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ANTIBODY RESPONSE TO *CULEX TARSALIS* SALIVARY GLAND ANTIGENS AMONG SENTINEL CHICKENS IN CALIFORNIA

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Abstract. The arboviral surveillance program in California depends in part on sentinel chickens to detect western equine encephalomyelitis virus, St. Louis encephalitis virus, and West Nile virus activity. From 2000 through 2002, 1,578 serum specimens from 34 sentinel flocks in northern and southern California were tested for antibodies to *Culex tarsalis* salivary gland antigens. Sentinel chickens that were seropositive for mosquito salivary gland antigens were more likely to seroconvert to St. Louis encephalitis virus than those seronegative for salivary gland antigens. Flocks with mosquito traps located < 50 feet away had a reduced antibody response to mosquito salivary gland antigens. The use of sentinel chickens and mosquito traps for arboviral surveillance should be standardized to ensure that surveillance data from different sites are comparable and that flocks have comparable opportunities for mosquito exposure. Sentinel chickens should be accessible to potential mosquito vectors to maximize their sensitivity for detecting arboviral activity.

INTRODUCTION

Sentinel chickens play an important role in California's surveillance program to detect western equine encephalomyelitis virus (WEEV), St. Louis encephalitis virus (SLEV), and West Nile virus (WNV) activity. Each spring, mosquito and vector control districts (MVCDs) place approximately 2,000 adult sentinel chickens throughout California and test them biweekly for antibodies to these viruses. The chickens are surrogate hosts for mosquito vectors that typically feed on wild birds, but their captive status allows the location and approximate time of arboviral infection to be determined.

Culex tarsalis is the primary vector of WEEV and SLEV in California, with *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, and *Ochlerotatus melanimon* playing secondary roles.[1–3] In 2003, WNV was first isolated in California from *Cx. tarsalis* collected in Imperial County; positive *Cx. quinquefasciatus* have since been detected in Los Angeles County.[4] When arboviral activity is present, sentinel flocks that receive the greatest amount of mosquito exposure should have a correspondingly greater risk of arboviral infection. Accordingly, testing the chickens for antibody to mosquito salivary gland antigens (SGA) should provide an indirect measure of their sensitivity for detecting arboviral activity.

Detection of antibody to SGA has been used to identify populations with arthropod exposure. For instance, persons in Japan with mosquito bite hypersensitivity had elevated IgG and IgE antibody levels to *Aedes vexans* SGA.[5] The tick engorgement index, a surrogate for tick saliva dose, and antibody levels to SGA were positively correlated among persons bitten by ticks in a highly Lyme disease–endemic area of New York.[6] Antibody levels to *Ixodes pacificus* SGA were elevated among residents of a Lyme disease-endemic community in northern California, and were correlated with seropositivity to *Borrelia burgdorferi*.[7] Elevated antibody levels to *Triatoma infestans* SGA were found among Chagas disease patients and individuals living in triatomine-infested areas of Brazil.[8]

The primary objective of the present study was to identify patterns of mosquito exposure among sentinel flocks in California using an enzyme immunoassay (EIA) to detect antibodies to SGA. The study flocks were selected to include locations that varied in mosquito abundance, arboviral activity, and ecologic features. A secondary objective was to determine if serologic evidence of mosquito SGA exposure was associated with risk of arboviral infection and/or environmental characteristics present at the flock locations.

MATERIALS AND METHODS

Serologic data collection. Specimens were obtained from sentinel flocks located in the Sacramento Valley (Sacramento-Yolo and Sutter-Yuba MVCDs), coastal (Marin-Sonoma MVCD), and southern California (Coachella Valley MVCD) regions. Whole blood was collected by pricking the comb with a lancet or by venipuncture; the serum fractions were stored at –80°C. A previously described indirect EIA was used to test specimens for antibody to *Cx. tarsalis* SGA, which yielded optical density values.[9] Briefly, salivary glands were dissected from colonized *Cx. tarsalis* (BFS strain), sonicated, and stored at -80°C until used for the assay. Ninety-six well plates were coated with SGA (5 μg of protein/mL). After blocking the wells with 2% casein, sera (diluted 1:100) were added, followed by biotinylated goat anti-chicken IgG (heavy and light chain) conjugate (diluted 1:1,500) (Vector Laboratories, Burlingame, CA). Avidin-biotin complex was added prior to developing the wells with 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD); the wells were read at 405 nm after 20 minutes. Negative control specimens were obtained from sentinel chickens in Sacramento-Yolo MVCD in April 2002, just prior to their placement at the flock locations. The mean optical density of the negative controls plus three standard deviations was the test cut-off value for a positive result.

Environmental data collection. All study flocks were surveyed to collect data on environmental factors that may have influenced the chickens' mosquito exposure. The sentinel flocks in Shasta MVCD were also surveyed, although no sera were obtained. A standardized questionnaire was used to collect data on factors such as habitat type, coop structure, and characteristics of nearby mosquito traps. Each questionnaire was completed in conjunction with a representative from the host MVCD. Data were also collected on mosquito abundance, sentinel chicken seroconversions to arboviruses, and virus-positive mosquito pools.

* Deceased.

Data analysis. Least squares regression was used to evaluate trends in the chickens' antibody response (optical density values) to *Cx. tarsalis* SGA. An *F*-test-to-remove, using the difference in the residual deviance between models, was used to compare models containing date of specimen collection as continuous linear, quadratic, or third-order polynomial functions. For flocks with arboviral seroconversions, a binary independent variable was used to denote the chickens' seroconversion status (1 = positive, 0 = negative).

To produce groups that were homogeneous in their antibody response to *Cx. tarsalis* SGA, the flocks from Sacramento-Yolo MVCD were classified into one of three groups, using the slope and intercept obtained when the mean antibody response was modeled as a function of month of specimen collection. The environmental characteristics of the flocks locations were compared among these three groups using a Pearson chi-square test to evaluate statistical independence for $2 \times k$ tables (binary variables) and one-way analysis of variance (continuous variables).

In other MVCDs, the association between selected environmental characteristics and the flocks' mean end-of-season antibody response to *Cx. tarsalis* SGA was evaluated using Pearson correlation coefficients (*t*-test). Correlation coefficients were also used to evaluate the association between the cumulative *Cx. tarsalis* trap counts and the antibody response to SGA among flocks in Sacramento-Yolo MVCD, assuming a two-week lag between mosquito exposure and the associated antibody response.

The association between the antibody response to SGA and arboviral seroconversion was evaluated by a Pearson chi-square test for proportions. The mean antibody response to SGA was compared among chickens according to their arboviral seroconversion status by an analysis of variance procedure (Kruskal-Wallis test).[10]

Significance probabilities less than 0.05 (*P* values) were considered a strong indication of a systematic influence (not chance variation). Analyses were conducted using S-Plus 2000 (Professional Release 3; MathSoft, Inc., Seattle, WA).

RESULTS

Serologic testing. From 2000 through 2002, 1,578 serum specimens were obtained from sentinel chickens at 34 flock locations. The overall end-of-season seroprevalence for *Cx. tarsalis* SGA varied among the three MVCDs in northern California (Table 1). In the Sacramento-Yolo MVCD, the seroprevalence at the beginning of the season was also measured.

TABLE 1

Antibody seroprevalence for *Culex tarsalis* salivary gland antigens at three study sites in northern California, 2000–2002

Mosquito and vector control district (no. of flocks)	Marin-Sonoma (7)	Sacramento-Yolo (9*)	Sutter-Yuba (8†)
Time of serum collections	No. positive/no. tested (%)		
October 2000		30/85 (35)	26/67 (39)
April 2001		0/80 (0)	
October 2001		28/80 (35)	37/77 (48)
April 2002		2/100 (2)	
October 2002	14/63 (22)	48/88 (55)	29/70 (41)

* An additional flock was added in 2002, making a total of 10 for that year.
† A flock was taken over by another district in 2002, leaving a total of 7 flocks for 2002.

From April through October 2002, the 10 sentinel chicken flocks in the Sacramento-Yolo MVCD were tested monthly for antibody to SGA. Each flock started with 10 chickens in April, although most flocks experienced some mortality over the season. The mean antibody response increased for each flock over the season, although it leveled off or decreased for some flocks by the end of the season (Figure 1). However, including a quadratic or third-order polynomial term for the month of specimen collection did not significantly improve the fit of the models for any of the flocks. Therefore, a simpler linear model with month of specimen collection as an independent continuous variable provided a parsimonious and useful representation of the data (Figure 2).

The Coachella Valley MVCD placed their sentinel chickens at the flock locations in early April. Specimens were acquired for anti-SGA antibody testing beginning in May, by which time the chickens had already received approximately one month of on-site mosquito exposure. Only the Adohr flock had enough specimens available to evaluate the seasonal trend in seroprevalence for *Cx. tarsalis* SGA. The seroprevalence for this flock was highest in May and June, and began decreasing in July (Table 2). The seroprevalence for the Desert flock also decreased in July, although the number of specimens was insufficient to evaluate the trend after this period.

Correlation between antibody to *Cx. tarsalis* SGA and SLEV. The Coachella Valley MVCD was the only study site with sentinel chicken seroconversions to arbovirus in 2001, with 51 (25%) of 207 chickens seroconverting to SLEV (State of California, Department of Health Services, unpublished data). During 2001, 356 serum specimens were obtained from 139 chickens in this district for anti-SGA antibody testing. Sera were available from 47 (92%) of the 51 SLEV-positive chickens and from 92 SLEV-negative chickens.

Fourteen (15%) of the 95 chickens with two or more serially collected serum specimens had a change in serostatus for *Cx. tarsalis* SGA. Of these, 13 were SLEV negative and one was SLEV positive. The SLEV-positive chicken was seropositive for *Cx. tarsalis* SGA at the time of SLEV seroconversion and seronegative 14 days later. All chickens with a change in serostatus for *Cx. tarsalis* SGA were classified as SGA positive for comparison of SGA serostatus between SLEV-negative and SLEV-positive chickens. Most (46 of 47) of the SLEV-positive chickens had one or more one specimen collected within 14 days of SLEV seroconversion; one chicken that was sampled only once, 42 days before SLEV seroconversion, was seropositive for *Cx. tarsalis* SGA. Of 71 chickens that were positive for antibodies to SGA, 43 (61%) were SLEV positive. In contrast, of 68 that were negative for antibodies to SGA, only 4 (6%) were SLEV positive. Chickens that were positive for antibodies to SGA were significantly more likely to be SLEV positive than chickens that were negative for antibodies to SGA ($\chi^2 = 44.0$, $P < 0.001$).

Sera were obtained from other sentinel flocks to evaluate the consistency of the association between antibodies to SGA and arboviral seroconversion. There were seroconversions to both WEEV and SLEV in Imperial County MVCD in 2002, particularly in August and September. Paired serum specimens were obtained in August and October 2002 from five SLEV-positive chickens at two flock locations. Three pairs were positive for antibodies to SGA at both times, one seroconverted, and one reverted to seronegative. Sera were also obtained in December 2000 from seven chickens in a Yuma

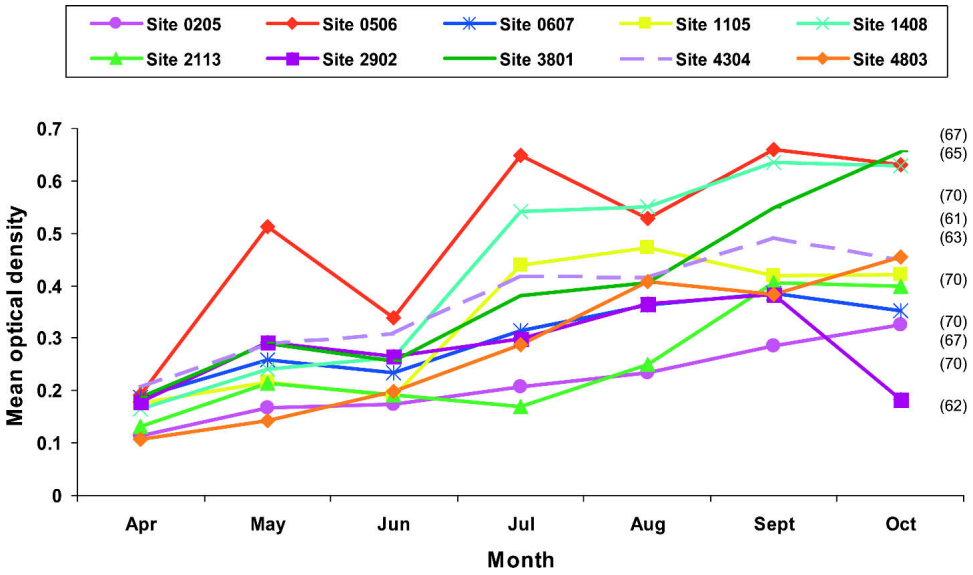

FIGURE 1. Mean antibody response of sentinel chickens in the Sacramento-Yolo Mosquito and Vector Control District to *Culex tarsalis* salivary gland antigens, by flock location and month, 2002. The total number of serum specimens tested from each flock is indicated in parentheses. This figure appears in color at www.ajtmh.org.

County, Arizona sentinel flock that seroconverted to SLEV in July 2000; all were positive for antibodies to SGA.

The modeled antibody response to *Cx. tarsalis* SGA is shown in Figure 3 for chickens from the Coachella Valley MVCD by SLEV seroconversion status. The fit of the regression model was modestly improved by including an interaction term for date of specimen collection and SLEV serostatus (coefficient = 0.0014, $P = 0.065$), reflecting a greater antibody response to SGA among SLEV-positive chickens that increased over the season.

The data were analyzed to determine if the antibody response to SGA among SLEV-positive chickens from the Coachella Valley MVCD was dependent on the time of specimen collection relative to SLEV seroconversion. Sera (n = 122) were available from SLEV-positive (n = 47) chickens from 76 days before and up to 56 days after SLEV seroconversion (mean and median = 14 days before). The district replaced chickens that seroconverted to arbovirus, resulting in fewer sera available post-SLEV seroconversion. The mean level of antibody to SGA (optical density) was calculated for the following: 1) SLEV-negative chickens and 2) SLEV-positive chickens with sera collected more than seven days before SLEV seroconversion; within seven days of SLEV seroconversion; and more than seven days after SLEV seroconversion. The mean antibody level was lowest for SLEV-negative chickens, and increased incrementally among SLEV-positive chickens sampled before, at, or after the date of SLEV seroconversion, respectively (Table 3). A comparison

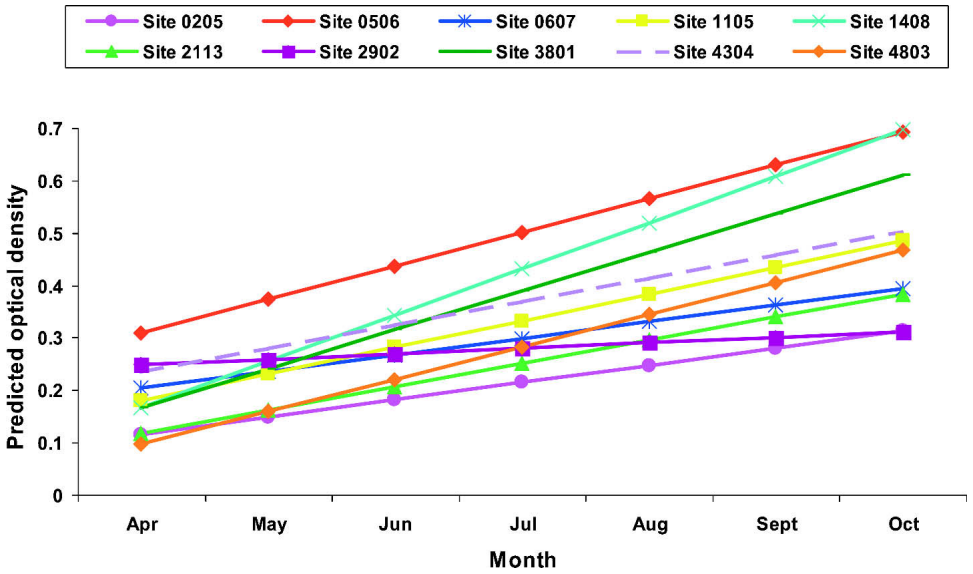

FIGURE 2. Predicted trends in the antibody response of sentinel chickens in the Sacramento-Yolo Mosquito and Vector Control District to *Culex tarsalis* salivary gland antigens, by flock site and month, 2002. This figure appears in color at www.ajtmh.org.

TABLE 2

Summary of serologic testing for antibody to *Culex tarsalis* salivary gland antigens (SGAs) and St. Louis encephalitis virus (SLEV) among sentinel chickens in Coachella Valley Mosquito and Vector Control District by month, 2001*

Flock	No. SGA-positive/no. tested (%)					Arboviral activity† no. SLEV-positive/no. tested (%)	*Cx. tarsalis* per trap-night‡ median (range)
	May	Jun	Jul	Aug	Sep		
Adohr	7/9 (78)	7/10 (70)	4/11 (36)	1/10 (10)	2/11 (18)	12/36 (33)	118 (12, 326)
Cook	0/10 (0)	1/10 (10)	0/4 (0)	1/4 (25)	1/4 (25)	0/16 (0)	2 (0, 13)
Desert	9/10 (90)	9/9 (100)	5/7 (71)	1/2 (50)	1/2 (50)	7/21 (33)	14 (0, 707)
Gordon	0/3 (0)	0/3 (0)	4/9 (44)	1/5 (20)	2/6 (33)	10/27 (37)	20 (0, 232)
Jessup	2/10 (20)	5/10 (50)	0/1 (0)	1/4 (25)	NA	3/15 (20)	74 (0, 1, 128)
Mecca	NA	NA	8/9 (89)	0/9 (0)	1/2 (50)	13/31 (42)	2.5§
SSSP	0/1 (0)	0/1 (0)	3/4 (75)	NA	NA	4/20 (20)	33 (1, 318)
Thermal	NA	0/3 (0)	1/5 (20)	1/2 (50)	NA	1/21 (5)	8 (0, 98)
York	0/1 (0)	0/1 (0)	0/2 (0)	1/4 (25)	1/4 (25)	1/20 (5)	9.5 (0, 49)

* The sampling of some flocks was incomplete or nonexistent (NA). SSSP = Salton Sea State Park.
† Source: State of California, Department of Health Services, unpublished data.
‡ Number of female *Cx. tarsalis* collected biweekly per trap-night in CO_2-baited traps from 3/19/01 to 9/23/01 (Adohr, Desert, Gordon, and SSSP) and from 5/10/01 to 9/14/01 (Cook, Thermal, and York).
§ 2.5 *Cx. tarsalis* were trapped on 8/29/01, the only night with available trap data.

of the groups by a non-parametric analysis of variance procedure (Kruskal-Wallis test) showed a significant difference between the group means ($P < 0.001$).

Environmental characteristics of flock locations. On-site surveys of the environmental characteristics present at 39 flock locations were conducted in the Coachella Valley (9), Marin-Sonoma (7), Sacramento-Yolo (10), Sutter-Yuba (8), and Shasta (5) MVCDs. The mean number of chickens per flock was 9.7 (range = 5–11). The observed flock characteristics are summarized in Table 4.

The flocks from the Sacramento-Yolo MVCD were classified into three groups on the basis of their modeled linear antibody response to SGA (Figure 4): group 1: low intercept and high slope (flocks 1408, 3801, and 4803); group 2: intermediate intercept and slope (flocks 0506, 1105, 2113, and 4304); and group 3: high intercept and low slope (flocks 0205, 0607, and 2902). The distribution of flock characteristics among these three groups is shown in Table 5. Group 3, which had the highest overall antibody response to SGA (high intercept value), had no mosquito traps or domestic animals present within 50 feet of the flocks. This group also had greatest average distance between the coop and the nearest mosquito trap. The low slope for this group indicated a low rate of increase in the antibody response to SGA.

Analysis of the correlation between flock site characteristics and end-of-season antibody levels to SGA was limited to flocks from the Sutter-Yuba MVCD due to the homogeneity of the flock characteristics and/or lack of sera for the other districts. There was no correlation between the percentage of the coop perimeter without barriers to mosquito entry and antibody levels to SGA (correlation coefficient [r] = 0.228, $P = 0.587$). The distance from the coops to the nearest mosquito trap ranged from five feet to more than one mile. There was a positive correlation between antibody levels to SGA and trap distance, which was not statistically significant ($r = 0.535$, $P = 0.172$).

Twenty-one (54%) of the 39 surveyed flocks had a trap within 100 feet of the coop; the percentage of the mosquito trap perimeter without barriers to mosquito entry was recorded for 18 (86%) of these traps. Seven (39%) of the 18 traps were accessible through ≤ 50% of their perimeter, usually due to their proximity to a wall that blocked the radius of light visibility or CO_2 diffusion.

The type of mosquito traps used at the sentinel flock loca-

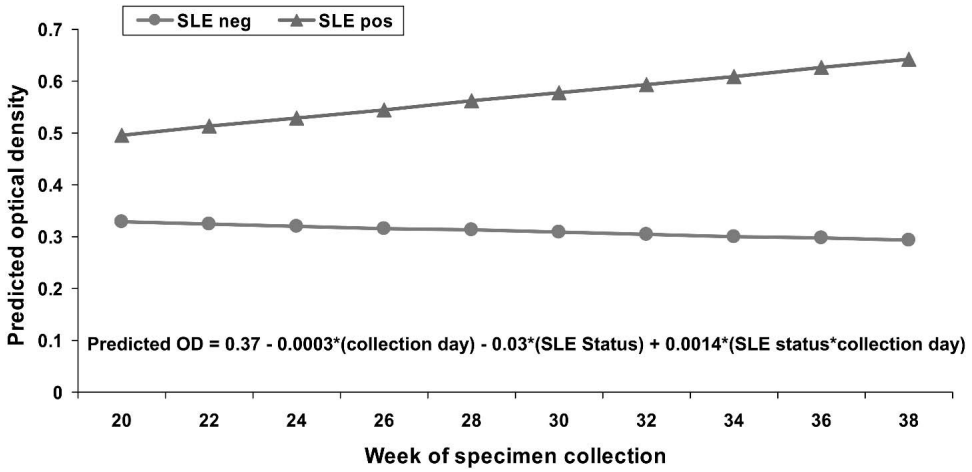

FIGURE 3. Predicted trends in the antibody response to *Culex tarsalis* salivary gland antigens among sentinel chickens in the Coachella Valley Mosquito and Vector Control District by St. Louis encephalitis (SLE) status, 2001. neg = negative; pos = positive; OD = optical density.

TABLE 3

Comparison of mean antibody response to *Culex tarsalis* salivary gland antigens (SGAs) by St. Louis encephalitis virus (SLEV) seroconversion status among sentinel chickens in Coachella Valley Mosquito and Vector Control District, 2001

SLEV status	Antibody response to *Culex tarsalis* SGAs		
	No. tested (%)	Mean optical density	Median optical density
SLEV negative	234 (66)	0.31	0.26
SLEV positive by time of specimen collection			
> 7 days before SLEV seroconversion	63 (18)	0.44	0.36
Within 7 days of SLEV seroconversion	15 (4)	0.56	0.69
> 7 days after SLEV seroconversion	44 (12)	0.71	0.71

tions varied among the MVCDs, with some using New Jersey light traps (Marin-Sonoma, Shasta, and Sutter-Yuba MVCDs) and others using CO_2-baited traps (Coachella Valley MVCD). In 2002, both trap types were used at the Sacramento-Yolo flock locations. The CO_2 traps typically yielded higher *Cx. tarsalis* counts than the light traps, although these differences were less apparent at flock locations with low mosquito counts. The mean monthly antibody response to SGA among the Sacramento-Yolo flocks was highly correlated with the cumulative *Cx. tarsalis* counts obtained from April through October using light traps ($r = 0.78$, $P < 0.001$) and CO_2 traps ($r = 0.69$, $P < 0.001$).

DISCUSSION

The sentinel chickens in the present study had measurable antibodies to *Cx. tarsalis* SGA. The observed seroprevalence pattern for *Cx. tarsalis* SGA was consistent with the mosquito abundance and historical arboviral activity at the study sites (State of California, California Department of Health Services, unpublished data).[11,12] For instance, sentinel chickens in the Marin-Sonoma MVCD, which is in the coastal region, had a low end-of-season seroprevalence for *Cx. tarsalis* SGA (Table 1) and low *Cx. tarsalis* abundance measures in 2002. This district also had less arboviral activity than the other study sites, with only six sentinel chicken seroconversions to WEEV and no virus-positive mosquito pools from 1990 through 2001. The Cook site in the Coachella Valley MVCD had the lowest *Cx. tarsalis* abundance, a low seroprevalence for *Cx. tarsalis* SGA and no seroconversions to SLEV in 2001.

TABLE 4

Summary of sentinel flock characteristics (n = 39)*

Flock characteristics	No. positive for characteristic (%)
Habitat type	
Agricultural	21 (54)
Suburban	8 (21)
Other†	7 (18)
Riparian	3 (8)
Shaded by trees and/or foliage	26 (69)
Domestic animals within 50 feet‡	23 (59)
Artificial light within 50 feet	21 (54)
Distance (feet) to nearest mosquito trap	
≤ 10	7 (18)
15–30	6 (15)
50–100	8 (21)
> 100	18 (46)
Coop perimeter that is barrier-free (%)§	
< 25	7 (18)
25–50	5 (13)
60–75	14 (36)
≥ 80	13 (33)

* Study sites were located in Coachella Valley, Marin-Sonoma, Sacramento-Yolo, Sutter-Yuba, and Shasta County Mosquito and Vector Control Districts.

† Marshland (2), desert (2), seasonal wetland (duck hunting club) (1), scrub oak (1), and urban (1).

‡ Cows, horses, sheep, pigs, dogs, cats, and other poultry.

§ The approximate percentage of the entire coop perimeter without outside barriers, such as an adjacent wall or fence, to prevent mosquito entry into the interior nest and roosting area of the coop.

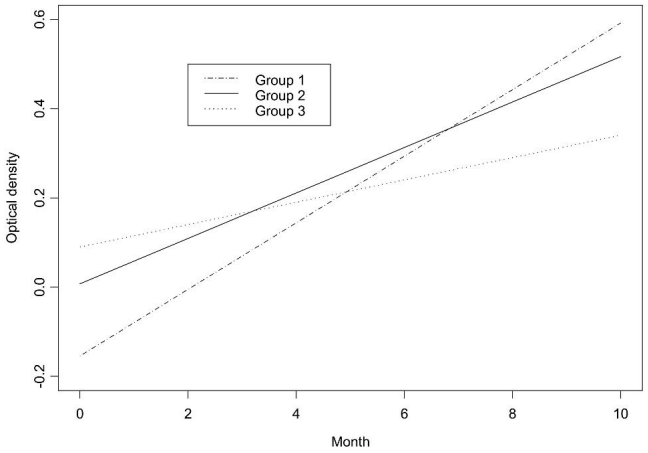

FIGURE 4. Predicted antibody response of sentinel chickens in the Sacramento-Yolo Mosquito and Vector Control District to *Culex tarsalis* salivary gland antigens, by group and month, 2002.

In the Sacramento-Yolo MVCD the chickens' antibody response to SGA increased over the 2002 season (Figure 1) and was positively correlated with the cumulative *Cx. tarsalis* trap counts at the flock locations. The slight tapering of the antibody response by the end of the season may indicate desensitization of some chickens following continuous mosquito exposure, although this decrease was not statistically significant. This could be equivalent to the Stage V reaction in humans following repeated mosquito exposure, when they no longer develop a measurable immediate or delayed-type hypersensitivity reaction following antigen exposure.[13] This suppression of cutaneous reactivity is believed to be associated with a decrease in the humoral antibody response.[14,15] Conversely, the observed decrease in the seroprevalence for *Cx. tarsalis* SGA among flocks in the Coachella Valley MVCD (Table 2) was likely due to the replacement of SLEV-positive chickens with new chickens that had less collective mosquito exposure.

The antibody response to SGA varied among individual chickens that were housed together within a flock, suggesting that their mosquito exposure differed. The source of sentinel chickens used for arboviral surveillance in California is limited to a few suppliers to help ensure that the chickens are uniform in age, breed, and genetic background. However, chickens that are seemingly identical in all other respects have been shown to differ in their attractiveness to mosquitoes.[16] It is possible that the hierarchical structure within a flock may determine the nightly roosting position of chickens, with resultant differences in opportunities for mosquito exposure. If

TABLE 5

Flock characteristics among sentinel chicken flocks in the Sacramento-Yolo Mosquito and Vector Control District grouped by predicted antibody response to *Culex tarsalis* salivary gland antigens*

Flock characteristics	Group 1 (%)	Group 2 (%)	Group 3 (%)	*P*
Present within 50 feet of flock				
Mosquito trap	3/3 (100)	2/4 (50)	0/3 (0)	0.050
Artificial light source	2/3 (67)	1/4 (25)	2/3 (67)	0.435
Domestic animals†	1/3 (33)	3/4 (75)	0/3 (0)	0.129
Lawn	1/3 (33)	2/4 (50)	1/3 (33)	0.870
Weeds and/or brush	2/3 (67)	3/4 (75)	2/3 (67)	0.961
Trees	2/3 (67)	3/4 (75)	3/3 (100)	0.564
Debris (i.e., old containers)	2/3 (67)	3/4 (75)	1/3 (33)	0.517
Flock in agricultural setting	2/3 (67)	3/4 (75)	1/3 (33)	0.517
Flock shaded by trees and/or foliage	2/3 (67)	3/4 (75)	1/3 (33)	0.517
Continuous variables				
Average distance to nearest mosquito trap (feet)	26.7	53.8	125	0.018
Average coop perimeter that is barrier-free (%)‡	66.7	68.8	75	0.363

* Group 1 = low intercept and high slope (flocks 1408, 3801, and 4803); Group 2 = intermediate intercept and slope (flocks 0506, 1105, 2113, and 4304); and Group 3 = high intercept and low slope (flocks 0205, 0607, and 2902).

† Cows, horses, sheep, pigs, dogs, cats, and other poultry.

‡ The approximate percentage of the entire coop perimeter without outside barriers, such as an adjacent wall or fence, to prevent mosquito entry into the interior nest and roosting area of the coop.

a portion of the flock tends to aggregate, creating a high host density, those chickens may be more protected from mosquito exposure than the others.[17] The activity level of individual birds has also been shown to influence the feeding success of *Cx. tarsalis*, with lower feeding success on more active birds.[18]

Variation was observed in the way flocks were set up, both between and within the MVCDs. While coop designs varied greatly between districts, within districts the coop design tended to be uniform. Some MVCDs used light traps at the flock locations while others used CO_2 traps. Many flock locations had no mosquito traps in the vicinity, precluding attempts to correlate mosquito abundance with the antibody level to *Cx. tarsalis* SGA at those locations. When traps were present, their distance from the chicken coops varied among the flocks, which complicated interpretation of the mosquito counts as an approximation of the chickens' mosquito exposure. In the Sacramento-Yolo and Sutter-Yuba MVCDs, trap distance was positively correlated with serologic evidence of mosquito exposure, suggesting that flocks with traps in the immediate vicinity experienced a reduction in their mosquito exposure.

In the present study, CO_2 trap and light trap counts of *Cx. tarsalis* were highly correlated with the antibody response to SGA. The four flock locations in the Sacramento-Yolo MVCD with the highest *Cx. tarsalis* CO_2 and light trap counts also had the highest mean antibody response to SGA for most or all of the season. In almost every case, CO_2 traps yielded higher mosquito counts than light traps. Because the CO_2 traps were only run for one trap-night per week, these counts were more likely to be influenced by extreme fluctuations in the counts than those obtained using light traps, which were averaged over seven trap-nights. The use of CO_2 traps over light traps has been advocated due to their higher selectivity for female mosquitoes and their lower susceptibility to interference by background illumination.[19]

Individual chickens placed outdoors in a trap attracted up to 1,500 *Cx. tarsalis* in one night, of which an average of 100 fed (range = 1–1,063).[20] In the present study, the maximum number of *Cx. tarsalis* trapped per night in Sacramento-Yolo MVCD differed among flock locations and according to whether CO_2 traps (range = 6–1,014) or light traps (range = 3–124) were used. Therefore, most chickens placed in the field over the course of the arboviral transmission season should have ample opportunity for mosquito exposure. Mosquito abundance measures are correlated with the arboviral infection rates in the human and sentinel chicken populations.[21,22] However, even when *Cx. tarsalis* abundance is high, microhabitat factors that affect mosquito host-seeking behavior and/or the accessibility of flocks to mosquitoes may influence the intensity of the chickens' mosquito exposure. This makes serologic testing for *Cx. tarsalis* exposure useful for evaluating the sensitivity of flocks for detecting arboviral activity. Such testing would not replace routine serologic testing for arboviral exposure, but could instead serve as a tool for MVCDs to evaluate the utility of their flock locations for arboviral surveillance. This could even be accomplished using a single seroprevalence measure for *Cx. tarsalis* SGA among sentinel chicken flocks, perhaps taken midway through the surveillance season, which would be more practical than conducting such testing on an ongoing basis. To ensure consistency of laboratory methods, such testing would ideally be done at a single reference laboratory.

The EIA used in the present study used a crude extract of *Cx. tarsalis* SGA. An important methodologic consideration is the potential for cross-reactivity of *Cx. tarsalis* SGA with antibody to other SGA from mosquito species such as *Cx. pipiens*, *Cx. quinquefasciatus*, and *Oc. melanimon*.[9] Western immunoblotting would be useful for evaluating the antibody response to antigens that are common to salivary glands from different mosquito species. The presently described method most likely reflects *Culex* spp. exposure among sentinel chickens, since avian hosts are typically fed on by these species and antibodies to SGA directed against *Culex* spp. would be expected to cross-react with *Cx. tarsalis* SGA.[23–25] However, serologic measures of *Cx. tarsalis* SGA exposure could be compared with mosquito population indices to evaluate the contribution of heterologous mosquito species to the serologic response.

The ability to evaluate statewide trends in arboviral surveillance data would be improved by standardizing the use of mosquito traps and sentinel chicken flocks. We propose the following recommendations to optimize the utility of data that are collected from sentinel flock locations: 1) standardize the type of mosquito traps used at sentinel flock locations; 2) avoid placing mosquito traps near barriers that block mosquito entry; 3) place mosquito traps approximately 100 feet from the coop to obtain counts that reflect the flocks' potential mosquito exposure, while not being so close as to interfere with that exposure; and 4) ensure that the roosting space inside the chicken coop is accessible to mosquitoes.

In the present study, detection of antibody to *Cx. tarsalis* SGA among sentinel chickens provided a means to evaluate the sensitivity of the flocks for arboviral surveillance. Standardizing the manner in which sentinel chickens and mosquito traps are used for arboviral surveillance would enhance the comparability of surveillance data collected from different districts and ensure that individual flocks have similar sensi-

tivity for detecting arboviral activity. As the data from the Coachella Valley MVCD demonstrated, serologic evidence of exposure to *Cx. tarsalis* SGA in sentinel chickens was associated with increased risk of SLEV infection. Ideally every sentinel flock should receive maximal mosquito exposure to ensure there is high sensitivity for arbovirus detection. With the recent detection of WNV in southern California, and the potential for introduction of new arthropod-borne agents though international commerce or bioterrorism, it is now more important than ever that arboviral surveillance be operated at uniformly high levels of sensitivity.

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