Partners: serendipity in arbovirus research

William C. Reeves

School of Public Health
University of California, Berkeley
Berkeley, California 94720-7360

Received 23 January 2001; Accepted 30 January 2001

ABSTRACT: A review of 60 years of research on mosquito-borne arboviruses in the Western U.S.A. revealed a number of instances when serendipity influenced the development of new concepts or novel approaches to solve ecological or epidemiological problems. Eight such events were selected as examples. The need for effective mosquito traps to collect live mosquitoes to be tested for virus infection posed design problems and also led to the use of CO₂ (dry ice) as a mosquito attractant. This research also led to identification of Culex tarsalis as a primary target for vector control programs in the western U.S.A. Attendance at a movie led to development of fluorescent dusts to mark mosquitoes for studies of their numbers, life tables and movements. Knowledge of vector-virus associations was used to influence state legislative action to provide funding for vector control and further discovery of vector-virus associations. Derivation of the term “Arbovirus” started as laboratory jargon and evolved into being the classification for over 500 vector-borne viruses. Sociobiological changes resulting from the use of television and air conditioning fortuitously decreased exposure of California residents to vector attack. These two changes were introduced into households in California in the early 1950s. The prime time when C. tarsalis, the primary vector of encephalitis, bites people is around sundown which also is primetime for television watching. These sociological changes are a valuable adjunct to vector control programs. Journal of Vector Ecology 26(1): 1-6. 2001.

Keyword Index: Arbovirus, Culex tarsalis, fluorescent markers, mosquito traps and CO₂ bait, serendipity

INTRODUCTION

Several months ago Dr. Robert Lane asked me to talk at the annual national meeting of the Society for Vector Ecology on some unique experiences I have had in 60 years of research on mosquito-borne arboviruses. I appreciated the opportunity this provided me to revisit some interesting events. I have selected occasions that led to development of new techniques or programs in the field of arbovirology and are considered now to be routine by current students of vector-borne diseases. For most of the users the origin of the procedure is unknown. I chose the title of the talk, “Partners—Serendipity in Arbovirus Research” as it describes the circumstances surrounding many of these developments. The dictionary definition of serendipity is “The faculty for making desirable discoveries by accident.”

HISTORICAL BACKGROUND

In 1930, only six viruses (Yellow fever, Louping ill, Blue tongue, African swine fever, Nairobi sheep disease and Vesicular stomatitis) were known to be transmitted by arthropods and virology was a newly emerging science. You will notice that only one of these viruses caused a disease in people; the others were diseases of domestic mammals. In the 1930’s, there were no virology classes at the University of California at Berkeley (UCB) and microbiology was focused on bacterial and fungal diseases. In 1940, a virus was jokingly described as “you can’t see it, smell it, touch it, or culture it, but it can kill you.” However, the field was growing and in 10 years, 10 additional arthropod-borne viruses were discovered, including Western equine encephalitis (WEE), Eastern equine encephalitis,
St. Louis encephalitis (SLE), Japanese encephalitis, Venezuelan equine encephalitis and West Nile. All of these viruses cause disease in humans and other animals and are still important. Today, 536 arboviruses are known in the world (Karabatsos 1985). In 1939, I was a graduate student in entomology at UCB and had developed a PhD thesis project on the biology of a tree-hole mosquito. I had it colonized but had difficulty hatching the fully embryonated eggs. I solved that problem but subsequently found my findings had been revealed to other workers who soon had a paper in press (Gjullin et al. 1941). Their title was, “The necessity of a low oxygen concentration for the hatching of Aedes eggs.” That had been my basic finding. It was a dark day; I no longer could use the topic for a thesis and had to start over. In retrospect, as you will see, it was the best thing that ever happened to me. I changed the direction of my research interests. In the 1940s WEE and SLE were epidemic in California and the Western U.S.A. (Hess et al. 1963). It was thought, but not proven, that these viruses were vectored by mosquitoes. I proceeded to learn the then primitive techniques of virology and started a new thesis project on the passage of WEE virus from guinea pig to guinea pig by mosquitoes. I was probably the only graduate student in the country studying such viruses in vectors at that time.

INTERRELATIONSHIPS OF SERENDIPITY AND RESEARCH

Studies in Yakima Valley, Washington

There had been an epidemic of WEE and SLE in the relatively isolated Yakima Valley of Washington in 1940 (Hammon and Howitt 1942). A project was planned at the Hooper Foundation at the University of California in San Francisco to study an anticipated epidemic in Yakima in 1941. I was invited to join that team as an entomologist and, if successful, to use my findings as a new thesis topic. Dr. Hammon was organizing a team of 11 scientists to cover a wide range of objectives. The team included two physicians, two veterinarians, three entomologists, one zoologist, and three virologists. This was an unusual breadth of workers to organize for a field project in that era.

Mosquito Trap

In May 1941, Dr. Hammon came to Berkeley to discuss the project. He asked me if I needed any special equipment. I said, “Yes, I need a special trap to collect hundreds, even thousands, of live mosquitoes of many species to be used for virus isolations.” Hammon said, “Where do I buy the traps?” and “How many?” I replied, “They haven’t been built yet or tested.” He tossed me a paper napkin and said, “Draw me a sketch.” I did, and he pocketed it though I didn’t include any dimensions (Reeves and Hammon 1942). The trap was simple! A funnel, a light bulb, a cage and a large exhaust fan. Two weeks later Hammon called and said, “Come to San Francisco to see your new trap.” When I arrived, there it sat made of large sheets of plywood and mounted on a trailer to be hauled to Yakima a thousand miles away (Figure 1). I had anticipated a small trap I could carry, so I dubbed this monstrosity “The Hooper Folly.”

Dry Ice

On arrival in Yakima I found an irrigated pasture with many mosquitoes in it. A cooperative farmer had an electric line into the pasture and he let me connect the “Hooper Folly.” After two nocturnal runs it was obvious the trap could collect hundreds of live mosquitoes. So I planned a third run. On the way I stopped in town and picked up 50 lbs. of dry ice to store frozen viruses and specimens at the field lab. I put the ice in the back of the car. On arrival in the field it was a warm night so I left the car doors open, checked out the trap and plugged in the electricity to start it.

When I returned and climbed into the car I was assailed by a cloud of mosquitoes that had occupied it and they were hungry. So, aggravated, I swatted some and headed for the lab to unpack and store the dry ice. As I did, a little light bulb came on in my head that said, “Do you remember an article by Rudolfs (1922) in New Jersey that said CO₂ attracts mosquitoes in the laboratory?” Suddenly, I realized I had an attractant - dry ice - to add to the light in my trap and perhaps increase its efficacy.

The next night I put dry ice in the funnel but the
Examples of the questions were: Where did you come from? How many of you are there? How old are you? Obviously, we needed new techniques and it was time to hope for more serendipity.

**Marking Mosquitoes**

Dr. Hammon and I went to a movie one night in Bakersfield. It was a black and white movie called, “The Thin Man” and starred William Powell, as a detective, his wife, Myrna Loy and their dog, Asta. The detective was called to solve a series of cash register robberies. He said, “No problem,” and had all the employees leave the room. He then took a small gadget (with a rubber bulb attached) from his pocket. He pushed the bulb and a small cloud of gray dust went into the cash register. He closed the register and put a small piece of tape across the drawer opening. He called in the manager and said, “Don’t use that register but call me when the tape is broken and some cash taken.” The next day the manager called—the cash register had been robbed. So Powell went over, pulled a little fluorescent lamp from his jacket and plugged it in. The employees were brought in. The hands of the first one went under the lamp and she was dismissed. The second person’s hands lit up brightly, they fluoresced, and Powell said, “Why did you rob the register?”

I jumped up from my movie seat and yelled, “By golly, Bill Hammon, now I can mark mosquitoes, release them and recapture them!” The audience shouted “Shut up and sit down.” I did, and the era of using fluorescent dusts to mark mosquitoes was born (Reeves et. al. 1948). Unfortunately, the film studio didn’t know what the dust was so I had to make my own with red rhodamine dye and gum arabic. Today a wide range of dusts in many colors can be bought. By marking a known number and species of mosquitoes to release and then recapture, we can determine how far mosquitoes move, how many there are, and their survival rates. During our first releases, marked female *Cx. tarsalis* were recovered 2.5 miles from their point of release (Reeves et. al. 1948). Subsequent studies of *Aedes* have made recoveries at up to 30 miles (Aarons et. al. 1950; Smith et. al. 1956). Today everyone takes such studies for granted. The lesson learned is—if you want to enroll serendipity as a partner, attend the right movie.

A few years ago a professor of sociology at UCB was studying the street people on Telegraph Avenue in Berkeley. He found they were not giving him true answers to his questions as to where they lived, where they went and how many of them there were. He took me to lunch to ask how I would do the study and get good data. Of course I said, “Mark them with a fluorescent dust, turn them loose, buy a large fluorescent light and find them again.” The Committee on Human
Subjects rejected the study. First they said the street people would wash the dust off. I said, “No way,” and then they said the study would be an invasion of privacy. I gave up and went back to study mosquitoes in the field where there was no committee to protect their privacy.

**California State Legislature and Malaria-Encephalitis Control**

At the end of World War II, the California State Legislature was concerned that thousands of veterans returning to California (many of them from the Pacific arena) would be infected with malaria. There was no cure for malaria infection then so there was a threat that malaria would be reintroduced into California where it had been eradicated. The State Legislature proposed to establish a subvention fund to stimulate increased control of *Anopheles* mosquitoes by local vector control districts and health departments. With our new knowledge of *Cx. tarsalis* as a vector, those of us concerned with encephalitis control and wanted the subvention to be augmented to include that problem. I was asked to present the encephalitis problem at a hearing on the “Malaria Bill” being held in January, 1945, by a joint committee of the Senate and the House. I accepted the appointment with some anxiety.

At the meeting I illustrated the current status of our knowledge of the cycles of WEE and SLE viruses with a colorful slide (Figure 2). The slide showed the basic cycle for WEE virus involving domestic and wild birds and *Cx. tarsalis* (Reeves 1985). It presented the divergence of infection to horses with a dead horse as a result. It also showed the divergence of infection to people with outcomes of death, a child on crutches and another with mental retardation as a result of encephalitis. To my amazement, a senator interrupted me and said, “That is my dead horse.” Not to be outdone, a legislator stood up and said, “That little boy on crutches is my grandson who is partially recovered from encephalitis.” Without further discussion of viruses and diseases, I presented a request for $400,000 for subvention of *Cx. tarsalis* control and $200,000 for further research to demonstrate that *Cx. tarsalis* control would prevent encephalitis. The bill was rewritten for control of both malaria and encephalitis and it was quickly passed and signed by the Governor. I felt very lucky to have been in the right place at the right time, another victory for serendipity.

**A Viewpoint of Research**

It was about this time that I was making a routine weekly collection of mosquitoes from the porch of a ranch in Button Willow, California. We had recently released several thousand marked *Cx. tarsalis* a few miles away and that site was a hot spot for mosquito and virus collections. The old farmer who lived there enjoyed sitting in a rocking chair and watching me as I worked. One day he suddenly said, “Doc, if you don’t know all the answers now you never will.” I had to think about that for a minute and then I said, “Yep, you are so right.” I still owe him a debt for predicting my future and providing me a new philosophy. I would never know all the answers to arbovirologic and vector ecologic problems. His statement has provided me with both a point of reference and a reality check on many occasions. Was this another act of serendipity?

**Derivation of the Term “Arbovirus”**

You all know something about “Arboviruses” but you don’t know that serendipity had something to do with the derivation of this word.

In the 1940s as the field of mosquito-borne viruses evolved, I educated my non-entomologist co-workers to vector ecology terminology. I started using the term, “The mosquito-borne viral encephalitides.” Quite a mouthful. Ticks and *Culicoides* were also found to be involved as vectors so I changed it to “Arthropod-borne virus encephalitides.” Still too long, and soon it became “Arboviruses,” a nice short name.

In 1958, the International Congress on Tropical Medicine and Malariology was held in Lisbon, Portugal. The World Health Organization and Rockefeller Foundation staff had organized a separate one-day meeting of some 50 attendees concerned with vector-borne viruses. This included many world leaders in the field and all the big names were there. The real objective was to discuss the unsolved problems in this relatively new field and to get a recommendation that WHO should...
take the responsibility for developing an international program. On the day of the meeting, I was stunned to learn I had been selected to chair the meeting and Telford Work, known to many of you, was to be “rapporteur.” The Rockefeller representative then told me that was why they paid Tel’s and my way to that meeting. I still don’t know why they selected two young upstarts for the job instead of the big names.

The meeting was a busy day. Many problems were discussed and some of the discussions disintegrated into shouting matches with great disagreement. I felt like I was running a three-ring circus and that I was caught in the middle of an uncontrollable mob of lions and tigers.

As the day ended, someone suggested the meeting should select a name for this field of study and for the specific viruses that were included. I thought, “Oh boy, now we will really have some turmoil.” Then, someone got the floor and I was stunned to hear him say, “Bill, tell us about the derivation of terms used for these viruses in your laboratory.” I did and ended with, “Arborviruses.” Immediately someone made a motion that this meeting and the WHO should accept that name and make it official worldwide. I held my breath and saw a hand wave. It was Dr. Anatol Smirodintsev from Russia. I thought, “Here we go—Russia vs. the U.S.A.” Anatol said, “The proposed name ‘Arborviruses’ has nothing to do with trees but ‘arbor’ does.” I said, “What if we take out the second ‘r’ and call it ‘Arbovirus’?” He said, “I like it. Accept that change and move for its acceptance by this group and after it’s approved, I also move that we terminate this meeting.” It was approved with no further discussion.

In the next few years, WHO organized its first formal meeting of an Advisory Committee on Arboviruses, adopted the term and requested that the International Committee on Virus Nomenclature approve it, which it did in 1963 (WHO 1985). Publication of the 3 editions of “International Catalogue of Arboviruses” (Taylor 1967, Berge 1975, Karabatsos 1985) exemplifies the widespread acceptance of this terminology. This gives you yet another example and another definition of serendipity, “Being at the right place at the right time and solving problems.”

Television and Air Conditioning

It is now time for my last and most recent example of serendipity. In the period 1960 to the early 1990s the incidence of proven clinical cases of WEE and SLE infection had been very low among the citizens of California as had the activity of the viruses in their basic cycles in vectors and birds. This was credited in large part to the successful control of Cx. tarsalis. In the early 1990s there were some wet years that had favored increased Cx. tarsalis. There was also the problem of increased genetic resistance of Cx. tarsalis to many insecticides, so control was difficult. By 1993, there was a widespread resurgence of WEE virus in Cx. tarsalis and infection of sentinel chickens in the Central Valley of California but no proven clinical cases in humans. The question was, how can so many sentinel chickens and mosquitoes be infected, but so few or no cases be detected in people in the same region? I was challenged by many people to explain this and I could not, nor could anyone else. We knew that the virus was virulent, transmission was high in the avian-vector cycles, few people were immune, and the vector was abundant and was still feeding each night starting one-half hour after sun-down. I could only think of one other avenue to pursue that might explain the absence of cases: Had people in the Central Valley changed their exposure to mosquitoes in some way since the early to mid-1950s? A most probable time for the change would be in the evening period after sundown, when the vector Cx. tarsalis feeds. From my personal experience in the hot Central Valley each summer, I finally focused on looking at air conditioning of houses and television use as possible variables that could affect exposure to mosquitoes. I had a student carry out a telephone survey of Kern County residents for such activities and to look at census data (Gahlinger et al. 1986). There was an amazing epidemiological fit as the number of proven cases of WEE and SLE per year that had varied from around 25 to over 150 per year in the period 1945 to 1958 had declined to less than five from 1958 onward. In the same time period, the percentage of households with one or more television sets in the Central Valley rose from 20% in 1952 to over 90% by 1960. Concurrently, the percentage of houses with central air conditioning increased from 10% in 1950 to 75% in 1984. Mosquito-biting time was prime time for television watching, and air conditioning gave indoor relief from hot summer nights and outdoor activities. These two changes in human activity are now considered to be a valuable adjunct to organized vector control programs by insecticides and environmental modifications. Again, my partner, serendipity, had come to my assistance—perhaps for the last time. It remains to be seen if two relatively recent changes, the use of computers and cell phones, may have an effect on the rate of mosquito feedings on people.

CLOSURE

In closing, I have enjoyed having serendipity as a partner for some 60 years. I thank Bob Lane for inviting
me to remember and present some of these events in
the life of a “Vector Ecologist” and for editing this
manuscript.

REFERENCES CITED

1950. Studies of the flight range of 

Berge, T. O. 1975. International Catalogue of
Arboviruses Including Certain other Viruses of
Vertebrates. U.S. Dept. of Health, Education and
Welfare, DHEW. Publ. No. (CDC) 75-8301.

Gahlinger, P. M., W. C. Reeves, and M. M. Milby. 1986.
Air conditioning and television as protective factors
Hyg. 35: 601-610.

Gjullin, C. M., C. P. Hegarty, and W. E. Bollen. 1941.
The necessity of a low oxygen concentration for
the hatching of Aedes mosquito eggs. J. Cell. Comp.

Hammon, W. McD. and Howitt, B.F. 1942.
Epidemiological aspects of encephalitis in the
Yakima Valley, Washington: mixed St. Louis and
Western equine types. Am. J. Hyg. 35: 163-185.

Hammon, W. McD., W. C. Reeves, B. Brookman, E.
M. Izumi, and C. M. Gjullin. 1941. Isolation of the
viruses of Western equine and St. Louis encephalitis
from Culex tarsalis mosquitoes. Science. 94: 328-
330.

Hammon, W. McD., W. C. Reeves, and P. Galindo.
1945. Epidemiologic studies of encephalitis in the
San Joaquin Valley of California, 1943, with the
isolation of viruses from mosquitoes. Am. J. Hyg.
42: 299-306.

Headlee, T. J. 1941. New Jersey mosquito problems.

Relation of temperature to activity of Western and

Karabatsos, N. 1985. International Catalogue of
Antonio, TX.

Mahaffy, A. F., K. C. Smithburn, H. R. Jacobs, and J.

Mitamura, T., M. Kitaoka, and M. Imai. 1950. Seasonal
occurrence of mosquito and its infectivity of
Japanese encephalitis virus in Okanaya City, 1942.
Relationship between the grade of epidemic and the

Reeves, W. C., B. Brookman, and W. McD. Hammon.
1948. Studies in the flight range of certain Culex
mosquitoes using a fluorescent-dye marker, with
notes on Culiseta and Anopheles. Mosq. News. 8:
61-69.

Reeves, W. C. and W. McD. Hammon. 1942.
Mosquitoes and encephalitis in the Yakima Valley,
Washington. IV a trap for collecting live

Rudolfs, W. 1922. Chemo tropism of mosquitoes. New

Investigations of a recurrent flight pattern of flood
water Aedes mosquitoes in Kern County, California.

operated light trap, an improved model. Mosq.

Viruses of the World. U.S. Public Health Service
Office, Washington, D.C.

WHO. 1985. Arthropod-borne and rodent-borne viral