1968

A Brief History of the NAMRU-3 Medical Zoology Program

Harry Hoogstraal
Naval Medical Research Unit 3

The Egyptian Public Health Association

U. S. Naval Medical Research Unit No. 3

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By

HARRY HOOGSTRAAL

Head, Medical Zoology Department

With the Author's compliments

Reprinted from:

SPECIAL NUMBER

The Journal of the Egyptian Public Health Association

SCIENTIFIC CONFERENCE

NAMRU-3 20th. Anniversary

27,28 June 1968

Volume XLIII **1968**

THE JOURNAL OF THE
EGYPTIAN PUBLIC HEALTH
ASSOCIATION
VOL. XLIII, SPECIAL NUMBER
1968
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HARRY HOOGSTRAAL
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The NAMRU-3 Medical Zoology program began during Easter week of 1950. We had just closed the NAMRU-3 field station at Torit, in Equatoria Province of southeastern Sudan, and were heading homeward to the United States. However, the offer to remain in Egypt for a year or 2 to develop a Medical Zoology department at NAMRU-3 provided an interesting challenge after working for several years in tropical forests and grasslands of Africa and other continents. We shall briefly describe subsequent events under 4 headings: (1) Medical Zoology in Egypt, (2) Ticks and Tickborne Diseases of the World, (3) Kala Azar in the Sudan, and (4) Miscellaneous Activities.

I. MEDICAL ZOOLOGY IN EGYPT

Our objective was to investigate inter-relationships between vertebrate hosts (wild and domestic mammals, birds, reptiles, and

* This talk was delivered extemporaneously and, with some additions, written in December 1968, for publication.

amphibians), ectoparasites (fleas, lice, ticks, mites, etc.), and disease agents (viruses, rickettsias, and bacteria) in the transmission and epidemiology of human and animal diseases in Egypt. We had earlier witnessed tragic loss of life and of working time among both local peoples and American personnel in the South Pacific because so little was known about vectors and reservoirs of arthropod-borne diseases. In Egypt, therefore, our approach would be to discover unrecognized disease cycles and foci and to provide biological information on zoonotic disease patterns in advance of, not following outbreaks of poorly known infections. Solid information on biological interactions between hosts, vectors, and pathogens would permit epidemiologists and health officials to plan and apply immediate prevention and control measures rather than waiting for results of time-consuming preliminary field studies. This program remains the heart of the NAMRU-3 Medical Zoology effort though inter-related branches have gradually developed elsewhere in the world.

In Egypt we soon were overwhelmed by the inability to recognize and distinguish satisfactorily between species in the local fauna. Only the birds were reasonably well known taxonomically. Classification of mammals, reptiles, amphibians, fleas, lice, ticks, and mites was chaotic. Our first concern was to learn to recognize and differentiate each host and each parasite species, to assure that each species was provided with a universally accepted scientific name, and to study the biological features that might influence the species' role in the epidemiology of disease.

Little information was available on the associations between Egyptian animals and zoonotic diseases and practically none on host-parasite inter-relationships, geographical distribution, ecology, population and seasonal dynamics, movement patterns and ranges, and behavior of hosts and ectoparasites.

Our job, therefore, was to explore all zoogeographical areas, all ecological habitats, and all biological niches in rocky hills, sandy deserts, and cultivated fields during each season of the year. Long series of vertebrate and invertebrate specimens collected for taxonomic studies were carefully annotated with field data and observations. These collections formed the basis first for a taxonomic monograph on each pertinent vertebrate and invertebrate group in Egypt, and later for reviewing data on species biology and host-parasite and ecological interactions that play determining roles in disease transmission.

Arrangements were made with specialists in each group to study NAMRU-3 collections, prepare systematic revisions, and report in scientific literature on the species in Egypt, how to recognize each, and how to distinguish it from related species. On the spur of the moment while making these arrangements, we decided to develop at NAMRU-3 a special competence in one group of parasites — the ticks — which infested most wild and domestic vertebrates, were often closely associated with humans, and were known to be potential reservoirs and vectors of numerous disease-causing agents. This casual decision ultimately led to expansion of the NAMRU-3 Medical Zoology program into the world-wide investigation of ticks in relation to diseases that we will mention in the second part of this discussion.

During the first 5 years we explored the northern and southern and eastern and western deserts, oases, coastal plains, mountains, hills, desert edges, cultivated fields, towns, and cities of Egypt. The present sturdy fleet of NAMRU-3 desert-going trucks and the storerooms full of excellent collecting and camping gear are in sharp contrast to our early gypsy-like caravans of exhausted, unspecialized vehicles loaded with World War II discarded gear and jerry-built equipment salvaged from dump heaps. Operating funds in those days, before NAMRU-3 had proved its scientific worth and commanded the respect it does today, were limited. Egyptian roads, today as good as any in the world, were then only in the planning stage. Technicians needed training and experience to develop into the smoothly functioning team members that today quickly and efficiently accomplish each field collecting mission.

Despite these problems, we collected tens of thousands of carefully annotated study specimens. The Egyptian vertebrate and ectoparasite faunas are now among the best known in the world. Each species can readily be identified and labelled with an accepted scientific name. The distributional limits and ecological characteristics of most species are well known. Preliminary papers showing numerous host-parasite inter-relationships have already been published and monographic reviews of available data are in various stages of completion.

For instance, the final monograph on the hundred or so mammal species of Egypt should be completed in about a year. This year, NAMRU-3 published a final annotated checklist and keys to the reptiles and amphibians of Egypt prepared by our herpetological consultant, who previously had written numerous papers clarifying the status of individual components of this fauna. Last year another consultant published a beautifully illustrated key to the 35 species of fleas now known from Egypt, where only about a dozen species had earlier been listed. This key was generously annotated with details on the host relationships and distribution of each Egyptian flea species. The lice, bed bugs, and bat flies of Egypt have also been reviewed and the parasitic mite collections are being studied for a final report. As most of you realize, the Egyptian tick fauna is now exceptionally well known. The limitations of time prevent us from presenting examples of even a few of the many fascinating facts that have been learned about this fauna.

Best of all, local vertebrates and ectoparasites are now actively studied in Egyptian university faculties of science,, agriculture, and human and veterinary medicine. Egyptian graduate students are undertaking a variety of experimental investigations on local vertebrates and ectoparasites. Egyptian governmental ministries and international organizations of the United Nations (W.H.O. and F.A.O.) collaborate in various parts of the Medical Zoology program, collate local findings with other information on zoonotic disease problems, and promote similar investigations in their own research units.

Research on arboviruses independently and collaboratively undertaken by the NAMRU-3 Virology and Medical Zoology departments has resulted in discovery of Sindbis, Quaranfil, Chenuda, Wad Medani, and other viruses in Egypt and the Sudan. Epidemiological and transmission factors of West Nile, Sindbis, Nyamanini, and Quaranfil viruses have been elucidated. Quaranfil and Nyamanini viruses, for instance, are the only tickborne viruses (except for those in the Russian spring-summer encephalitis complex) that have been studied the year around in nature and experimentally in the tick vectors, in other tick species in which multiplication and transmission does not occur, and in vertebrate hosts. These investigations are being extended in the recently established Medical Zoology virus-vector laboratory. Similar studies are underway with newly discovered Egyptian viruses, and the search for other viruses that may affect human and animal health is being widened. Identification of these viruses is a collaborative project between NAMRU-3 and the Rockefeller Foundation Arbovirus Research Unit of the Department of Epidemiology and Public Health in the Yale University School of Medicine. Most of the viruses already discovered in Egypt and the Sudan are now considered by specialists to be the prototype of groups of associated viruses recently isolated from many other countries in Africa, Asia, and Europe.

Rickettsial disease research is undertaken in co-operation with the Egyptian Ministry of Health (Virus Research Laboratory) and the United States Public Health Service (National Institutes of Allergy and Infectious Diseases — Rocky Mountain Laboratory). Q fever, which was first shown by NAMRU-3 investigations to be present in Egyptian people, domestic animals, and ticks, has been the subject of continuing studies by Egyptian and NAMRU-3 researchers. The extensive but irregular distribution of Q fever in Egypt is reflected in local incidence rates. Evidence of Q fever as a zoonotic infection of wild and domestic animals, ticks, and other ectoparasites is increasing but the epidemiological pattern in the field and the interplay between transmission factors in nature and by human activity has not yet been studied definitively. The incidence and distribution of murine typhus, transmitted by fleas has also been studied. The agent causing tick typhus was shown to occur in Egypt but, surprisingly, this infection is known here only in certain localized desert rodents. Elsewhere in Africa, southern Europe, and the Near East, tick typhus is often a common infection of numerous mammals and ticks in a variety of ecological situations.

A rickettsia-like organism, *Wolbachia persica,* first isolated from bird-parasitizing ticks in Egypt, is now extensively used as a prototype for experimental studies among rickettsial and rickettsia-like groups. A causal relationship between *W. persica* and certain diseases is highly suspect and will be investigated when time permits. The characteristics of *W. persica* and its infection pattern in ticks were originally studied in detail by an Egyptian student in England and by an American student in U.S.A., both working with materials furnished by NAMRU-3.

A rickettsial disease presently being intensively investigated collaboratively by this Unit and Egyptian and American rickettsiologists is epidemic typhus. This disease has long been considered to have only a man-louse-man transmission cycle, without involvement of other vertebrate reservoirs or invertebrate vectors. Certain features of this classic cycle are biologically unusual. We find it especially interesting that no vertebrate reservoir is known and transmission to man occurs through contamination by infected insects (lice) rather than through their bite. Considerable evidence has been assembled by Egyptian colleagues to suggest that another cycle of epidemic typhus may occur among domestic animals and may be transmitted by other bloodsucking arthropods. The incidence, dynamics, and nature of this domestic or wild animal infection and the question of the relationship of this cycle to various parasites and to the man-louse-man cycle in the epidemiology of epidemic typhus is now being investigated. We hope to determine whether apparent domestic animal infections are merely an exceptionally interesting biological phenomenon or a real factor in the recurrent and sometimes devastating outbreaks of epidemic typhus among humans.

Plague, another threat that concerns Egyptian public health authorities, was once common but now appears to be absent. Some years ago when a plague focus was thought to smoulder near Asyut, personnel of the NAMRU-3 Medical Zoology department and the High Institute of Public Health in Alexandria formed a team to search for evidence of plague in the parasites and vertebrate animals of towns, cultivated fields, desert edges, and nearby deserts of the Asyut area. The results were entirely negative.

Relapsing fever spirochetes, *Borrelia persica,* were found in *Ornithodoros tholozani* ticks that we collected in caves and small shelters in the western desert of Egypt. These highly virulent spirochetes may cause serious illness and death of persons bitten by infected ticks. With the increasing human population and utilization of desert areas, the presence of this disease agent is a cause for concern.

We have also made an extensive study of *Ornithodoros erraticus* group ticks and their spirochete, *Borrelia crocidurae,* in Egypt. Infection is common in these ticks, which are widely distributed in certain desert biotopes and in sandy cultivated areas of northern Egypt, but are absent in southern Egypt and mountains of Sinai. Usual hosts are a variety of rodents, hedgehogs, carnivores, and ground-inhabiting birds such as the Egyptian little owl. Humans may be bitten when they rest or sleep on the ground.

Infected *O. erraticus* ticks were sent to Germany for experimental studies. After human lice were inoculated with *B. crocidurae* from these ticks or after lice were fed on infected rodents, the tick spirochete developed in these lice and could be transferred to new hosts on which the lice fed. Thus, we postulate that louseborne relapsing fever, which sometimes erputs in fierce epidemics among humans and then completely disappears from the scene for many years, may in fact start when a spirochete-infected tick bites a louse-infested human. The spirochete becomes adapted in the louse population, is spread among the crowed human population, and is transmitted as louseborne relapsing fever. Biologically and epidemiologically this source of human infection and spread by lice is quite possible.

The presence of various *Salmonella* organisms in Egyptian ticks has also been shown. Tularemia infection has not been found in Egyptian mammals. Aside from human health, the life cycle of a jerboa blood and tissue protozoan, *Hepatozoon balfouri,* has been demonstrated in its vertebrate hosts and mite vectors, as well as the distribution, incidence, and host-relationships of this infection. Veterinary problems in Egypt have not been studied in this department but direct or indirect support for a number of investigations has been given to local veterinarians.

2. TICKS AND TICKBORNE DISEASES OF THE WORLD

In 1950 we began to study Egyptian ticks and their disease relationships, as well as numerous ticks that we had already collected incidental to other research in the Sudan and in Madagascar. Today, approximately 100 species, or one-eighth of the world's described tick fauna, is known from the area covered by Egypt and Sudan, where about 20 species were listed in 1950. Only a few endemic species had been collected in Madagascar; today's list of about 25 species in Madagascar includes much important biological data and morphological criteria for identification of larvae and nymphs of most species. But comparative numbers of described species alone cannot elucidate their role in the epidemiology of disease. Clarification of ambiguous species relationships has uncovered new fields and foci to explore and new epidemiological concepts concerning the roles of ticks in disease transmission.

The initial difficulties and uncertainties of correct identification and true species differentiation were enormously greater than we had anticipated. In the process of solving local tick species problems, we needed to go further and further afield for comparative study materials. At last, we found ourselves involved in numerous inter-related tick problems around the world.

Results of the Sudan tick study were published in 1956 in a monograph that is frequently used as a reference by other specialists, biologists, parasitologists, and educators. Sixty-five species were recorded from the Sudan, together with much biological data and extensive literature reviews, in an area where only 12 or 15 species had previously been listed with little or no biological information. Similarly in Egypt, the tick fauna of about 10 poorly known species has been expanded to 40 species together with a large volume of data on distribution, ecology, life cycles, seasonal dynamics, host preferences, and disease relationships. Details from numerous papers already published on Egyptian ticks and their biology are being collated with unpublished experimental and field data for a final monograph on this tick fauna. Results of the Madagascar tick study, published in 1953, revolutionized knowledge and concepts of the fauna of this remarkable island and provided new clues to principles of tick speciation, distribution, and host-parasite relationships. This work initiated a continuing, world-wide interest in the genus *Haemaphysalis,* which forms a conspicuous part of the Madagascar tick fauna. Since 1953, an even greater variety of endemic ticks has been discovered and, in co-operation with scientists in Madagascar, we are also preparing a final monograph on this fauna.

The nature of our tick studies is based on (1) the unusual capacity of many tick species to harbor and transmit a wide variety of organisms pathogenic to humans and to other animals, and (2) recognition of the individual species as a unique physiological and biological entity. Thus each species plays a specific role, differing from that of every other species, in the natural history of each arthropod-borne disease. Each species must be clearly recognizable morphologically (for identification purposes) as well as biologically, and must be evaluated separately for its characteristic role in the cycle of infectious disease transmission. Failure to appreciate these principles and confused species concepts have negated or seriously reduced the value of a considerable part of the world's scientific literature dealing with experimental or applied studies on ticks. In this age of molecular biology and of rapid human occupation of sparsely settled lands, it is especially necessary that both the researcher and the public health official be «species-conscious», and that systematists provide the best possible taxonomic information. To do this, the biological features of each species must be investigated and elucidated.

In attempting to solve local taxonomic problems, we soon learned the necessity of becoming intimately acquainted with many, or all, species of the genus in other parts of the world in order to understand individual species at the local level. This requirement has been reflected in worldwide revisional studies by ourselves and colleagues of certain tick genera or of species complexes in different parts of the world. From stressing the importance of biological information associated with each sample of ticks collected by ourselves or others, and careful analysis of these data and observations, we have frequently recognized clues leading to more intensive morphological studies that have revealed new structural-taxonomic criteria. These, in turn, have led to wider studies and to revised species concepts in distant geographical and ecological areas. Certain problems have been solved only after 10 or 15 years of gradually acquiring more samples and data until at last we found that we had sufficient evidence for meaningful answers.

The genus *Haemaphysalis* is a case in point. Two *Haemaphysalis* species were previously reported from Madagascar. We found that one of these was badly confused with 2 different species, that several species from this island required description and naming, and that each is modified structurally in association with specialized spines, hairs, or feathers of its specific host animal group. Surprisingly, no Madagascar haemaphysalid showed relationships to African species. Thus we studied Asiatic species and in the fauna of Southeast Asia found a close relative of each species from Madagascar. But the imperfectly known Asian haemaphysalid fauna needed infinitely more investigation before these apparent relationships could be clarified satisfactorily. During the Sudan and Egypt studies we were troubled by a unique distributional concept, stated in literature, in which a single haemaphysalid species was considered to range from the Cape to Cairo and into Southeast Asia and Taiwan. And in Egypt, taxonomic confusion masked the relationships between a haemaphysalid species infesting coastal desert hedgehogs and other species in North Africa, the Near and Middle East, Central Asia, and Pakistan.

These problems meant reviewing voluminous literature, studying type materials in museums around the world, and vastly augmenting our *Haemaphysalis* collections by expeditions to other countries and continents to fill obvious gaps in knowledge. Meanwhile, an epidemic of Kyasanur Forest disease erupted in southern India. We were requested to assist in identification and differentiation between species and immature stages of 14 haemaphysalid species inhabiting the infected area in and near Kyasanur Forest and numerous other species in potentially infected deserts, cultivated areas, forests, and mountains of the Indian subcontinent. As these studies were extended from southern India northward, the fantastic proliferation of *Haemaphysalis* species in the Himalayas first became apparent. More recently, one Himalayan haemaphysalid species has been circumstantially associated as a possible vector of an often fatal, still poorly known infection of humans, which we named Himalayan hemorrhagic disease. In order to clarify the status of certain Indian haemaphysalids, we were forced to look more closely at samples of several more or less similar species reported from New Zealand, Australia, New Caledonia, numerous Pacific islands, Japan, and northeastern China and USSR. These were found to represent a single species that had been transported on Japanese cattle to Australia late in the nineteenth century, and had thence been distributed on cattle to other islands. Tick populations reported under 3 different names from China and USSR proved to represent a single species, the same as that in Japan. The Indian species, previously thought to be the same as that in Australia and southeastern Asia, was shown to be confined to the Indian subcontinent and Ceylon, with an introduced population in Malaya and Borneo, where it is restricted to parasitizing domestic animals and seldom if ever infests wild animals in humid forests. The Indian species is now easily differentiated from the Australian-Japanese species by numerous taxonomic, ecological, biological, and chromosomal differences. Meanwhile, increasing evidence of *Haemaphysalis* species involvement in the epidemiology of several human and animal diseases demanded urgent reappraisal of numerous other species concepts, some of which are still in progress. For instance, we yet have not satisfactorily clarified the problem of the one species once thought to be present in both Africa and Asia, but we do know that in this range, 5 or more separate species form a complex that we had not previously understood.

Eighteen years after starting to study the ticks of Egypt, Sudan, and Madagascar, we find that the genus *Haemaphysalis* consists of about 150, rather than 60, species around the world. From laboratory-reared materials, we can recognize the larval, nymphal, and adult stages of more than half of these species. Now that immature stages can be identified, surveys of infested animals in nature and study of their potential association with disease organisms become more meaningful and the volume of field-acquired biological-epidemiological data increases dramatically. Numerous papers on individual *Haemaphysalis* species and groups have already been published during this investigation and an effort is being made to complete a monograph of some 2000 pages on this genus as rapidly as possible. As a forerunner to this monograph, a volume on the *Haemaphysalis* ticks of India is being prepared in collaboration with Indian colleagues for publication by the Indian government.

Another tick genus, *Hyalomma*, presented a different type of problem. Some 100 species names were available in literature. Almost all of these were inadequately defined. Taxonomic chaos had resulted from the exceptional range of structural variation observed in most large collection samples. This variation is a reflection of the physiological adaptation of hyalommas for survival in the often inclement drier environments of Africa, southern Europe, and southwestern and central Asia. Few sectors of this geographic range were represented by the extensive collection samples required for comparative taxonomic study. Few species had been laboratory-reared to reveal criteria for species recognition and differentiation, the characteristic morphological form, and limits of variation. It was generally appreciated that many *Hyalomma* species are vectors of several human disease agents and are important economically in animal husbandry of developing nations. However, literature directly or indirectly concerning these ticks was hopelessly confused by a welter of meaningless species names.

To solve these problems, we built up a collection of carefully annotated samples of *Hyalomma* ticks exceeding a thousand-fold in scope and volume all others in the world combined. Personally and with the aid of interested colleagues we systematically collected hyalommas from many parts of Africa, Europe, and Asia, reared numerous specimens in the laboratory, and attempted to cross-breed certain species to determine the potential of this phenomenon in nature. While the last word on the genus *Hyalomma* has not been written, we have been able to show that only about 25, rather than about 100 recognizeable species comprise this genus. Most if not all other *Hyalomma* names in literature are synonyms. During this study, it has been necessary to describe and name only a single new *Hyalomma* species, a rare, relict parasite from Somali Republic closely related to 3 other species of the Indian subcontinent.

The fauna of Egypt and the Sudan, the focal point of this study, is now known to include more than half of the world list of *Hyalomma* species. Many occur over extensive areas of Africa or Asia. One species, however, is so localized that we did not discover it until searching an unusual desert habitat in Egypt 2 years ago.

An especially interesting aspect of the *Hyalomma* study has been the investigation of these and other ticks carried to and beyond Egypt by birds migrating southward during the fall from Europe and western Asia into tropical Africa and northward during the spring from Africa into Eurasia.

During the fall migration periods of 1959, 1960, and 1961, 32,086 birds (comprising 72 forms) were examined for ticks in Egypt. Of these, 40 forms, represented by 31,434 birds, were tick infested. The 1,040 bird hosts (3.31% of the tick-infested bird forms examined) bore 1,761 ticks, or 1.69 ticks per host. Over 20 strains of pathogenic viruses were isolated from these birds and their ticks during the 1961 migration period. In the fall of 1962, when 11,036 birds were examined, the prevalence of tick infestation was much higher than previously and ticks were found on 5 additional bird species. In this year, 881 infested birds (24 species, represented by 10,612 individuals) yielded 1,442 ticks. Always during the fall migration, larvae and nymphs of *Hyalomma marginatum marginatum* were most common on these birds.

On their spring migration from Africa to Eurasia, birds were most commonly infested by larvae and nymphs of *Hylomma marginatum rufipes,* less frequently by several other tick species. During the spring of 1962, 1,774 birds (representing 44 species) were examined at Burg El Arab; of these 56 specimens (13 species with a total of 867 individuals) yielded ticks - the prevalence of infestation of tickhost species was 6.46%. During the spring of 1960, 959 birds (representing 29 species) were examined near Cairo; of these 128 specimens (13 species with a total of 786 individuals) yielded ticks — the prevalence of infestation of tickhost species was 16.3%.

Thus, we see the tremendous opportunity for intracontinental transfer of organisms pathogenic to man and lower animals. Bird-borne ticks actually do molt to adults and parasitize local animals thousands of miles away from where they originated, as has been demonstrated on numerous occasions in Africa, Europe, USSR, and the Near East. These ticks may cause infection in animals that eat them after they detach from birds, may interbreed with related forms, and may occasionally establish temporary populations far outside the normal range of the species. While critical extremes in envirnomental conditions usually prevent permanent establishment of tick species in foreign zoogeographical climes, these factors may not necessarily limit the pathogens infecting migrant-carried ticks.

Both *Hyalomma marginatum marginatum* in Eurasia and *H. marginatum rufipes* in Africa are notorious vectors of numerous agents causing human and animal diseases. Among the most interesting of these tick-disease relationships is Crimean-type hemorrhagic fever, a serious, often fatal infection of humans in southwestern USSR and Bulgaria, carried and transmitted by *H. marginatum marginatum*. This summer, through collaborative work by American (Yale Arbovirus Unit) and Soviet (Institute of Poliomyetitis and Viral Encephalitis, Moscow) scientists, the virus causing Crimean-type hemorrhagic fever has been characterized and identified for the first time. Comparative study of this virus and others isolated from ticks, cattle, humans, and hedgehogs in West Pakistan, Kenya, Congo, and Nigeria reveals the common identity of each strain.

The so-called Central Asian hemorrhagic fever of Uzbekistan may also be caused by the same virus. The Central Asian disease, with an even higher mortality rate than that in southwestern USSR and Bulgaria, is transmitted by *Hyalomma anatolicum anatolicum,* a mammal-infesting tick distributed from Central Asia north of the Himalayas and East Pakistan and northern India south of the Himalayas through the Middle East Arabia, and northern Africa to the Atlantic Ocean.

The immense geographic range of the virus causing this disease leads us to wonder about the role of migrating birds and infected ticks in its spread, to intensify our bird-tick-virus investigations, and to attempt to obtain more information and blood samples for virological study from African and Asiatic cases of human illness in which hemorrhagic symptoms are prominent and may otherwise be misdiagnosed. NAMRU-3's geographical vantage point in Egypt, and its unique facilities for integrated biological, virological, and medical investigation provide an exceptional opportunity for assisting in further elucidation and prevention of this disease. Human infections in various parts of Eurasia and Africa may have long been classified as other diseases. A sequence of biological events in these areas may result in epidemics similar to those in southwestern USSR. Clinicians and public health authorities should be alert to this potential hazard to human health and life.

For similar reasons, we have made a special study of the *Hyalomma* ticks of India and those in the inter-related fauna of Pakistan, Afghanistan, and Nepal. You will recall that epidemics of Kyasanur Forest disease occur only in Mysore forest areas. However, there is serological evidence of infection in other ecological zones, especially from those in drier regions of northwestern India where several *Hyalomma* species commonly infest wild and domestic animals. To appreciate the potential for further spread of clinical Kyasanur Forest disease, one has only to look at the contemporary pattern of Rocky Mountain spotted fever distribution in USA. Rocky Mountain spotted fever incidence has faded from one continental area — the Far West — and increased in a new area — the Eastern Seaboard — with a different combination of tick vectors, vertebrate reservoirs, and environmental factors that influence human exposure to the infection.

Another genus of ticks — *Argas* — began to concern us epidemiologically when Quaranfil fever virus, which had originally been isolated from the blood of a febrile child near Cairo, was frequently isolated from *Argas* ticks living in trees bearing egret nests near Cairo. We identified these ticks as *Argas persicus*. At that time, *A. persicus* was considered to be a widely distributed parasite of domestic chickens and of wild birds in Asia, Africa, Europe, the Americas, Australia, and Madagascar. But we were never successful in isolating Quaranfil virus from *A. persicus* samples from chicken houses near Cairo. It was also observed that while hatching larvae from chicken house samples crawled downward those from egret samples crawled upward. More intense study of the larvae, nymphs, and adults from these samples revealed definite, constant morphological criteria showing that we were dealing with 2 separate species, which we had previously confused as one species. Later, we learned that the chicken parasite, the true *A. persicus,* is an inefficient reservoir and vector of Quaranfil virus but that the second species, from egret rookeries, is an efficient vector.

Applying the morphological criteria to *Argas* samples from other parts of the world, we quickly learned that the true *A. persicus* is much more limited geographically and in adaptation to wild hosts than was previously thought. *A. persicus* infests domestic chickens in some parts of some continents, where it has been brought by humans on imported fowl, but rarely parasitizes wild birds outside of its original range of distribution in Central Asia. Most or all populations previously attributed to this species in Asia, Africa, North and South America, and Australia are in fact distinctive different species, each with a characteristic distribution pattern and favorite host. The Australian «*A. persicus*» proved to be 2 different species, and the true *persicus* is not known to occur there. The American «*A. persicus*» consists of 4 other species plus a few scattered populations of imported *A. persicus*. We now recognize about a dozen different species in the subgenus *Persicargas,* which we erected for this group. Other presently unrecognized *Argas* species will undoubtedly be discovered as the search widens. Some Asian *Argas* species related to those we found in Egypt are also infected with Quaranfil virus, others harbor different viruses which are being studied more intensively.

During collaborative studies on American *Persicargas* species, with colleagues at the Rocky Mountain Laboratory, we became worried by lack of biological information on species whose larvae were recorded to infest a wide variety of birds during each season of the year in several western states and Mexico. These data did not conform to the usual subgeneric pattern of nest-infesting species feeding only during the host's nesting period and only on a single host species or on a small variety of host species. Thus, after attending an Entomological Society of America seminar on tickborne diseases at Dallas, we called on old friends at the University of Arizona Entomology Department and at the Arizona — Sonora Desert Museum to tell us where flocks of birds slept the year around in trees in the desert near Tucson. They advised us to visit certain groves of mesquite and other trees beside streams and cotton-field irrigation ditches. In these groves we searched for bird droppings and scattered feathers on tree branches and on the ground below. Within a few hours we had collected dozens of *Argas* adults, nymphs, and larvae hidden under the bark of these trees. Thus, from clues first found in Egypt and used to expand knowledge of these ticks (and viruses) in Africa, Asia, and Australia, we were able to pinpoint a previously unrecognized tick habitat in the United States. From the interest aroused by this finding, we feel certain that American entomologists and virologists will soon add much to the information we gained on this 2-day trip to Arizona.

Necessary and important as these taxonomic-biological investigations may be, we also need to know more about the tick's internal mileau and the physiological and biochemical factors that influence the ability of each species to support and transmit viruses, rickettsias, and other pathogens. Tissue culture of tick cells, which could be a valuabletool for research on tick-pathogen interrelationships, has not been successfully advanced beyond the primary culture stage, to the continuous cell development stage, because guidelines from physiological and biochemical studies of tick fluids are lacking. With support of the U.S. Public Health Service's National Institute of Allergy and Infectious Diseases, the NAMRU-3 Medical Zoology Department is analyzing the bloodmeal digestion and utilization by different tick species, how ticks are able to survive starvation for so many months or years, the nature of metabolism during egg development, the role of factors influencing development of pathogenic organisms in different species, and how extreme changes in environmental temperature and humidity are met metabolically. Even in the earliest stages of this investigation, fascinating results are being obtained. For instance, in the chicken and egret-infecting *Argas* species that we originally could not tell apart, we now know that hemolymph (blood) concentration is lower in one species than in the other and that during the first day of blood digestion the total protein in the gut contents of one species is about one-tenth that of the other. By disc electrophoresis, the gut content shows 3 bands of ironbound proteins in the first species but only 1 band in the second. Total lipids in eggs of one species are twice those in the second species and nearly all of the lipids in the 10-day old egg are carried over to the hatching larva. Lipid levels in the gut of engorged female *Argas* are 10-fold those in engorged males, but levels in female hemolymph and coxal fluid are only 5-fold those of males. Thus, we trust we are well on the way to identifying tick groups and species physiologically and biochemically, as well as externally, and to providing answers for improvement of tick tissue culture research.

3. KALA AZAR IN THE SUDAN

In 1959, the Minister of Health of the Sudan Republic, seriously concerned over recurring, often fatal epidemics of kala azar that had been sweeping the eastcentral grasslands, called on NAMRU-3 to investigate the cause of the disease and to recommend measures for its prevention and control. Agricultural development schemes were held in abeyance, plans to resettle the population of Wadi Halfa (soon to be flooded by waters held behind the new Aswan High Dam) were endangered, and entire tribes were being wiped out by explosive outbreaks of kala azar. The infection was undoubtedly transmitted by one or more of the many sandfly (*Phlebotomus*) species in the area, but the vector species and its distribution, ecology, seasonal dynamics, flight pattern, and breeding and resting places were not known. Attempts to control the disease by insecticide spraying of village huts were fruitless and no one could tell public health officials where, when, or how people became infected.

By early 1960, NAMRU-3 medical zoologists, parasitologists, and clinicians were sent into the field to begin a four-year study of kala azar epidemiology. The headquarters laboratory was established in Upper Nile Province at Malakal; a «Forward Laboratory» was improvised in a kala azar focus at Paloich, about a hundred miles noth of Malakal; and round huts with mud walls and peaked grass roofs were constructed in a nearby Dınka village to house personnel for year round disease surveillance. Exceptionally harsh environmental and climatic conditions characterize this area. After a hot 6-month dry-season, the parched, burned-over grasslands are turned into endless swamps by the annual rains, forcing closeure of all roads for the rest of the year. Great distance from supply bases and communication centers, and the need to process delicate laboratory cultures, living insects, and experimental hamsters under ideal, carefully controlled conditions presented many operational, logistic, and scientific problems.

Initially we spent weeks in search of man-biting sandflies in riverside and savannah forests, grasslands, swamps, villages, and towns. Then one night in late March in an *Acacia-Balanites* forests typical of the area, we were bitten by sandflies. From subsequent research we learned that we had found the correct ecological situation early in the season of activity of the vector species, *Phlebotomus orientalis*. Adults of this insect become extremely common in *Acacia-Balanites* forests in the late dry season (late March to late May) and bite humans from dusk to an hour after dawn. They occur only in these forests and in nearby villages, almost nowhere else, and only at this season. Over a three-year period, we found from 1.9 to 5.0% of the female *P. orientalis* collected while biting humans to be infected by *Leishmania donovani* the causative agent of human kala azar. Results of experimental studies showed that this organism developed in the anterior gut of *P. orientalis,* migrated to the head and mouthparts, and was transmitted to a new vertebrate host when this sandfly species fed a few days later. When *P. orientalis* were fed on a human kala azar patient and later inoculated into hamsters, these animals developed fatal *L. donovani* infections. Only 2 other sandfly species frequently bit humans in this area. One, *P. heischi,* localized in distribution and erratic in annual incidence, was not found to be infected by L. *donovani.* Another, *P. papatasi,* was a common, vicious pest of humans in many villages but was not infected in nature and experimental studies showed it to be an inefficient vector of the kala azar agent.

Leishmania donovani was also recovered from 2 rodent species and from 2 carnivore species in the same *Acacia-Balanites* forests, thus establishing these specific habitats as zoonotic disease foci with a sandfly species that was extremely common and readily bit humans and actively transmitted the infection from wild reservoir mammals to humans. Notably, the domestic dog, which is an important kala azar reservoir in certain other parts of the world, was shown to play no epidemiological role in the central Sudan. In Malakal city, 2 rodent species were found infected but in the absence of man-biting sandfly species the rodent-sandfly-rodent cycle does not present a hazard to human health in this urban center. *L. donovani* was also isolated from Sudanese patients with typical kala azar symptoms and from NAMRU-3 staff members who participated in sandfly collecting but had atypical symptoms. The *L. donovani* strain from each source, sandfly, rodents, carnivores, and Sudanese and Americans with typical and atypical disease symptoms, all proved to be identical to each other when tested comparatively by the World Health Organization Leishmaniasis Reference Center.

From this evidence, we were able to recommend that all persons passing through or living near *Acacia-Balanites* forests be advised to avoid sandfly bites from dusk to dawn during the second half of the dry season. Localized application of insecticides to control adult sandflies would be ineffective because large swarms of *P. orientalis* move extensively through these forests.

However control might be achieved through spreading insecticides from knapsack sprayers, vehicles, or airplanes. Luckily, the forests harboring sandflies are not extensive and during the few weeks of peak vector activity, trees are leafless and there is little if any ground vegetation. Technically, therefore, the problem of insecticide distribution to the lower levels of the forest, where adult sandflies rest between flights, is comparatively simple.

4. MISCELLANEOUS ACTIVITIES

One of the most pleasant and rewarding functions of the NAMRU-3 Medical Zoology department is provision of research facilities and scientific guidance to numerous Egyptian students working for advanced degrees. At the moment, 8 students, 4 for the Ph.D. degree and 4 for the Masters degree, are doing their research in this department. We have also served on graduate committees for students in India, provided facilities for American Ph.D. students in Egypt and abroad, and sent a flow of research materials from Egypt to Egyptian graduate students at universities in England and U.S.A. These students later teach in Egyptian universities or become investigators in various local ministries and research institutes. One recent graduate has helped make notable contributions to knowledge of tickborne viruses as a member of a Rockefeller Foundation research team at the University of Ibadan, Nigeria. We have also lectured in several faculties of Egyptian universities and participated in graduate examinations.

We serve on the Expert Panel on Tickborne Diseases of the Food and Agriculture Organization (F.A.O.) of the United Nations, on various special panels of the World Health Organization, and on editorial committees of 6 scientific journals. We have frequently organized and chaired symposia or special sessions for international or American congresses on tropical disease, entomology, and parasitology. Numerous manuscripts and books are sent to this department for prepublication critique or for review and each year we write one or more invited review papers for special scientific volumes. In 1965, we participated in a

United States Public Health Service Delegation on Hemorrhagic Fevers to the USSR. In other years we were invited to join a travelling symposium on scrub typhus sponsored by the University of Niigata in Japan, and headed an East African expedition of international specialists in ticks and tickborne diseases. We have been called on to lecture or present seminars at over 50 universities and scientific societies around the world and in 1967 delivered the 29th annual Theobald Smith Memorial Lecture to the New York Society of Tropical Medicine.

A departmental activity of major proportions is identification of ticks for numerous institutions and research projects. For instance, during one 10-month period we identified 2,949 collections containing over 100,000 ticks from 54 nations. During the same period, we presented about 20,000 identified ticks, including many exceptionally valuable specimens, to a dozen scientific institutions. Numerous living or preserved specimens representing a variety of tick species have been sent to research institutions for electron microscope studies and for studies in embryology, chromosomes, physiology, tick tissue culture, viruses, rickettsia, etc...

For teaching purposes, each year we send hundreds or thousands of specimens of ticks, fleas, lice, mites, bedbugs, sandflies, scorpions, and other medically or biologically interesting arthropods to universities around the world. Special vertebrate animals, mammals, birds, reptiles, and amphibia, are also donated for this purpose.

Hundreds of vertebrate animals are also sent abroad each year for medical research. Practically all of the fat sandrats (*Psammomys*) used for diabetes investigations in the United States, Canada, and Europe have been collected and shipped by this department. Thousands of rodents have been sent to various institutions for physiological research, especially on physiological adaptations of desert-inhabiting animals. Others have been provided for attempts to develop new kinds of laboratory animals. Snakes have been shipped for venom studies. Collections of mammals, birds, and reptiles have sometimes been sent to zoological gardens for exhibit or breeding, or to museums for preparation of exhibits. Tens of thousands of study skins of mammals and birds, and thousands of preserved reptiles and amphibians have been deposited in museum research collections; these have been collected in many parts of the world in connection with host-parasite-pathogen research, chiefly in Egypt, Sudan, Turkey, Madagascar, Ethiopia, Kenya, and Tanzania.

About 250 papers, including some of book length, published or now in press in scientific literature, have been written by NAMRU-3 Medical Zoology staff members and more than 100 others by collaborating scientists. Numerous others are in an advanced stage of preparation. Over 300 foreign books and papers on ticks and tickborne diseases and on leishmaniasis have been translated into English and 400 to 1000 copies of each have been distributed to interested scientists around the world.

The departmental library on ticks and tickborne diseases, housed in a new fireproof building, is by far the most complete and extensive of its kind in the world. This library forms the basis for a Bibliography on Ticks and Tickborne Diseases, on which we have been working, with the aid of several dedicated assistants for several years. The first bibliographic volume, consisting of titles arranged by author and year of publication, will soon be published. This will be followed by several cross-indexed subject volumes being prepared in Cairo under the direction of Miss Mildred Doss, who for many years was the chief editor of the U.S. Department of Agriculture Index-Catalogue of Medical and Veterinary Zoology.

The tick collections housed here are the largest in the world. Tick collection functions are closely integrated with those of the Rocky Mountain Laboratory in Hamilton, Montana, which now contains representatives of close to 90% of the world's known tick fauna. Both collections are meticulously curated and preserved as a scientific heritage for future generations of investigators and as a readily available source of precise information for all contemporary students of ticks and tickborne diseases around the world. The Cairo collection is ably curated by Mrs. Kawther Mohammed el Kammah, MS.

We are proud of the hundreds of handsome, scientifically accurate illustrations of ticks that enhance NAMRU-3 Medical Zoology publications. Indeed, we are sometimes chagrined when a colleague says: «I saw your last paper in the Journal of Parasitology; what wonderful illustrations.» Most of these drawings were made by Mr. Roman Strekalovsky, who has had long experience as an artist for the Entomology Department of the Cairo University Faculty of Science and is unsurpassed as a scientific illustrator. Recently, another outstanding artist, Mrs. Rifkea Abu Bakr, joined the departmental staff. Mrs. Abu Bakr's initial experience was gained in illustrating antiquities for her professor husband, an accomplished student of Egyptian dynastic history.

Much credit for the success of this department's effort is due to Markram N. Kaiser, a graduate of Cairo University Faculty of Agriculture, who came to NAMRU-3 some 17 years ago, later obtained a Ph.D. degree with distinction at Emory University, and now heads the tick biochemistry and physiology project and functions as the Deputy Head of the Department. Dr. Kaiser has been especially concerned with the *Hyalomma* studies, has won acclaim for his biological research on tick viruses, and directs some of the most dynamic and rewarding investigations underway at NAMRU-3.

Mr. Sobhy Gaber Abdel Malek, the departmental research assistant, is living proof of the inaccuracy of the quip that no one person is indispensable. Already a highly experienced entomological technician when I came to NAMRU-3 from the Sudan in 1950, Mr. Sobhy ably and efficiently supervises a myriad of medical zoology research activities and is the first person on whom we call when a new problem arises.

Several other technicians have played an important role in departmental activities for many years. Mr. Sayed Ahmed Metwally, son of a NAMRU-3 night guard, joined the staff as a youngster. He has now earned a B.A. degree at Cairo University and is a highly qualified virology technician. Mr. Ezzat Guindy Ayoub, with a background in veterinary medicine, specializes in bird identification and blood protozoology. Mr. Ibrahim Helmy

Mohammed is a highly talented and successful leader of field expeditions. Mr. Ibrahim Soliman Khedr is a jovial, hardworking, efficient jack-of-many-trades in the field as well as in laboratory. Laboratory Aides Mr. Gad el Sied Abdel Baien and Mr. Hassan Touhamy Hassan have long and ably supported numerous research activities. This experienced, smoothly running team is the backbone of the NAMRU-3 Medical Zoology department.

Other departmental mainstays are Mrs. Lola Strekalovsky, head of the translation unit, Mrs. Anna L. Gahin, head of the library and bibliographic unit, and Mrs. Annie Massabki and Miss Barbara Z. Kymbriti, secretaries. Dr. Robert E. Williams heads the newly organized virus-vector division and Professor Wadie Tadros is the Supervisor of the fine team of able young people who are doing graduate research under the auspices of the NIH supported tick biochemistry project.

The high morale of departmental members is a reflection of the warm co-operation of NAMRU-3 administrative divisions and directors, the naval Bureau of Medicine and Surgery, Office of Naval Research, and Public Health Service. Equally important to the environment necessary to achieve a smooth flow of scientific results has been the splendid and friendly co-operation of Egyptian government officials and scientists at all levels in numerous ministries, universities, and scientific institutions. Only through this rare international combination of administrators, scientists, and helpers, whose great interest is the increase of biomedical knowledge for the general welfare of mankind, could the NAMRU-3 Medical Zoology program develop as it has.