Journal of the American Mosquito Control Association, 17(2):118–123, 2001

COST EFFECTIVENESS OF THREE ARBOVIRUS SURVEILLANCE METHODS IN NORTHERN CALIFORNIA

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ABSTRACT. We compared the cost effectiveness of enzootic arbovirus surveillance in northern California by antibody detection in sentinel chickens, virus isolation from mosquitoes, and antibody detection in wild avian hosts. Total and annual recurring costs were determined for each method based on estimated personnel and actual material and travel costs for biweekly surveillance at 3 sites in the Sacramento Valley from May 1 through mid-October 1997 and 1998. Serologic detection of antibodies in wild birds was the most expensive method. Total costs associated with sentinel chickens and mosquitoes combined were less than half of those for the wild bird program. Recurring annual costs for the wild bird and mosquito methods were only slightly less than expenses for those methods during the 1st year of operation, which included nonrecurring setup costs. Recurring costs for sentinel chickens were reduced ~40% from total costs during the 1st year of the program and were <14% of recurring costs for wild bird serology. Exceptions and caveats of our analysis are discussed. When considering data from a companion paper on detection of enzootic virus transmission using the 3 methods, we concluded that the current system that combines sentinel chickens and virus isolation from mosquitoes is the most cost-effective and efficient surveillance program and should be retained. Future research efforts should investigate the costs and surveillance efficiency of modifications in the frequency of specimen collection and the placement of chicken flocks and mosquito traps.

KEY WORDS Arboviruses, California, surveillance, cost

INTRODUCTION

Surveillance for western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses is a key component of the Vector-Borne Disease Surveillance Program for the State of California (Eldridge 1987, Walsh 1987, Reisen 1995). The program has 2 principal components: detection of enzootic virus transmission and monitoring of mosquito vector abundance. Recognition of enzootic virus activity is based on detection of antibodies against WEE and SLE in sentinel chickens and isolation of virus from pools of the mosquito vector *Culex tarsalis* Coquillett captured in CO_2-baited traps. Goals of the program are to document local enzootic virus transmission and to predict the threat of virus transmission to people (i.e., to function as an early warning system). Critical decisions regarding public health risk and initiation of control measures are based on the density of female *Cx. tarsalis* captured in New Jersey light traps. Data on enzootic virus activity and mosquito abundance are augmented by passive surveillance for clinically ill humans infected with WEE or SLE and equine illness from WEE infections.

Although comprehensive and well supported, there are limitations to the California arbovirus surveillance system. For example, when virus transmission surpasses critical threshold levels, control measures should be implemented to interfere with the infection of humans and domestic animals. In practice, however, the relationship between enzootic transmission and human infection is unclear, and amplification of virus in enzootic transmission cycles complicates predicting risk of human infection, compared to a system like dengue, where humans are the only vertebrate hosts (Eldridge 1987; Reeves 1990; Eldridge et al. 1998, 1999).

In an effort to improve the existing surveillance program, a collaboration was established among the University of California at Davis, 10 California mosquito abatement districts, and the California Department of Health Services. The intent was to develop a model system for improved arbovirus surveillance. Herein we address the portion of the model surveillance program that involves monitoring enzootic virus transmission. Specifically, we examined the relative costs of 3 methods for monitoring enzootic arbovirus activity in northern California. We added antibody detection in wild avian hosts (McLean et al. 1983; Gruwell et al. 1988, 1989; Bennett et al. 1993; Reisen et al. 2000) to the standard methods of antibody detection in sentinel chickens and virus isolation from mosquitoes. Wild bird serology was added because a component of the model surveillance program is evaluation of alternative methods for detecting and estimating the extent of enzootic virus transmission (Eldridge et al. 1998, 1999). Our comparison of 3 surveillance methods was not intended for establishing thresholds; instead, we sought to determine which system was most cost effective, sensitive, and specific for detection of virus transmission. It is important to consider cost effectiveness during the evaluation of surveillance methodologies so that recommendations can be made that will help mosquito abatement districts function as efficiently as possible within their budgetary limitations (Phillips et al. 1993).

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MATERIALS AND METHODS

Surveillance methodology: Our analysis was based on a comparison of 3 methods for monitoring enzootic arbovirus transmission carried out at 3 sites in Sacramento and Yolo Counties in northern California during 1997 and 1998. Detailed descriptions of study sites, specimen collection and processing, assay procedures, and results are provided by Reisen et al. (2000). Briefly, we compared 1) detection of antibodies in the blood of wild birds by enzyme-linked immunosorbent assay (ELISA) with confirmation of positive specimens by plaque reduction neutralization (PRNT), 2) detection of antibodies by ELISA in the blood of sentinel chickens with confirmation of positive specimens by the indirect fluorescent antibody test (IFA), and 3) virus isolation in cell culture from pools of female *Cx. tarsalis.* Justification for differences in our procedures for detecting antibodies in avian sera is as follows. We screened chicken and wild bird sera with an ELISA to rapidly identify potentially positive specimens. Because antibodies detected by ELISA can be cross-reactive (i.e., produce a false positive result), positive ELISA results require confirmation with a more specific assay. We used an IFA to confirm ELISA-positive chicken sera because anti-chicken reagents were available for that specific, sensitive, cost-effective, and relatively rapid procedure. Species-specific reagents were not available for IFA testing of wild bird species. Therefore, we were required to confirm wild bird antibody reactions with PRNT, which is sensitive and specific but more labor intensive and expensive than the IFA. Had we been able to assay wild bird sera with an IFA, the cost of testing their sera would have been reduced. The time required and personnel, reagent, and supply costs prohibited assaying all specimens by IFA or PRNT.

We did not begin capturing wild birds and mosquitoes until June 1. However, for comparison with sentinel chickens, which began a month earlier, we calculated costs for all 3 methods beginning May 1. During both years of study, all 3 surveillance methods were terminated in mid-October.

Wild birds were captured in mist nets, a blood sample was collected from their jugular vein by vein puncture, and birds were marked with a leg band prior to release. We collected wild birds in 10 mist nets for 4 h (0600–1000 h) each day of surveillance activity. Based on our experience, attempting to capture birds in mist nets after 1000 h during the summer in northern California does not result in sufficient captures to merit the extra effort (S. A. Wright and T. W. Scott, unpublished data).

Sentinel chickens were >18 wk old at the beginning of each year of study. Their combs were pierced with a lancet and blood from each chicken was collected in a separate sterile screw-top tube. One sentinel flock of 10 chickens was located at each of the 3 sites.

At each site, mosquitoes were collected in 5 encephalitis virus surveillance (EVS) traps (Pfuntner 1979) supplemented with dry ice. Ten or fewer pools of ≤50 female mosquitoes from each collection day were triturated and assayed for virus.

Surveillance activities were carried out at 2 sites during the 1st week and at the 3rd site during the 2nd week of each month; each site was sampled biweekly. Wild birds were captured and bled on 2 consecutive days. Mosquitoes were captured during the evening between the 1st and 2nd day of mist netting. Sentinel chickens were bled during 1 of the 2 days of mist netting.

Collection and sampling of birds was conducted under federal master bird banding permit 21615, State of California Scientific Collecting Permit 801194-04, and University of California Animal Care and Use protocol 6729.

Study sites: Our 3 study sites were Stone Lakes National Wildlife Refuge, Beach Lake Wildlife Area, and Fremont Wildlife Area (Reisen et al. 2000). All 3 are located in the Sacramento River flood plain, which is dominated by Fremont cottonwoods (*Populus fremonti*), valley oaks (*Quercus lobata*), several willow species (*Salix* spp.), tule (*Scripus acutus*), cattails (*Typha latifolia*), and various wetland grasses.

During the past 3 decades, transmission of WEE in the northern Sacramento Valley of California has been sporadic, punctuated by periods of 2–6 years of transmission interspersed with periods of 2–4 years of no detectable virus activity (Reisen et al. 1995). Enzootic SLE transmission has not been detected in the Sacramento Valley since 1974. Enzootic WEE transmission was detected at our study areas in 1997, but not in 1998 (Reisen et al. 2000).

Cost estimates: Time spent at a given task was estimated as the average time required for the 2 years of study. To simplify and standardize our analysis, all travel and work time estimates were held constant across study sites even though travel time from the Sacramento–Yolo Mosquito and Vector Control District in Elk Grove, CA, to the Fremont Wildlife Area was approximately twice that required for Stone Lakes and Beach Lake. Estimates of mean times for processing specimens in the field and laboratory were calculated from combined times for the 3 sites. Costs of supplies and equipment were the actual amounts paid per year. Vehicle expenses were based on vehicle logs and shop records. Costs associated with items that required construction (e.g., EVS traps and chicken coops) included materials and labor. Labor costs ($25/h) were based on average salaries for participating personnel from the Sacramento–Yolo Mosquito and Vector Control District. Actual expenses incurred each year (e.g., personnel, mist nets, chickens, syringes, and needles) were used to calculate recurring costs. Total costs included recurrent costs plus items paid for only once during the 1st year of study and used in the subsequent years

Table 1. Time spent per day and personnel costs associated with 3 different arbovirus surveillance methods.[1]

Activity	Wild bird serology	Sentinel chickens	Virus isolation from mosquitoes
Travel to site	60 min × 2	60 min	60 min × 2
Set-up and -down	60 min × 2	20 min	30 min × 2
Field processing	240 min × 2	30 min	0
Lab processing	40 min	10 min	90 min
Total time (10 samples)	12.7 h	2.0 h	4.5 h
Personnel costs/day ($25/h)	$317.50	$50.00	$112.50
Personnel costs/month	$3,810.00	$300.00	$675.00

[1] Some categories were doubled (× 2) to account for the 2 people required to complete that task.

without additional expense (e.g., chicken coops, EVS traps, and banding pliers). We report costs according to the value of the U.S. dollar in 1998. No attempt was made to account for projected inflationary effects on total versus recurrent costs.

RESULTS

Time requirements and personnel costs directly related to each surveillance method are summarized in Table 1. Travel, mist netting, and mosquito collection times were doubled to account for personnel costs associated with the 2 people required to complete those tasks. One person bled sentinel chickens. During our field study, we captured wild birds and mosquitoes and bled chickens during the same trip, but for purposes of cost comparison, travel time was considered separately for each method. Time required to prepare to carry out a method and to clean up afterward was greatest for mist netting wild birds, followed by collection of mosquitoes and was least for sampling sentinel chickens. Processing specimens (i.e., time spent collecting, labeling, dispensing) at the field site was 16 times longer for wild birds than sentinel chickens. There was no field processing time associated with mosquito collections beyond carrying traps to the field vehicle, which was accounted for in clean-up time. Laboratory processing time was greatest for mosquito collections, which needed to be identified to species, placed in vials, and frozen prior to virus assay. Time required for laboratory processing of wild bird and chicken sera was considerably less than for mosquitoes. Total time required to carry out a surveillance method at a single site for 1 day was longest for the wild bird serology. Mosquito collections required <½ the time needed for wild birds, and specimens from a single chicken flock were processed in <½ the time required for mosquitoes.

Personnel costs in Table 1 reflect time-related differences required to carry out each activity. Personnel costs per day were highest for surveillance based on wild birds, mosquitoes were the next most expensive, and a program using sentinel chickens incurred the least personnel expense. Monthly personnel costs of each method reflect 2 collecting periods at the 3 sites: 4 days of mist netting, 2 nights collecting mosquitoes or 2 collections of blood from chickens at each site.

Transportation costs included all expenses associated with the field vehicle during the periods of study (e.g., gasoline, maintenance, insurance). Delivery of specimens for testing to the Center for Vector-Borne Disease Research, University of California, Davis, accounted for the higher costs for wild birds and mosquitoes than for chickens.

Total and recurring costs associated with the 3 different methods are summarized in Table 2. Salary costs were products of monthly figures in Table 1 times the 5.5 months of study.

For wild birds, nonrecurring costs were for bird banding pliers; a book that was used to determine the species, age, and sex of captured birds; metal poles to support mist nets; and bird bags to hold birds prior to bleeding. Recurring costs included new mist nets purchased each year due to wear and tear, cryogenic vials to freeze avian sera prior to assay, syringes and needles to bleed wild birds, and the fee for a State of California Collecting Permit. Assay costs were based on testing 10 sera/site/day at $8.10/specimen, which was the average daily number of specimens tested and the charge for serologic testing by the Center for Vector-Borne Disease Research, University of California, Davis.

For the sentinel chicken system, nonrecurring costs were for materials and labor (two 8-h days/coop at $25/hr) to construct 3 chicken coops and the transfer coop for holding chickens prior to collecting blood. Chickens were purchased each year along with enough chicken food to feed 30 birds for the entire study period. Tyvek suits, gloves, lancets, and cryogenic vials were required to handle and bleed chickens. Postage was for shipping chicken sera to the Viral and Rickettsial Disease Laboratory, California Department of Health Services, Berkeley, CA, where each specimen was examined for $3.85.

For the collection and analysis of mosquitoes for virus the only nonrecurring costs were for the parts and labor to make the 10 EVS traps. Batteries and dry ice were needed to operate the traps, and cryogenic vials were used for frozen storage of mosquito pools prior to virus assay. We estimated that

Table 2. Total and recurring costs from May through mid-October for 3 arbovirus surveillance systems.

Wild bird serology	Cost ($)	Sentinel chickens	Cost ($)	Mosquitoes	Cost ($)
Mist nets (10)	930	Coops (3)	1,397	EVS traps (10)	400
Cryogenic vials	290	Labor for coops	1,200	Batteries	125
Syringe and needles	288	Transfer coop	45	Dry ice	300
Bird bags	50	Chickens (30)	150	Cryogenic vials	60
Banding pliers	33	Chicken food	384	Transportation	264
Bird identification book	45	Tyvek suits and gloves	55		
Mist net poles	20	Lancets and cryogenic vials	87		
Transportation	528	Transportation	250		
Collecting permit	42				
		Postage	45		
Assay sera[1]	5,346	Assay sera[1]	1,271	Assay pools[1]	1,848
Personnel ($25/h)	20,955	Personnel ($25/h)	1,650	Personnel ($25/h)	3,713
Total costs	28,527		6,534		6,710
Recurring costs	28,379		3,892		6,310

[1] Wild bird sera were assayed for $8.10/specimen, and 60 specimens were examined biweekly. Sentinel chicken sera were assayed for $3.85 each, and 30 blood specimens were examined biweekly. Mosquito pools were assayed for $8.00 each, and 21 pools were assayed biweekly.

7 pools/site/biweekly collection were assayed. The Davis Center for Vector-Borne Disease Research charged $8.00/pool to determine if mosquitoes were infected with WEE or SLE.

Serologic detection of arbovirus infection in wild birds was the most expensive surveillance method (Table 2). Total costs associated with sentinel chickens and mosquitoes combined were <½ of total costs for the wild bird program. Annual recurring costs were only a modest reduction compared to total costs for the wild bird and mosquito methods. However for sentinel chickens, recurring costs were ~60% of total costs during the 1st year and <14% of the recurring costs for the wild bird program on an annual basis.

DISCUSSION

Our comparison of costs indicates that the most cost-effective enzootic WEE and SLE surveillance system in northern California is serologic detection of transmission in sentinel chickens followed by detection of virus in vector mosquitoes. Deploying mist nets, checking nets, removing captured birds for 4 h each day, and collecting blood from wild birds is considerably more labor-intensive, and thus more costly, than collecting blood from captive flocks of sentinel chickens or retrieval of captured mosquitoes from traps. Labor costs were the principal savings for the sentinel chicken and mosquito methods over wild bird serology. Total costs during the 1st year were similar for the sentinel chicken and mosquito methods. However, there were considerable savings during subsequent years when using sentinel chickens, indicating that sentinel chickens were the single most cost-effective surveillance method. A surveillance program that used both sentinel chickens and virus isolation from mosquitoes would cost <½ that of a wild bird serology program alone. As a preventive measure to evaluate the risk of human infection with WEE or SLE, a sentinel chicken and mosquito surveillance program costs less than the economic burden associated with a single person suffering from a transient arboviral infection (~$21,000) and considerably less than 1 person with residual sequelae ($3 million) (Villari et al. 1995).

Costs associated with the 3 methods may vary from 1 location or type of mosquito abatement program to another. For example, most mosquito abatement districts in California that use sentinel chickens to monitor arbovirus activity deploy more than 3 flocks. A typical scenario is to collect blood from chickens in 9–10 flocks on the same day at 2-week intervals (Reisen et al. 2000). The Sacramento–Yolo District where we worked uses 9 flocks. The entire process of managing 9 flocks, including travel, collecting specimens, and processing specimens in the laboratory, takes 2 people a total of ~50 h/month. Operating 9 sentinel flocks, rather than 3, would increase total costs to $21,237 and recurrent costs to $13,401, which during the 1st year of operation would save >$7,000; in subsequent years, recurrent costs would be ~$15,000 less than for wild bird serology. Not only is this a budgetary savings, but the less expensive 9-flock system will allow districts to assess transmission in 6 more sites than when capturing and bleeding wild birds at only 3 sites.

A mosquito abatement district might similarly want to collect and assay more mosquitoes for virus infection than we included in our analysis. Our protocol resulted in collection of fewer specimens (≤350) than is typically needed to detect a single infected mosquito. In California, minimum infection rates for WEE and SLE in pools of *Cx. tarsalis* characteristically range from 1.0 to 1.5 per 1,000 mosquitoes tested (Reeves 1990, Reisen et al.

2000). If mosquito collections were increased from the 1 night every 2-weeks that we considered in our analysis to 3 nights biweekly at each site, total costs for mosquito surveillance would increase to $20,130 and recurrent costs to $18,930. Tripling the mosquito surveillance effort would still save >$8,000 in total costs during the 1st year of the program and >$9,000 in recurrent cost during succeeding years compared to wild bird serology. Unlike sentinel chickens, which are a predictable surveillance resource, it is difficult to know with certainty the number of mosquitoes that one can capture. We would expect, however, that 3 nights of mosquito collection would significantly increase the chances of collecting enough specimens to detect an infected mosquito at the infection rates cited above. An unusually large number of mosquitoes captured would increase processing time and assay costs, but expenses associated with those parts of the mosquito surveillance program would not be expected to exceed the savings noted.

Other variable expenses include labor costs, which may differ from our calculations depending on the salary of the person performing each task. Similarly, costs associated with sentinel chicken flocks and mosquito collections will increase if chickens are bled more frequently than biweekly or if mosquitoes are collected more often than 3 times every 2 weeks. Less expensive chicken coops can be constructed, but based on our experience, they do not protect chickens from canine predators as well as the design used in the current study (coop design is available from D. A. Brown upon request).

Analysis of the relative merits of arbovirus surveillance methods must consider, in addition to costs, their relative effectiveness for detection of virus transmission (Phillips et al. 1993). For the specimens we collected, as well as material from 2 southern California locations, Reisen et al. (2000) concluded that sentinel chickens were the most sensitive and specific indicators of enzootic arbovirus transmission. Wild bird serology did not provide early warning of subsequent sentinel chicken seroconversions, and in several cases, virus was not isolated from mosquitoes before or after chickens seroconverted in the same area. Most wild birds were not recaptured frequently enough to accurately determine when or where they were infected. Of the 20,192 wild birds sampled in southern and northern California, the blood of only 0.7 and 0.1% contained detectable antibodies against WEE and SLE, respectively. The blood of few wild birds contained antibody; in fewer still was seroconversion detected during a single transmission season, and detection of arbovirus antibody was spread over 149 different avian species with various behaviors and ecologies. Reconstructing the infection history of stationary, captive sentinel chickens and mosquito vectors captured in EVS traps was much less problematic. During the 2 years of study, 24 and 7% of the chickens in 56 flocks seroconverted to WEE and SLE, respectively. Isolation of virus from mosquitoes should in theory be detectable at the same time or prior to chicken seroconversion, although in practice that was not always the case. An additional important reason for retaining assay of mosquito pools in an arbovirus surveillance system is that, should exotic viruses (those viruses that would not normally be expected to occur in California) be introduced into the state, they will be more rapidly and efficiently detected by virus isolation from mosquitoes than by antibody assay of avian sera.

As an alternative to capturing birds in mist nets, one could use avian traps that capture birds without restraining them over an extended period of time (e.g., a modified crow trap or ground trap [Gruwell et al. 1988, 1989; Bennett et al. 1993; Reisen et al. 2000]), which might have the added benefit of reducing personnel costs. Unfortunately, in rural areas, effectiveness of these kinds of traps is often compromised by extensive damage from large animals such as wild pigs, dogs, and coyotes and by unacceptably high bird mortality rates from predators such as opossums, rats, and snakes (W. K. Reisen, personal communication). Future studies should make cost comparisons among different kinds of avian traps in urban settings. To be efficient for surveillance, those methods would need to result in frequent recapture of wild birds so that seroconversion is detected between captures and the timing and location of infection can be pinpointed.

We recommend that the current surveillance system for WEE and SLE, which employs sentinel chickens and collection of mosquito vectors for virus isolation, be retained for establishing local virus transmission, determining risk of human infection, and making decisions regarding the initiation of control measures. Future efforts should be directed at improving the application of these methods by investigating the costs and efficiency of modifications in the frequency of specimen collection and flock or mosquito trap placement.

ACKNOWLEDGMENTS

We thank John Hileman, Kim Knerl, Jason Rasgon, and Linda Styer for their technical assistance in carrying out field studies. William K. Reisen, John D. Edman, Laura D. Kramer, and John DeBenedictis made constructive comments on an earlier version of the manuscript. This research was supported by the generous support of the Sacramento-Yolo Mosquito and Vector Control District, Coachella Valley Mosquito and Vector Control District, Contra Costa Mosquito and Vector Control District, Greater Los Angeles County Mosquito and Vector Control District, Kern Mosquito and Vector Control District, Marin-Sonoma Mosquito and Vector Control District, Northwest Mosquito and Vec-

tor Control District, Orange County Vector Control District, San Joaquin County Mosquito and Vector Control District, Sutter-Yuba Mosquito and Vector Control District, and the University of California Mosquito Research Program.

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