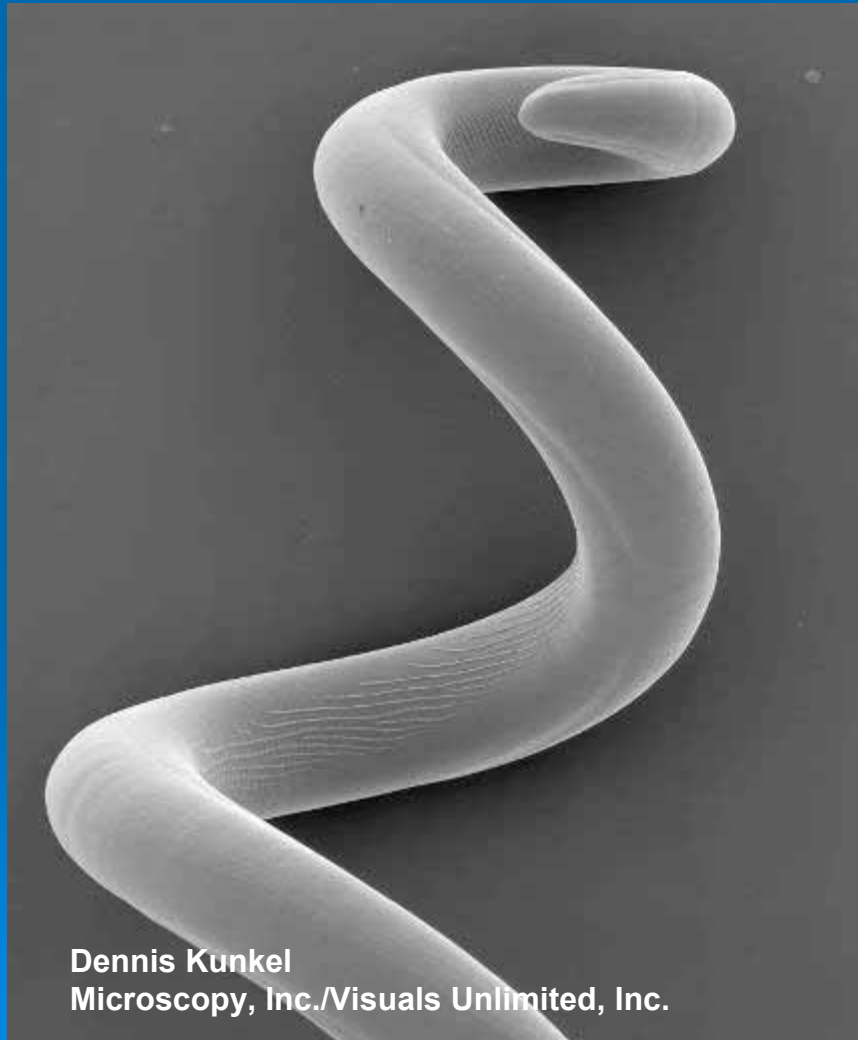
Cross-Town Animal Hospital

Year	2004	2005	2006	2007	2008	2009	2010	2011
Cases				12	6	1	4	

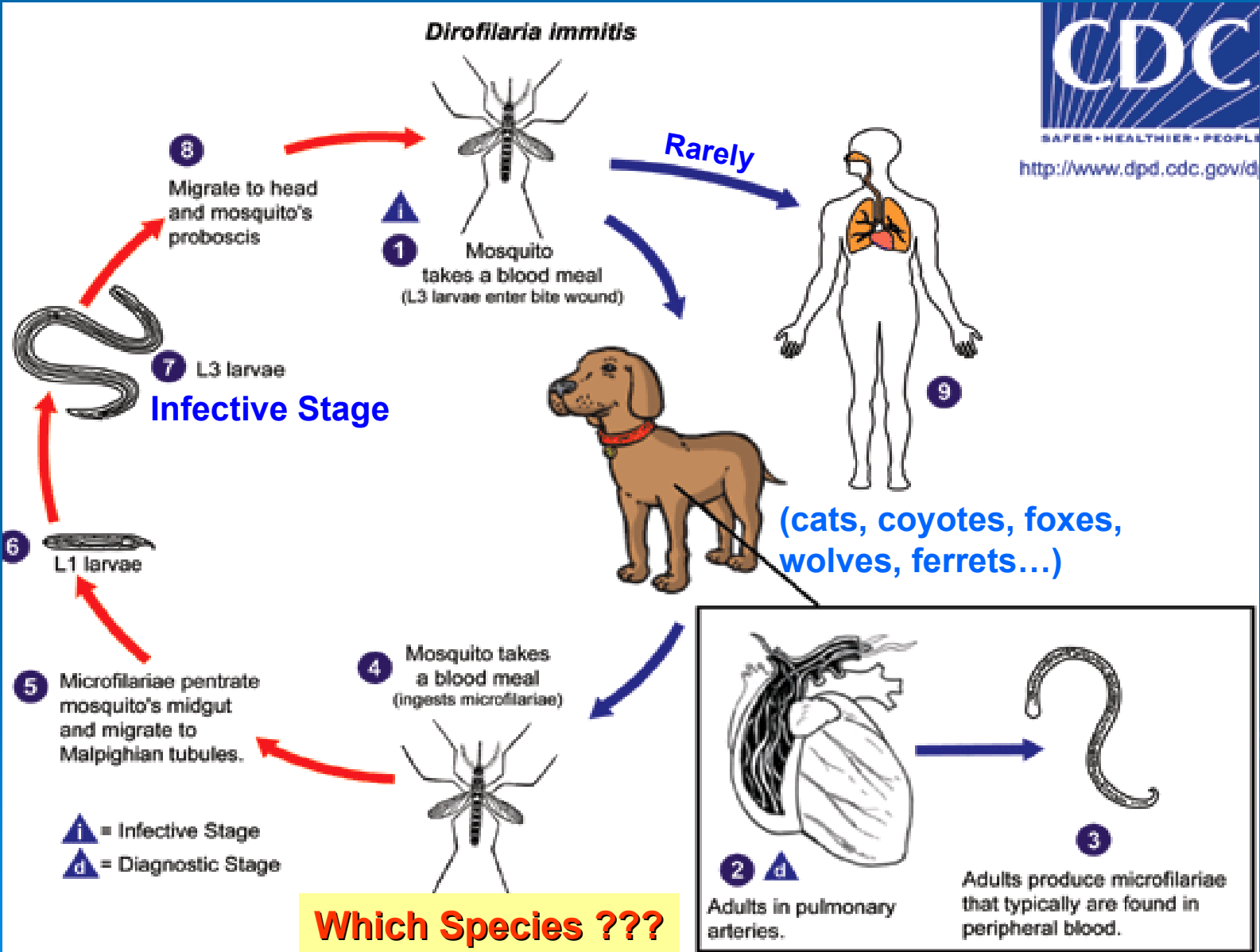
(No cases in 13 years until 2007)

What is Dog Heartworm *D. immitis*?

- Parasitic nematodes living in the heart and blood vessels of animals



Transmission Cycle



Mosquito Species Vectoring *D. immitis*

World wide > 60

US ≥ 24

CA 3 +1

San Joaquin County ?

Table 1

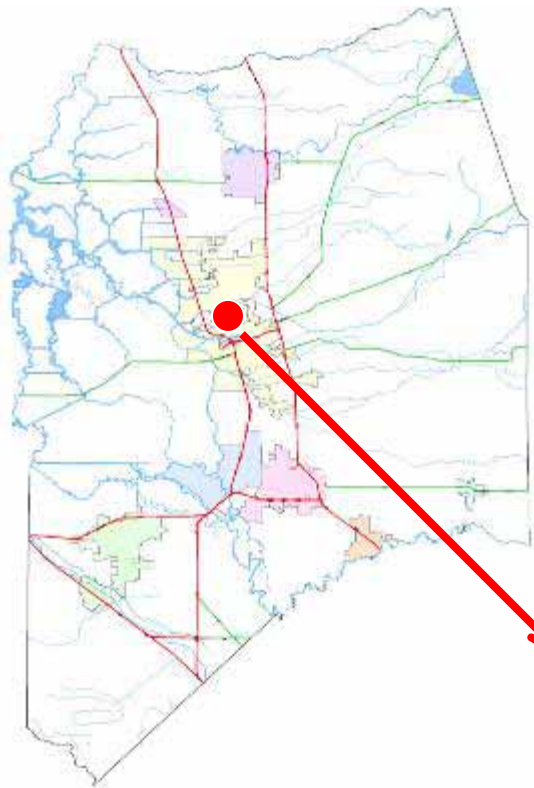
Mosquito species collected naturally infected with filariids presumed to be *Dirofilaria immitis* in the United States

Species	Locations (State)	Total Reports ^a	Number of Reports with L ₃ ^b
<i>Aedes albopictus</i>	FL, LA	3	1
<i>Aedes canadensis</i>	CT, FL, MA, NJ	4	4
<i>Aedes cantator</i>	NJ	1	1
<i>Aedes excrucians</i>	CT, MA	3	2
<i>Aedes infirmatus</i>	FL	1	1
<i>Aedes sirrensis</i>	CA	1	1
<i>Aedes sollicitans</i>	CT, NC, NJ	4	2
<i>Aedes sticticus</i>	AL, MA	3	3
<i>Aedes stimulans</i>	CT, MA	2	1
<i>Aedes taeniorhynchus</i>	FL, NC	3	3
<i>Aedes triseriatus</i>	IN	1	1
<i>Aedes trivittatus</i>	AL, IA, IN, OK, TN	6	6
<i>Aedes vexans</i>	AL, CA, CT, FL, IN, LA, MD, MI, MN, NH, NY, OK	16	10
<i>Anopheles bradleyi</i>	NC	2	1
<i>Anopheles crucians</i>	AL, FL	2	1
<i>Anopheles freeborni</i>	CA	1	1
<i>Anopheles punctipennis</i>	AL, IA, KY, MA, MD	7	3
<i>Anopheles quadrimaculatus</i>	LA, MA, MI, NY	5	4
<i>Culex nigripalpus</i>	FL	1	1
<i>Culex pipiens</i>	MI	1	1
<i>Culex quinquefasciatus</i>	AL, FL, LA	3	2
<i>Culex salinarius</i>	MD, NC, NJ	3	2
<i>Psorophora columbae</i>	LA	1	1
<i>Psorophora ferox</i>	CT, FL	2	2

→ *Culiseta incidens* Strongly competent in laboratory

Critical Factors Determining Vector Capacity for Heartworm *D. immitis*

1. Geographic Proximity
 2. Abundance
- 



2007:

- 25 cases reported
- 11 addresses given
- Many cases appear clustered



Credit: Stacy Bearden

5-year (2006-2010) total mosquito collection from areas near University of Pacific, Stockton

(EVS and Fay traps at Monterey, Pixie Wood park and Rural Cemetery)

Mosquito Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
<i>Aedes melanimon</i>					1		1	1	3	37	15		58
<i>Aedes nigromaculis</i>							1						1
<i>Aedes sierrensis</i>			3	406	115	12					1		537
<i>Aedes vexans</i>				5	1	3			1	1			11
<i>Aedes washinoi</i>				2	1								3
<i>Anopheles freeborni</i>		1	1		2	11	1		3		1		20
<i>Culex erythrothorax</i>										27	1		28
<i>Culex pipiens</i>		16	32	86	394	973	671	408	501	369	81		3,531
<i>Culex tarsalis</i>			6	27	156	1,085	1,058	375	291	70	1		3,069
<i>Culiseta incidens</i>	10	136	351	783	1,013	1,170	353	196	106	100	128	16	4,362
<i>Culiseta inornata</i>	1	55	28	15	8	13	1	2	2	15	29	4	173

Mosquito Species in SJC	Geographic Proximity	Abundance (5yr)
<i>Aedes dorsalis</i>	minimal	Very low (185)
<i>Aedes melanimon</i>	minimal	
<i>Aedes nigromaculis</i>	minimal	
<i>Aedes sierrensis</i>		
<i>Aedes vexans</i>	minimal	
<i>Aedes washinoi</i>	minimal	Very low (136)
<i>Anopheles franciscanus</i>	minimal	Very low (131)
<i>Anopheles freeborni</i>	minimal	
<i>Anopheles punctipennis</i>		Very low (163)
<i>Culex pipiens</i>		
<i>Culex tarsalis</i>		
<i>Culex erythrorhax</i>	minimal	
<i>Culex stigmatosoma</i>		Very low (55)
<i>Culiseta incidens</i>		
<i>Culiseta inornata</i>		
<i>Culiseta particeps</i>		Very low (93)
<i>Orthopodomyia signifera</i>		Very low (2)

3. Temporal Proximity

Heartworm transmission requires minimal temperature

- Heartworms do not develop at temperature below 14 °C (57 °F)
- Heartworms require at least 130 Hearthworm Development Unites (HDUs) in 30 days to become infective

Heartworm Development Unites (HDUs):

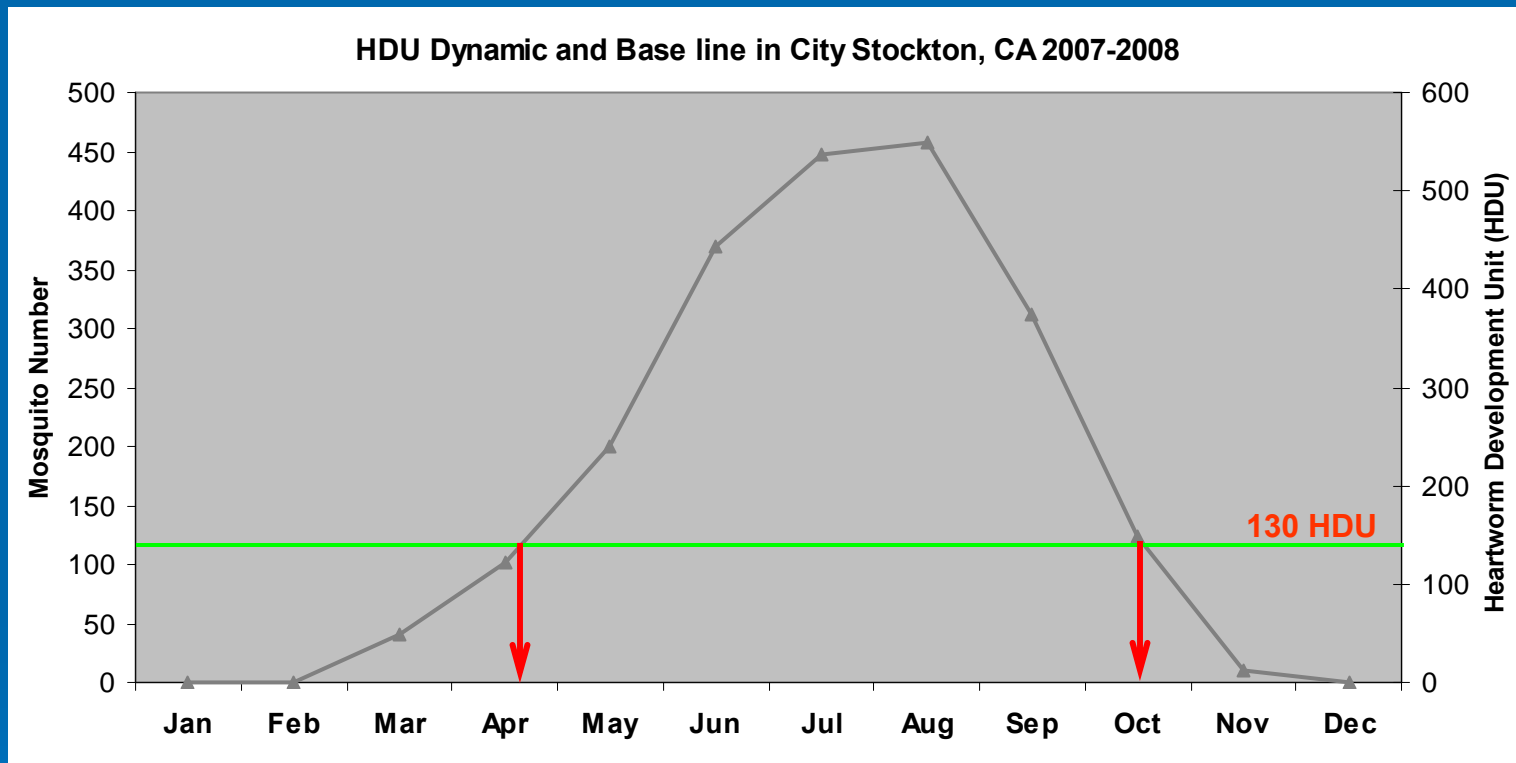
The number of degree days that the larval heartworm is about the minimal temperature for development

$$15\text{ °C for 24 hr} = 15 - 14 = 1\text{ HDU}$$

$$15\text{ °C for 12 hr} = (15 - 14) * (12/24) = 0.5\text{ HDU}$$

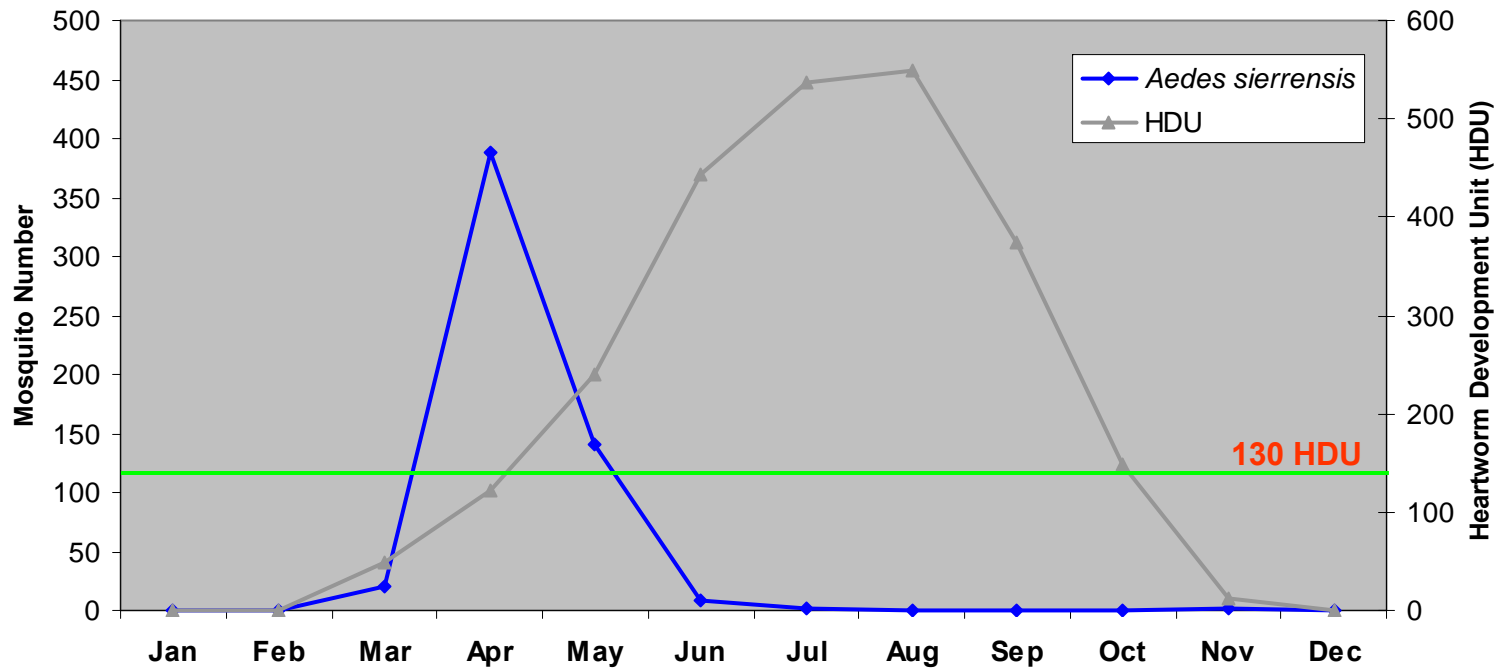
$$20\text{ °C for 24 hr} = 20 - 14 = 6\text{ HDU}$$

...

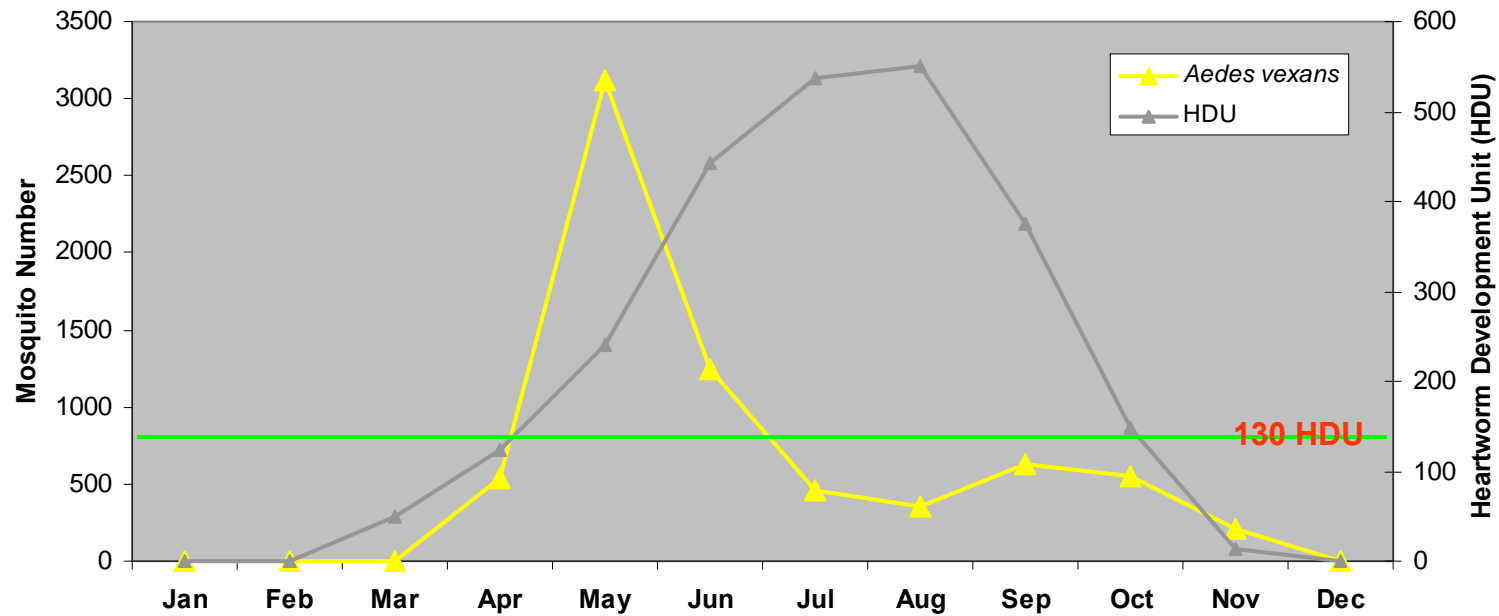


Transmission of dog heartworms may occur only when the HDU curve (grey line) surpasses the 130 HDU baseline (green line). This period approximately corresponds to May - October.

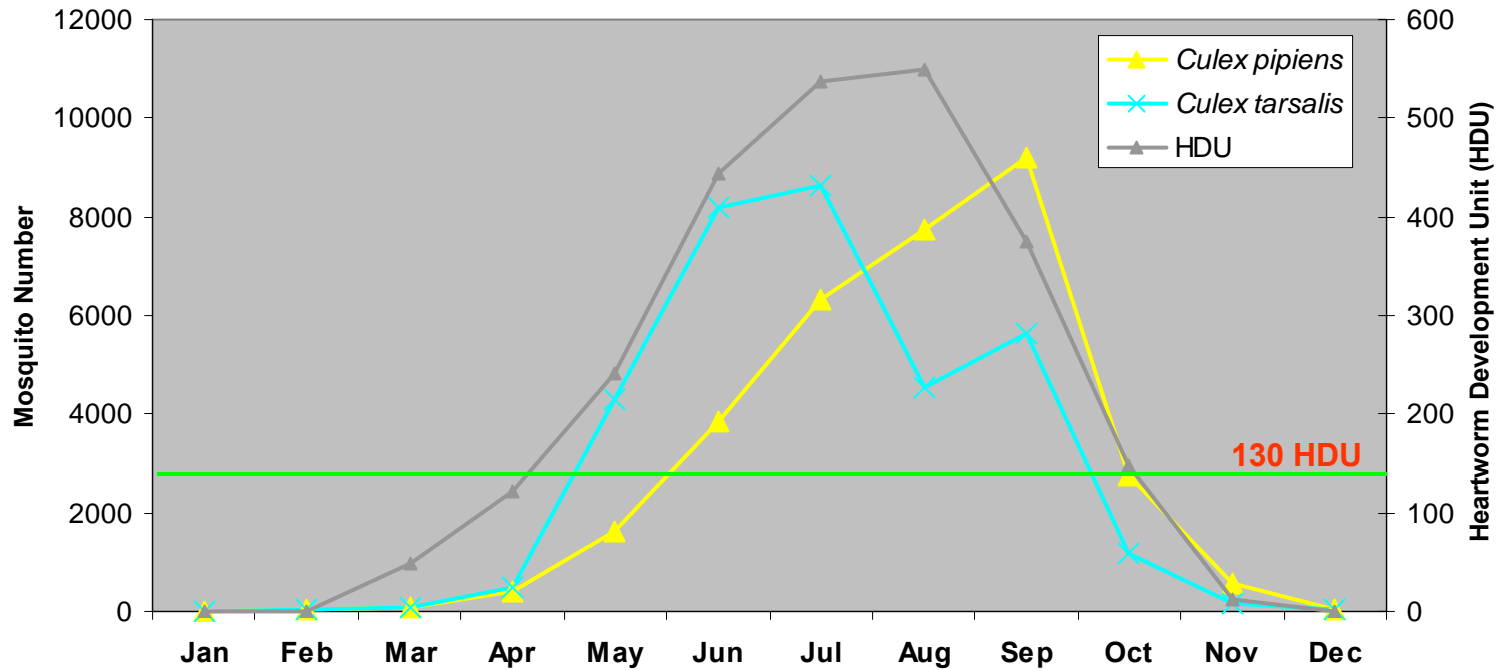
Average Monthly Mosquito Collection in Last Five Year (2006-2010)



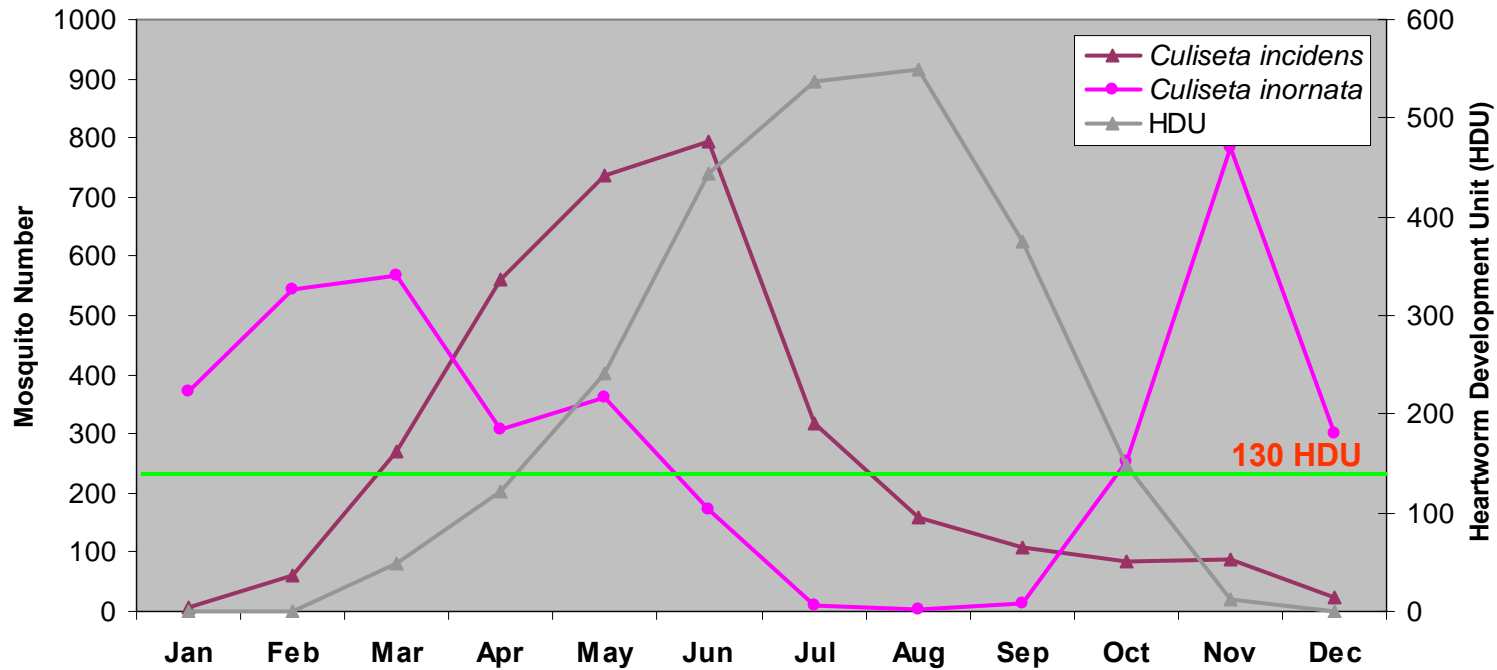
Average Monthly Mosquito Collection in Last Five Year (2006-2010)




Average Monthly Mosquito Collection in Last Five Year (2006-2010)



Average Monthly Mosquito Collection in Last Five Year (2006-2010)



Target Species

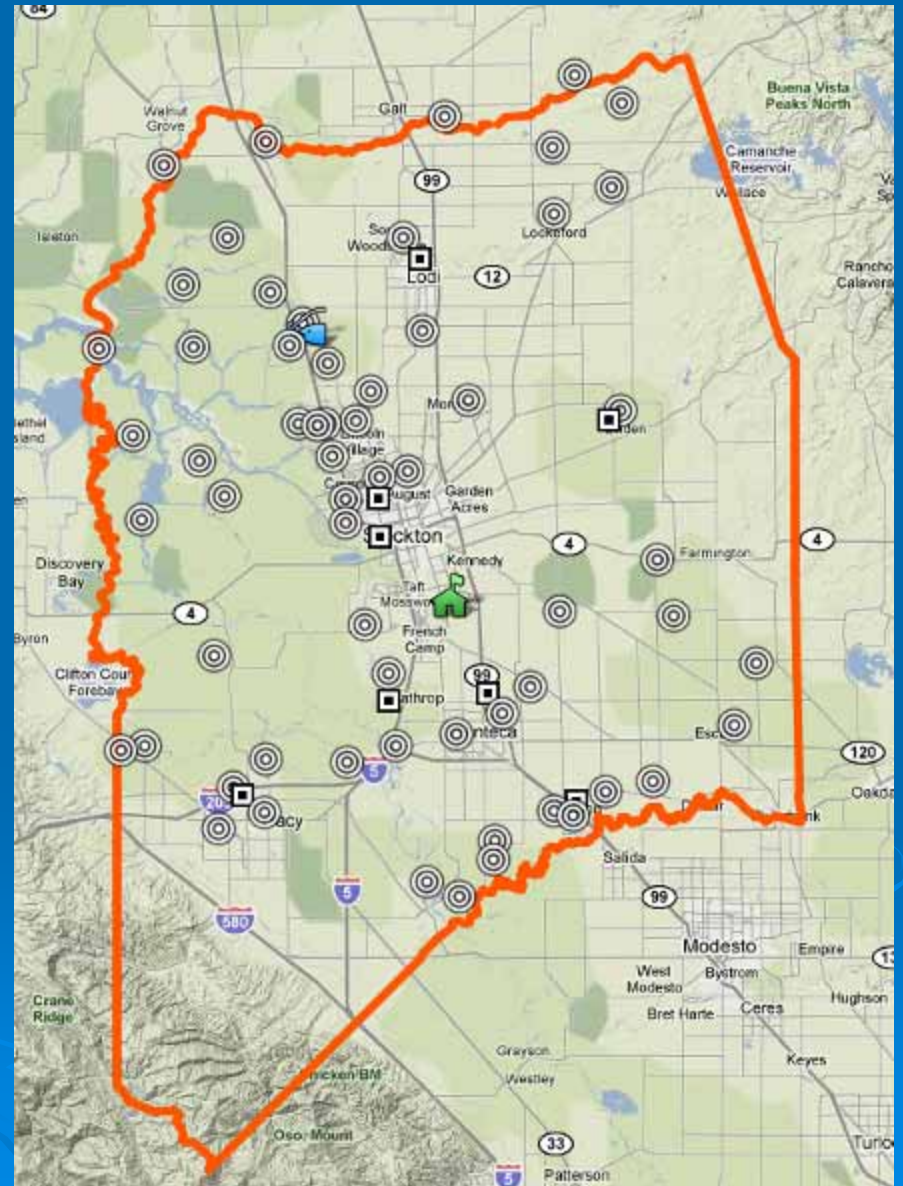
- *Aedes sierrensis*
 - *Aedes vexans*
 - *Culex pipiens*
 - *Culex tarsalis*
 - *Culiseta incidens*
- 

Materials and Methods

Mosquito Collection

WNV Surveillance:
63 EVS traps + 8 Gravid Traps

- Test all mosquitoes collected
- *Culex pipiens* + *Culex tarsalis*: pooled in 1-5 for WNV & *D. immitis* testing
- All other species: pooled in 1-10 for *D. immitis* testing
- Test mosquito heads and thoraxes for infective L3 *D. immitis*
- Test mosquito abdomen for presence of non-infective *D. immitis*



Detection Method

What is PCR (Polymerase Chain Reaction)?

“a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating millions of copies of a particular DNA sequence”

Traditional Microscopic Dissection VS. PCR

Disadvantages of microscopic dissection:

- Time consuming: one mosquito in 5-10 min
- Accuracy: miss worms
- Specificity: other species of worms

PCR:

- Fast: can process 200-500 mosquitoes in a day
- Accuracy: can detect a single worm in 200 mosquitoes
- Specificity: assure correct *D. immitis* identification

This Project / Study Will Continue And Work Toward Answering These Questions:

- **What species are the primary vectors in urban and rural areas ?**
- **Where and when do the vectors occur ?**

Contact Information

For additional information or questions regarding the San Joaquin County Mosquito & Vector Control District Canine Heartworm Study: (209) 982-4675 or district@sjmosquito.org