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ECOLOGY AND EPIDEMIOLOGY OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS TRANSMISSION IN THE REPUBLIC OF SENEGAL

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Progress continued during the fourth year of studies on the ecology of tick-borne Crimean-Congo hemorrhagic fever (CCHF) virus in the West African savannah. Prospective observations of tick and virus activity were maintained in northern Senegal to describe the seasonal activity of vector ticks. Adult tick abundance was low relative to previous years; seroconversion of sheep was correspondingly diminished. Immature ticks on small mammals and birds were most abundant during or after the rainy season. Laboratory studies of immature Hyalomma truncatum and H. marginatum rufipes feeding on various native vertebrate hosts demonstrated that the dropoff pattern ("2-host" or "3-host") depended on the host. Transmission studies have shown that adult male H. truncatum inoculated with CCHF virus transmitted virus to females during mating and cofeeding. Survival of uninfected, unfed adult ticks, monitored during more than one year, depended on temperature and humidity. In other studies, laboratory mice inoculated with CCHF virus differed in survival, viremia, and antibody depending on route, age titer of infection. CCHF virus was isolated from Boophilus decoloratus.

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SUMMARY

The ecology of transmission of Crimean-Congo hemorrhagic fever (CCHF), a life-threatening tick-borne viral zoonosis, remains poorly understood despite considerable recent research. Human disease or enzootic transmission occur in parts of southern U.S.S.R, central Asia, southern Europe, the Middle East, and throughout much of the African continent (Watts et al. 1988). At least 30 ixodid tick species (Camicas et al. 1990), most notably of the genus Hyalomma, have been found to be infected by CCHF virus, however, little is known of their importance in maintaining transmission in nature. Numerous wild mammal and bird species show evidence of CCHF virus infection, yet the role of these vertebrates in horizontal transmission or amplification of the virus remains undefined.

During the fourth year of our project to study the factors that contribute to transmission of CCHF virus in Senegal, observations and experiments were undertaken on the feeding behavior of vector ticks, their ability to become infected with and to transmit the virus, and vertebrate responses to infection. Study of the population dynamics of the important vertebrates and ticks indigenous to our study sites in northern Senegal were continued at Dahra, Yonofere, and Bandia. Ticks under study included species of Hyalomma, as well as Rhipicephalus, Amblyomma and Boophilus species, all potential vectors of CCHF virus. Immature ticks and serum samples were again sampled from candidate reservoirs including various birds, rodents hedgehogs and hares. Domestic ungulates were studied regularly in order to define adult tick seasonal activity, density and host associations.

New laboratory observations on CCHF virus infection of vertebrates included studies of viremia, disease and antibody responses of laboratory mice inoculated by different routes and virus titers. To complement this, studies of adult H. truncatum infected with CCHF virus, and of their ability to transmit by cofeeding and during mating, also were undertaken. The survival of other, uninfected adult ticks was monitored during more than one year and found to depend on temperature and humidity. Finally, laboratory studies of the response of immature H. truncatum and H. m. rufipes to feeding on different bird and mammal species demonstrated that drop-off depended upon the host.

FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 86-23, Revised 1985).

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I. INTRODUCTION

Crimean-Congo Hemorrhagic Fever (CCHF), is a member of a group of arthropod-borne viral zoonoses producing acute, sometimes fatal febrile and hemorrhagic symptoms. Human disease often involves initially the nervous system and in severe cases may progress to vascular disorders such as profuse diapedetic hemorrhages, brain edema, general malaise, and ultimately cardiac arrest. The first recognized epidemic occurred in the Crimea, U.S.S.R. in 1945 (Chumakov 1945, 1947) after which the viral agent was isolated from ixodid ticks (reviewed by Chumakov 1974). CCHF virus, family Bunyaviridae, genus Nairovirus, was later found to be identical to that of "Congo virus" from Africa (Casals 1969).

Transmission of CCHF virus occurs over an extremely large area of the world, including the southern U.S.S.R, central Asia, southern Europe, the Middle East, and the entire African continent (Hoogstraal 1979, Watts et al. 1988). In West Africa, Senegal and southern Mauritania have been studied for CCHF virus infections in certain vertebrate and tick species, an more recently human disease (Saluzzo et al. 1985a, Gonzalez et al. 1990). Early work by Chunikhin et al. (1969) first demonstrated evidence of infection in domestic animal of Senegal. Later, ticks from Senegal abattoirs were studied and numerous strains of CCHF virus were isolated (Robin & LeGonidec 1972, Robin 1972, 1973, 1974, 1975). More recent observations of human and domestic animal sera identified other foci of transmission in various regions of Senegal (Saluzzo et al. 1984, 1985a, 1985b, 1986, Camicas et al. 1986). Thus, CCHF virus transmission is well-known in this region, making it ideal for field study of this threat to human health.

More than 30 species of ixodid ticks have been shown to be capable of supporting infection by CCHF virus (Camicas et al. 1990); however, it seems that only a few species are important in maintaining the transmission cycle in nature. Epidemiological reports have implicated certain Hyalomma species that vary according to geographic region, particularly H. m. rufipes which has been associated with intense transmission in western Africa (Hoogstraal 1979). Whether this association is justified requires extensive studies of host associations and vectorial capacity such as those that we have undertaken. CCHF virus has also been isolated from H. truncatum and H. impeltatum, species that are abundant in our region. Host associations and population dynamics of the adult stage of these ticks have been studied in Senegal (e.g. Camicas et al. 1986, Gueye et al. 1986, 1987, 1989); much less is known about the ecology, population dynamics and host-associations of the immature stages of any of the Hyalomma species. Similarly, the vertebrates that might serve as maintenance reservoirs of the virus need to be defined.

Furthermore, the importance of transovarial transmission of the virus between tick generations in nature, relative to that of horizontal transmission, deserves to be analyzed. Finally, we need to understand the manner in which the numerous individual components of the CCHF virus cycle interact in nature.

The objectives of our project have been to address many of the abovementioned needs by investigating certain poorly understood variables that are probably important to the CCHF virus transmission cycle. Ultimately, we hope to develop a more complete understanding of the complex interactions of the dynamics of the enzootic cycle and the epidemiology of human disease. This report summarizes accomplishments during the fourth year of study.

Objectives

The principal objectives for 1990 included:

1. Continuation of longitudinal field observations of tick-host associations and seasonal patterns of tick abundance.
2. Systematic serosurveillance of animal CCHF virus infection prevalence and incidence to elucidate risk factors, and environmental or vectorial correlates.
3. Further development of laboratory models of CCHF virus transmission by observations of infection using laboratory and natural hosts.
4. Analysis of feeding patterns of probable vector ticks on natural and laboratory hosts.
5. Studies of tick infection and the possibility of direct horizontal transmission between adult ticks.

The research described herein is the effort of a team of investigators comprised of scientists and technicians from numerous institutions. This collaboration involves ecologists, entomologists, immunologists, epidemiologists, and virologists from a variety of different organizations (Table 1). In addition, various presentations at scientific congresses, reports, and publications have resulted from research under the grant; the most significant of these, for the period 1988-1990, are noted in Table 2.

Study Sites

Field sites for prospective studies were chosen from information on previous research undertaken at the Institut Pasteur (Camicas et al. 1986, Saluzzo et al. 1985a) and unpublished data). The sites have not changed since the project began in 1987. Details of the 3 sites chosen for prospective observations were previously reported (Wilson and

Digoutte 1988, 1989). Here, we briefly describe the villages of Yonofere, Dahra and Bandia which we continued to study throughout this grant-year.

Dahra is located about 100 km. west of Yonofere and 200 km. east-northeast of Dakar (Fig. 1). The region is classified as Sahelo-sudanian savannah (Bille 1971, Bille and Poupon 1972), a dry "thorn-brush" habitat dominated by grasses and widely dispersed trees, particularly Acacia species (Barral 1982). Rainfall occurs principally during July through September and may vary considerably from year to year (Fig. 2), averaging about 400 mm. annually (Leroux 1983). Two different but comparable sites are under study. We are working inside the Dahra "Centre de Recherche Zootechnique" (CRZ), a national research station for domestic animal husbandry that is part of Senegal's "Institut Scientifique de Recherche Agricole. (This site is designated "Dahra-CRZ"). Outside that station we are working with cooperating resident herdspeople who provide us access to their animals and land.

Yonofere is a small village of roughly 700 inhabitants occupying a few hundred widely dispersed huts about 300 km. to the east-northeast of Dakar (Fig. 1). The habitat, rainfall and geoclimatic characteristics are similar to those in Dahra. Residents grow millet during the rainy season and herd sheep, goats and cattle year-round.

The site in Bandia is located about 20 km. from the Atlantic coast, some 60 km. southeast of Dakar (Fig. 1). On the edge of the Bandia forest, this more heavily vegetated region receives, on average, more rainfall (ca. 700 mm.); fluctuations in both daily and seasonal temperatures are somewhat modulated by the proximity to the ocean. This station has been the site of numerous previous studies of mammals (e.g. Hubert 1977), arthropod vectors (Camicas et al. 1986) and virus isolation (e.g. Digoutte 1985); this site offers an extensive history of observations for comparison.

The majority of results reported herein cover the period from 1 January, 1990 through 31 December, 1990. Certain data are still being analyzed; therefore, results have been summarized in a form that may be further modified. Studies are organized by topical questions divided into 3 sections: Tick Ecology and Behavior, Vertebrate-Virus Interactions, and Virus Transmission.

I. TICK ECOLOGY and BEHAVIOR

Various systematic observations designed to understand the ecology of the ticks and vertebrates that are important in CCHF virus transmission were continued. Among the arboviruses that cause zoonotic disease in humans, CCHF virus is noteworthy for the ecological diversity of potential vectors and vertebrate hosts with which it is associated. Either by inference or direct evidence of infection, numerous studies have implicated various species of African ticks as CCHF virus vectors (reviewed by Hoogstraal 1979). In Senegal, at least 8 such ticks are found (Camicas et al. 1990), including 5 species of the genus Hyalomma, 2 species of Rhipicephalus and Amblyomma variegatum. These ticks are being studied at our 3 permanent sites. In addition, new laboratory studies of the feeding behavior of immature ticks have been undertaken.

Adult Tick Seasonal Activity

The seasonal pattern of activity and population density of the principal tick species again were characterized at the 3 major study sites. This work is being undertaken in collaboration with Drs. Jean-Paul Cornet and Jean-Louis Camicas.

Samples from sheep. A herd of sheep is chosen by chance encounter, in Yonofere and Dahra, and 10 randomly selected individuals are carefully examined for the presence of ticks. Particular attention is focused on the tail, perianal and abdominal regions, feet and the head (ears and eyes). All ticks are removed with forceps and stored for later identification and virus isolation. Five herds are examined at each site about every month. Samples were first taken beginning in May 1987 and have continued through December 1990. In this manner, more than 4,400 sheep have been examined. From these animals more than 17,000 adult H. truncatum, H. impeltatum, H. m. rufipes, H. dromedarii, Rhipicephalus evertsi evertsi, and R. guilhoni have been sampled. Three species were most abundant: H. truncatum, H. impeltatum, and R. guilhoni.

As in the past year, adult ticks on sheep during 1990 were relatively sparce as compared to 1987 and 1988. H. truncatum and R. guilhoni were most abundant at Yonofere (Fig. 3). In addition to these 2 species, H. impeltatum and R. e. evertsi were found in moderate numbers at Dahra (Fig. 4). Seasonal patterns of activity were difficult to discern, however H. truncatum appeared to be most abundant during the dry season. H. impeltatum, found only at Dahra, also exhibited most activity during the dry season. The pattern for R. guilhoni was less clear: little variation was obvious at Yonofere, while this tick was somewhat more active during the dry season at Dahra. Similarly, R. e. evertsi was most abundant during the dry season.

Sentinel Animals. A second type of sample is made of adult ticks on privately-owned sheep and cattle in Yonofere and Bandia. These animals have been tagged but are maintained as part of their original herds. About 40 sheep, 12 goats and 2 cattle in Bandia and more than 200 sheep in Yonofere are being sampled at regular intervals. These observations permit comparisons with a somewhat different ecological regions (Fig. 1). Furthermore, repeated samples from the same individual animals allow us to consider differences in infestation rates that may be correlated with immune status or CCHF virus infection risk. As with the samples above, the abundance of adult ticks during 1990 was low, making analyses of seasonal patterns of activity difficult. Results appeared to be similar, however. Observations such as these are being compared over the course of 4 years to determine patterns that should contribute to our understanding of the long-term temporal dynamics of CCHF virus transmission (see Section III) and to the focal nature of epizootics.

Immature Tick Population Ecology

Our studies of the ecology and population dynamics of immature stages of the Hyalomma and Rhipicephalus ticks of interest continued with samples taken from wild-caught vertebrates. In general, larvae and nymphs of these ticks feed on birds and small mammals and are rarely found on the ungulates that serve as hosts to adults. Thus, our investigations of the host associations and seasonal activity of these potential vectors have continued during 1990 at the long-term field sites.

Immature ticks on birds. In an effort to better define the role of birds as hosts to larvae and nymphs, as well as to characterize seasonal activity and densities of these ticks, monthly samples of birds at Yonofere again were undertaken. Birds were trapped using Japanese mist-nets or a locally constructed ground net. Each bird was carefully examined for ticks by blowing air through a tube to separate the feathers and view the skin. Attached immature ticks were placed into live vials until molting and were then identified.

A total of 260 birds were examined at Yonofere during 1990, of which 6 (2.3%) harbored 14 ticks (Table 3). All ticks except for 1 were immature H. m. rufipes (4 larvae, 9 nymphs); 1 Argus sp. was also removed from a golden sparrow (Passer luteus) in November 1990. H. m. rufipes was the principal tick feeding on birds as in previous years, however few specimens were recovered making comparisons difficult. Four of the 6 infested birds were either grey-headed sparrows (P. griseus) (44 sampled) or their close relative, the golden sparrow (56 sampled). In addition, 1 of 15 laughing doves (Streptopelia senegalensis) and 1 of 13 chestnut-bellied starling (Spreo pulcher) harbored immature

H. m. rufipes. It would appear that the paucity of ticks on birds reflects the fact that relatively fewer adult ticks were found feeding in 1990 and 1989 than in previous years. In addition, the uneven sampling among months combined with the apparent temporal pattern of tick activity make it difficult to implicate any particular bird species as important hosts. As observed previously, most immature H. m. rufipes appeared at the end of the rainy season early into the dry season.

Immature ticks on small mammals. From our studies, the role of small mammals as hosts to immature Hyalomma ticks is becoming more apparent. Certain mammal hosts generally are more often and more heavily infested, although this varies among sites. At Yonofere, modified Manufrance live-capture traps, baited with peanut butter, are placed 10m apart in lines located near suitable habitat. More than 100 trapnights per month produce rodents at these sites. In addition, hares (Lepus whytei) are shot or netted from a vehicle, and hedgehogs (Erinaceus albiventris) are trapped or captured by hand. These small mammals are carefully inspected for ectoparasites by blowing against the fur to view the skin surface. As with birds, attached ticks are removed with fine forceps; in addition, these small mammals are held for 7 days in cages over water where engorged ticks that detach may be recovered.

Small mammal abundance at Yonofere during 1990 was very low (Table 4), making any estimates of tick attachment rates difficult. Of the few hares and hedgehogs that were examined there was evidence of greater infestation by immature ticks, especially H. truncatum. In addition, H. m. rufipes immatures and Rhipicephalus guilhoni adults were captured. No evidence of seasonal variation was discernible.

Host-Mediated Feeding by Immature Ticks

In addition to the diversity of vertebrate hosts fed upon by Hyalomma ticks, the number of times that these vectors feed strongly influences the probability of transmission of disease agents. Ixodid ticks normally take 3 bloodmeals, once as larva, nymph and adult. Thus, pathogens that are not transmitted transovarially, can infect vector ticks only during the larval or nymphal bloodmeal. And only then can the resulting infected nymph or adult transmit to other hosts. Such a constraint on transmission of tick-borne agents may be even further restricted by host choice. For example, infected nymphal ticks that feed on vertebrate species different from those used by larvae cannot participate in horizontal transmission of a disease agent.

Of about 650 recognized ixodid tick species, more than 90% are considered to exhibit a "3-host" feeding pattern in which each of the 3 active stages typically attaches, feeds and

detaches from a different individual animal. At the other extreme are perhaps 20 or more tick species that exhibit a "1-host" pattern in which individuals remain attached to and feed on the same individual host, as larvae, nymph, and adult. In these ticks, immature stages molt on the host, and only the engorged adult female drops to the ground. An intermediate pattern, termed "2-host," has been reported for a few species of Hyalomma and Rhipicephalus ticks in which larvae and nymphs take their blood-meals on the same individual host; only adults feed upon a different animal. Sometimes, however, certain of these species behave as "2-host" ticks, sometimes as more typical "3-host" ticks (Hoogstraal 1979). Because of the potential importance of these feeding patterns to CCHF virus transmission ecology, we undertook studies of the dropoff patterns of larval and nymphal Hyalomma ticks feeding on laboratory vertebrates and natural hosts.

Feeding of ticks. Larval ticks were reared from eggs laid by wild-caught females found engorging on cattle or sheep. Larvae for any single experiment were normally taken from the same egg batch to maximize uniformity. Ticks were infested by shaking them onto the head or back of each animal. The number of ticks introduced onto each host was estimated. Beginning day 2 post-infestation, water in the tray beneath each cage was checked, and all detached ticks were removed and counted. To approximate natural conditions, hosts were held under ambient temperature, humidity and daylight. They were free to move, eat and groom. In addition to laboratory vertebrates from Institut Pasteur stocks, we studied wild-caught animals including Mastomys and Arvicanthis rodents, hedgehogs, hares, guinea fowl, and various passerine birds.

Dropoff patterns of H. truncatum. Preliminary observations on the dropoff rhythm of larval H. truncatum from guinea pigs, undertaken with Dr. Thomas Logan of USAMRIID, demonstrated that unfed larvae engorged and detached within 3 to 7 days post-infestation, in a manner typical of a "3-host" tick. Hyalomma truncatum is considered usually to be "3-host" (Hoogstraal 1979) and more recent observations confirmed that when feeding on guinea pigs, all unfed larvae dropped-off as engorged larvae (Fig. 4).

This pattern changed when H. truncatum larvae were allowed to feed on other hosts. On laboratory rabbits, H. truncatum larvae took longer to engorge, and a proportion of ticks remained on the host and dropped-off as newly molted nymphs. Some nymphs remained attached and, later still, detached as engorged nymphs (Fig. 5).

In order to explore further this observation, and to consider the possible implications for transmission of CCHF virus, we allowed larval H. truncatum to feed on vertebrate hosts that are locally abundant and frequently parasitized in the local West African environment. The methods by which ticks were fed was as previously described.

When feeding on multimammate rats, slender gerbils and Nile rats, which are abundant and frequently infested small mammals of the region, larval H. truncatum behaved in a manner like that observed with guinea pigs (Fig. 7). These hosts produced the response of a typical "3-host" tick: all larvae dropped off following their first blood-meal. Thereby, these wild rodents produced tick responses like those observed seen on a laboratory rodent, the guinea pig.

This pattern, however, was not observed with other hosts that were studied. Hedgehogs, also an native vertebrate often found heavily infested by these ticks in nature, produced a different detachment pattern when fed upon by larval H. truncatum. The majority fed as "3-host" ticks, but, consistently a few ticks remained attached to feed as nymphs (Fig. 8). Thus, larval H. truncatum from the same egg batch behaved either as "2-host" or "3-host" ticks, even while feeding on the same individual host. This same phenomenon was observed even more dramatically in experiments on hares. Here, however, the majority of larvae followed the "2-host" pattern, feeding once as a larvae and again as a nymph on the same individual hare (Fig. 9). Thus, the general feeding pattern of larval H. truncatum appeared to depend on the host species to which it attached. Even on the same host, some of these ticks behaved differently than did others.

Dropoff patterns of H. m. rufipes. The other major Hyalomma species that is found at our study sites and throughout much of the African continent, and which has been implicated in enzootic and epidemic transmission of CCHF virus, is H. m. rufipes. Most authorities consider this species to be a "2-host" tick, but anecdotal claims of occasional "3-host" feeding have been made. Here again, the phenomenon has not been systematically investigated. The few published life-cycle studies have been undertaken using laboratory animals.

Because immature H. m. rufipes frequently are found parasitizing birds, we infested various species of passerines with larvae of this tick. The results from a few experiments in which a consistent "3-host" pattern was observed are shown (Figs. 10,11). Regardless of the bird species studied, all larval H. m. rufipes remained attached to feed as nymphs on the same individual. Thus, bird-feeding larvae of this potential CCHF virus vector would appear to infrequently feed as nymphs on mammals or even other birds.

When H. m. rufipes were allowed to feed on various mammals known to harbor naturally immature stages of this tick, yet another host-determined feeding pattern emerged. Larvae infesting guinea pigs detached entirely as engorged larvae, behaving as a "3-host" species (Fig. 12). However, when feeding on hares, a few engorged larvae dropped off, while the vast majority remained attached to refeed as nymphs. H. m. rufipes that fed on hedgehogs responded in a similar, principally "2-host" manner, although the time to repletion appeared delayed.

The explanation for these observations awaits further study. Perhaps anti-tick immunity of certain hosts that repeatedly are infested affects the rate and success of engorgement of various ticks. However, when we repeatedly infested the same individual hares or hedgehogs, the tick feeding patterns did not change. Perhaps intense infestations produced aberrant responses from the ticks. Yet, our field observations indicate similar levels of parasitism in nature. Unlike many other laboratory studies, our hosts were free to groom, and engorged larvae may have been physically removed by their hosts, thus producing the mixed "2-host" and "3-host" response. Yet this would also be the case in nature. Indeed, we attempted to simulate as much as possible the conditions and tick-host interactions that have been observed in nature.

These results may have implications for the maintenance of vector-borne disease agents. Because adult Hyalomma ticks typically feed on potential CCHF virus reservoir species that are different from those parasitized by larvae and nymphs, horizontal transmission of disease agents from adults to immatures is negated. Thus, except for transovarial infection, the "2-host" feeding pattern that we observed should serve to decrease the intensity of CCHF virus transmission. Transovarial transmission has been demonstrated for a few Hyalomma species under experimental conditions and may occur in nature. Unless it occurs with 100% efficiency, however, some other form of amplification or reintroduction is necessary. Ticks following the "3-host" pattern could maintain the virus cycle through horizontal infection of larvae by nymphs, via a common vertebrate host. Detaching, infected larvae would then molt to nymphs and could infect yet another host, thereby continuing the cycle. In West Africa, engorged larval Hyalomma rapidly molt to nymphs and do not undergo diapause, thereby permitting both immature stages to feed during the same period. Thus, even a short period of viremia would still provide a window of time during which nymphs could infect other cofeeding larvae. This process, however, could not occur among ticks following the "2-host" pattern.

Our findings suggest that laboratory studies of transmission by immature Hyalomma ticks should be carefully designed to avoid mistaken assumptions about the tick's behavior in nature. Fortunately, the host-dependent feeding pattern described here apparently is restricted to a handful of tick species. Transmission studies involving these ticks should consider using hosts other those laboratory vertebrates that are so often studied.

Temperature- and Humidity-Mediated Survival of H. truncatum

Ticks are notorious among arthropods for their longevity when unfed. The environmental conditions that influence survival rates in this state have not been investigated for the Hyalomma species that are likely vectors of CCHF virus in nature. Survival in hot and dry climates such as those experienced by these ticks is undoubtedly influenced by temperature and humidity. We undertook a laboratory study of male and female adult H. truncatum to determine the influence of temperature and humidity on their survival in the absence of hosts.

Tick rearing and treatment. H. truncatum adults were reared from the eggs of a single engorged female removed from a sheep in Yonofere. This tick was held at 28°C and 75%RH until eggs were laid and larvae eclosed. Larvae then were allowed to feed on a captive Whyte's hare (Lepus whytei) producing a cohort of sibling ticks of uniform age and condition. The majority of feeding larvae remained attached to feed again as nymphs; engorged nymphs that detached were recovered from a water pan beneath the hare. These fed nymphs were weighed and confined individually to plastic mesh-covered tubes maintained as above until adults emerged. Following eclosion, the sex and weight (Sartorius electronic balance, type H51-F1) of each tick was determined.

Twelve groups of 20 ticks each (10 males; 10 females) were selected at random from among the lighter half (5m;5f) and the heavier half (5m;5f) of the lot. Each group of ticks was assigned randomly to a regime of temperature (5, 17, 24, or 30°C) and humidity (10%, 50%, or 80% RH) that was held constant throughout the experiment. Humidity was maintained by placing the ticks in sealed glass jars containing different concentrations of potassium hydroxide (Winston and Bates 1960); jars were kept in refrigerators or incubators. The ticks were examined individually each 4 to 7 weeks for signs of life. Those not responding to breath or light pressure with movement were considered dead and were discarded. Observations were continued for 64 weeks.

Survival of ticks. Overall, the number of male H. truncatum that died [103/118 (87.7%)] was similar to that of females [107/122 (87.3%)]. The initial weights of males (110.4 mg) averaged less than that of females (136.5 mg). Nevertheless, survival did not differ between the sexes: males lived for an average of 37.4 weeks and females for 38.2 weeks.

Different temperatures and humidities, however, did affect the survival of these ticks. At all temperatures, survival was greatest at the highest humidity and least at the lowest humidity (Fig. 13). When humidity regimes were combined, the death rate appeared elevated at the highest temperature and least at the lowest temperature (Fig. 14). Optimum survival occurred at moderate temperatures and high humidity. The weight of ticks was unrelated to their survival. Similarly, males and females did not differ in their response to different conditions.

The lenghty survival of unfed adult H. truncatum suggests that this tick may survive long periods in the absence of a host. Despite the hot, dry conditions that exist in many regions where CCHF virus is transmitted, adult ticks probably remain sequestered in subterranean or arborial microclimates that are much cooler and more humid. This tick thereby may survive long periods and continue to be capable of reproducing and perhaps transmitting CCHF virus. The effect of virus infection on tick survival under various conditions deserves study.

III. VERTEBRATE-VIRUS INTERACTIONS

The variables influencing vertebrate responses to CCHF virus infection contribute to transmission dynamics in a complex manner. Although numerous factors such as host population density or vector-host contact influence the significance of a given level of tick infestation, hosts must be capable of maintaining infection for a sufficient period of time to transmit to the vector. Inhibition of vector feeding or viral pathogenic effects on the host or vector also may influence these interactions. Similarly, ticks infesting vertebrates that express only a low-titer viremia might be of little consequence to CCHF virus transmission. These interactions are difficult to study in natural vertebrate populations. As a result, we have increasingly turned to laboratory studies that simulate certain natural conditions. However, observations on the natural prevalence of infection continue.

Prevalence of Infection in Domestic Ungulates

Prospective serological studies of individually-identified sheep from herds in Yonofere, and of sheep, goats and cattle at the Dahra-CRZ provide us with measures of incidence of new transmission of CCHF virus in these areas. Numbered eartags on more than 200 sheep in 4 herds from Yonofere permit individual identification of sheep that were sampled every 2 months. The antibody status of more than 300 sheep and goats is being studied every 3 months at Dahra. During 1990, samples from 156 cattle and 982 sheep or goat samples from Dahra were tested. Among 20 to 30 cattle sampled monthly, IgG antibody prevalence averaged 14.7% and varied by the age of the group sampled; no IgM was apparent. These results are similar to those obtained previously from other sites in this bioclimatic region (Wilson et al. 1990b). Prevalence among sheep and goats there was less variable, as the entire flock of more than 300 tagged animals was periodically tested. IgG seropositivity remained elevated, though less so than immediately following the 1988 epizootic (Wilson et al. 1990a); differences were observed among the sample periods, with prevalence varying between 35.0% and 24.2% as the sample population varied with births and deaths. Two (0.2%) new infections were suggested by seroconversion and the appearance of IgM.

Antibody prevalence among sheep at Yonofere also remained low during 1990. From the 4 herds of sheep examined every 2 months, a total of 287 individuals were sampled and 1232 blood samples were tested. Prevalence of anti-CCHF virus IgG ranged from 3.8% to 12.2%. Only 1 new infection was observed. Herd immunity thereby declined during the year as infection-immune individuals died. As opposed to 1987 and 1988 when tick

abundance was many-fold greater and CCHF virus transmission was epizootic, little transmission was apparent during 1990.

Development of a Mouse Model

Laboratory studies of CCHF virus transmission are hampered by the risk of human infection, the lack of severe symptoms in most vertebrates, and the absence of a suitable animal model. Furthermore, vertebrate animals likely to be natural hosts are difficult to study under laboratory conditions. Laboratory mice are susceptible to CCHF virus infection (Karabatsos 1985), and initial efforts to use them in transmission studies involving Hyalomma ticks were promising (Logan et al. 1989). Studies under the direction of Dr. Jean-Paul Gonzalez, and in collaboration with Ms. Linda Keyes, a visiting medical student from Yale University, were undertaken to examine the feasibility of using white mice as a model rodent in laboratory studies of CCHF virus transmission. The serological and virological responses of mice infected at various virus titers by different routes were studied. Virus and antibody transfer to newborn (NB) mice from their mothers also was examined.

Antibody and antigen detection and evaluation. Sera were tested for evidence of anti-CCHF virus IgG using an ELISA test (Niklasson et al. 1984) modified slightly by adding a saturating solution of PBS with 0.05% Tween 20 and 1% non-fat bovine milk. In this direct ELISA test, 96-well plates (Immulon II, Dynatech Laboratories, Alexandria, VA) were coated with diluted CCHF virus hyperimmune mouse ascitic fluid. CCHF virus in crude suckling mouse brain was heat inactivated at 60°C for 1 h. and then added. Test sera, diluted 1:400, followed by test-species specific anti-immunoglobulin conjugated with horse radish peroxidase (Biosys, Compiegne, France) was used to detect the IgG. A chromogenic substrate (ortho-tolidine, Sigma, LaVerpilliere, France) was added for colorimetry. All plates included a control of crude suckling mouse brain without CCHF virus antigen. Differences in optical density (OD) between the test and control wells were measured at 450 nm using an automatic reader (Multiscan MCC/340, Flow Labs, Irvine, Scotland) coupled to a microcomputer. By iterations of the distribution of OD values, the mean of the negatives was calculated. Sera were considered positive if the OD was greater than 3 standard deviations above this mean.

IgM antibodies were detected by immunocapture ELISA (Saluzzo and LeGuenno 1987). Plates were coated with anti-u-chain specific for the species being tested. The test serum, followed by CCHF viral antigen were then added. The detecting antibody was a high-titered mouse ascitic fluid against CCHF virus antigen. Anti-mouse immunoglobulin conjugated with horse

radish peroxidase and the chromogenic substrate were added as above. Evaluation and criteria were as for IgG.

An antigen capture ELISA (Saluzzo and LeGuenno 1987) also was employed to test for presence of CCHF virus antigen in sera. Plates first were coated with anti-human IgM u-chain specific antibody, followed by human sera with high titer IgM. The test serum was added next and then a high-titered anti-CCHF virus monoclonal IgG was added to bind to any antigen captured from the test serum. An anti-mouse IgG, conjugated with horse radish peroxidase and the chromogenic substrate were used for colorimetry.

Virus isolation was attempted by intracranial inoculation of suckling mice and by inoculation of Vero cells using undiluted and 10-fold diluted sera. Virus identification was made by an indirect immunofluorescent test on Vero cells, using polyclonal and monoclonal antibodies. The identity of virus isolates was confirmed by a complement fixation test at the "Centre Collaborateur OMS de Reference et de Recherche pour les Arbovirus" at the Pasteur Institute in Dakar.

Adult and NB white Swiss laboratory mice also from the stock maintained at the Pasteur Institute were used. The CCHF virus strain (Dak H49199) had been isolated in 1988 from a fatal human case in Rosso, Mauritania (Gonzalez et al. 1990). The working stock virus had been mouse brain passaged 3 times. Mice were inoculated by intracranial (i.c.), intraperitoneal (i.p.), or subcutaneous (s.c.) injection of 0.02 ml of viral dilutions in Hanks' medium. Adult mice were inoculated with a volume of 0.02 ml by i.p. or s.c. injection. Hanks' medium alone was used to inoculate control subjects. Infected mice were observed daily for clinical signs of infection. Blood samples were collected by intracardiac puncture of adult mice and carotid puncture of NB mice. Sera were tested for IgG antibody by the direct ELISA test mentioned above. Virus reisolation from adult mouse sera was attempted by i.c. inoculation of day-old mice. To confirm the presence of CCHF virus in NB mice, we used the antigen capture ELISA.

In Experiment 1, the survival of day-old mice (groups of 10), inoculated i.p. or i.c. with viral concentrations ranging from undiluted to 10^{-7}, was monitored during 20 d. Experiment 2 studied the survival of mice ranging in age from 1 d to 16 d (10 groups of 10 mice each) inoculated i.p. with CCHF virus (diluted 10^{-1}). In Experiment 3, the antibody response of 84 adult male mice (4 groups of 21 mice) that were inoculated i.p. with CCHF virus concentrations ranging from undiluted to 10^{-6} was monitored. In Experiment 4, pregnant mice were inoculated s.c. with CCHF virus diluted 10^{-1} from 1 to 6 d before giving

birth; the antibody responses and viremia of mothers and babies then were monitored.

Survival of day-old mice that were inoculated i.p. or i.c. with different virus dilutions (Experiment 1) varied according to route and titer of inoculation (Fig. 15). The 50% lethal dose (LD_{50}) was calculated at 5.9 LD_{50}/0.02ml by i.p. and 5.8 LD_{50}.0.02ml by i.c. At doses between undiluted and 10^{-4}, mice that were inoculated i.c. appeared to die earlier and more rapidly than those inoculated i.p., although in all cases 100% of mice eventually died (Fig. 16). At viral dilutions 10^{-5} to 10^{-7}, mortality patterns were virtually the same for both groups.

To determine the time course of development of resistance to CCHF infection (Experiment 2), young mice were inoculated i.p. with CCHF virus at different ages (Fig. 17). Virtually all mice aged 1 to 3 d died following inoculation; mice inoculated at older ages, however, increasingly survived. All surviving mice were tested at 21 d PI for presence of IgG against CCHF virus. Antibody was detected in mice injected age 3 d and older (Fig. 18). Antibody titer increased with age of inoculation. Mice which survived infection from the virus titration experiments were also tested for presence of anti-CCHF virus antibody (Fig. 19).

The antibody response to 4 different doses of CCHF virus infection (undiluted, 10^{-2}, 10^{-4}, 10^{-6}) was measured over 23 d (Experiment 4). All mice but 1 survived, and none exhibited signs of illness. IgG was first detected at 3 d PI in mice infected with undiluted virus, 5 d in those infected with 10^{-2}, 7 d in the 10^{-4} group, 10 d in the 10^{-6} group (Fig. 20). Maximum titers were observed between 10 and 18 d. Antibody titers declined below detectable levels by 23 d PI except in mice inoculated with undiluted virus. Viremia was detected by reisolation of virus from sera inoculated i.c. into suckling mice. Only those mice infected with undiluted virus were tested. Viremia was detected from 3 d to 7 d PI.

Pregnant mice that were infected s.c. with CCHF virus diluted 10^{-1}, and their offspring that were born 1 to 6 d PI, were tested for the presence of antibody and virus. Of 18 infected mothers that were tested, 16 (88%) exhibited a positive antibody response (Fig. 21). Anti-CCHF virus IgG was detected in 46 of 123 (37%) offspring from sero-positive mothers and in none of the offspring from sero-negative mothers. CCHF virus antigen was detected neither in the brains of offspring from infected mothers who were sero-positive, nor from the offspring of sero-negative mothers.

These studies demonstrate that the pathogenic effects of CCHF virus on NB mice depended on the titer of virus that was inoculated and the route of delivery. Mice inoculated either i.p. or i.c. with higher titers of virus died from 1 to 12 d PI, however they died sooner with increased virus concentration. Inoculation by the i.c route appeared to be pathogenic more rapidly than that by i.p. for most virus titers. The final mortality rates for both routes, however, were similar at each viral concentration. Mortality rates differed, however, among NB mice depending on the concentration of virus that initially was inoculated. Similarly, the age at which mice where infected also appeared to influence the pathogenic effects of this CCHF virus strain: older mice generally were better able to survive infection from a dose that was usually fatal to younger mice. The antibody response of adult mice that were inoculated i.p. with CCHF virus appeared to vary with virus concentration. IgG rose rapidly in mice inoculated with undiluted virus, and more slowly with decreasing viral concentration. Babies born to female mice that were inoculated s.c. while pregnant showed evidence of maternal antibodies, presumably passed transplacentally or through the milk, in a manner that is consistent with that typical of rodents (Tizard 1982).

Viremia seems to persist only briefly in most vertebrates thusfar studied; therefore amplifying, horizontal transmission may be limited to a short period during which infectious and uninfected ticks would be feeding simultaneously. Studies of potential vector ticks feeding on inoculated hosts (e.g. Logan et al. 1989) and of the reinfection of immune hosts are needed to elucidate the impact of a brief viremia on transmission. Investigation into how CCHF virus-infected ticks alter host physiological status and receptivity (Ribeiro 1987) and perhaps enhance virus transmission. Logan et al. (1989) recently reported that immature Hyalomma truncatum ticks which fed on suckling mice that had been infected by CCHF virus successfully transmitted virus to and from ticks and mice. These results provide further insight into the potential of laboratory mice as a model vertebrate in studies of the transmission dynamics of CCHF virus infection to and from the immature stages of vector ticks.

IV. VIRUS TRANSMISSION

Natural Prevalence of Virus in Ticks

Vectorial capacity is partly a function of the frequency and intensity of CCHF virus infection in populations of ticks. Collections from vertebrates continue to be analyzed systematically by inoculation into suckling mice and by cell culture. Under the direction of Dr. Camicas, ticks from Yonofere, Dahra and Bandia are identified and pooled and then tested for virus. Virus isolation efforts are coordinated by Dr. Zeller. Species-specific pools from individual animals or herds were held at -70°C until testing, at which time they were ground for virus isolation. All pools were tested by suckling mouse inoculation, and, in collaboration with Mrs. Mondo, most were also tested either in Vero or SW-13 cell lines. Identity of viruses from ticks or eggs was confirmed using a CF test following mouse passage, studies undertaken in collaboration with Mrs. Calvo-Wilson.

Tick collections were made from 3 sources: nonselective collections by herdspeople from their cattle and sheep, randomly selected sheep being studied for evidence of tick activity patterns, and individually identified sheep, cattle and goats that are being bled for evidence of antibodies and virus. Each month ticks were collected by herdspeople in Yonofere and surrounding villages who were given tubes for ticks that they have removed from their animals. In addition, those ticks collected from sheep sampled in Yonofere and Dahra each month for determination of tick seasonality also were tested for the presence of virus. Finally, ticks removed from sheep and cattle at the Dahra-CRZ, sheep in Yonofere, and goats and cattle in Bandia which are part of studies on the incidence of CCHF infection also were tested for virus.

During 1990, a total of 9,494 ticks were captured from ungulates in Yonofere, Dahra and Bandia and tested in 1,074 pools (Table 5). The tick species tested were Hyalomma truncatum, H. m. rufipes, H. impeltatum, H. impressum, Rhipicephalus. guilhoni, R. e. evertsi, R. sanguineus, Amblyomma variegatum and Boophilus decoloratus.

One strain of CCHF virus was isolated from a pool of B. decoloratus. Although CCHF virus has been isolated from this tick elsewhere in Africa, this represents the first such isolation in Senegal. In addition, other viruses have been isolated from various species of ticks including Wad Medani, Bhanja, and Jos viruses. Numerous strains of Wad Medani virus have been isolated from ticks in Yonofere, particularly from H. truncatum (32 strains), R. guilhoni (11) and H. m. rufipes (1).

Curiously, Wad Medani is less often found at other nearby sites. Bhanja virus (3 strains) was isolated from B. decoloratus taken in Bandia as was Jos (3 strains) from A. variegatum obtained at that same site. The curious absence of CCHF virus from these sites, particularly Yonofere where it appeared abundant during 1987 and 1988, cannot be explained by the isolation methods being used. Other viruses were isolated suggesting that CCHF virus circulation has, for unknown reasons, declined during the recent period.

Experimental Transmission Between Ticks

Natural infection of adult ticks with CCHF virus in endemic sites appears to be rare; similarly, field and experiemental evidence suggests that transovarial transmission is inefficient. Furthermore, viremia in vertebrates generally lasts only for a few days. The contribution of various forms of transmission to the maintenance of CCHF virus remains enigmatic; the frequency of animal and human infection suggests that other transmission mechanisms may function to amplify transmission. For these reasons, experiments designed to explore the possibility of "sexual" transmission between male and female H. truncatum were performed under the direction of Drs. Gonzalez and Cornet.

Tick colony and manipulation. Ticks were reared from a single egg batch laid by an adult H. truncatum removed from a sheep in Yonofere. Immatures were fed on a captive Whyte's hare and held as adults at 75%RH and 28°C. Adult males were pre-fed on laboratory rabbits for 11 days before hypostomectomy in order to prepare them for mating. These males were immobilized on their scutum using a cork board and overlapping pins. After spreading the palps, the hypostome was cut using opthalmic scissors; a hemolymph clot formed shortly thereafter. The chelicerae were not altered. In the case of female ticks, the gonopore was sealed using Cyanolit glue: females were held with fine forceps, and a drop of glue was placed over the genital aperature and allowed to harden.

Infection and feeding of ticks. A suspension of CCHF virus strain Dak H49199, titered at 6.5 log LD_{50}/ml, was used to inoculate adult H. truncatum. Adult ticks were inoculated as previously described (Gonzalez et al. 1989) by the intra-anal route with 2ul of CCHF viral suspension diluted (10:1) in Hanks' medium. Supernatant from a centrifuged sample of this suspension was tested by inoculation into suckling mice and verified at the Pasteur Institute. Ticks were fed on naive rabbits by shaving the rabbit's back and applying a depiliary cream (Veet, Ricket & Coleman Co.), over which an open-bottom, aluminum tube (30mm diameter) was glued (Pattex, Henkel Co.). Ticks were placed into the tube which was sealed using a mesh

cloth. Six to 8 male and female ticks were placed together in tubes and held for 6 or more days until engorged females detached. Males were then tested for virus while females were held at 75%RH and 28°C until egg laying was complete.

Virus assay and antigen-capture. Individual adult ticks and pools of ca. 50 larvae or ca. 50 nymphs were ground in Hanks' medium (10% wt/vol) then centrifuged (10,000 RPM, 10 m). The supernatant was assayed for virus reisolation by intracranial inoculation into suckling mice. Tick egg batches (ca. 1,000 eggs per batch) were ground in Hanks medium (50% wt/vol) and centrifuged. The supernatant was assayed for CCHF virus in Vero cell culture. Virus detection and identification in ticks was attempted by ELISA antigen capture as described above.

CCHF virus transmission was detected both during cofeeding and by direct transfer during copulation (Table 6). In the first "mating" trial, CCHF virus was isolated at >3.2log LD_{50}/ml from 2 of 3 surviving inoculated, hypostomectomized males, demonstrating successful introduction of the virus. 45 of 6 females that mated with these males showed virus titers of >2.2log LD_{50}/ml. Transovarial transmission was observed by virus isolation from 2 of 4 egg batches that were virus positive. One of 6 pools of larvae from these eggs were positive but none of 15 subsequent nymphal pools produced evidence of infection (Table 6).

In the second "cofeeding" trial involving hypostome-intact males and gonopore-occluded females, the later became infected. No eggs were produced by these females. Since males were able to feed, yet females were incapable of being mated by these males, CCHF virus transfer apparently occurred by transmission via the host. Indeed, the rabbit on which these ticks fed showed antibody against CCHF virus following infestation, suggesting further that female H. truncatum had been infected through cofeeding. In the control trial, noninoculated males tested negative, and none of the female ticks that fed and mated with them were infected. Similarly, the rabbit did not seroconvert.

This experiment suggests that H. truncatum males are capable not only of infecting other ticks that feed during the same time, but also that they may efficiently transfer CCHF virus directly to females during mating. Transovarial transmission was observed, suggesting that this may be a means by which virus is transferred to eggs. The extent to which this may be important in nature will require further investigation.

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APPENDICES

Figure Legends

Figure 1. Map of Senegal indicating the principal surrounding countries (in capitals), larger cities (dot), and 3 major study sites (italics, underline) discussed in this report. Rainfall isoyhets are also indicated.

Figure 2. Average monthly rainfall at the Dahra CRZ Research Station during 1959-1986, and total monthly rainfall during 1987, 1988, and 1989 at that site.

Figure 3. Adult ticks collected monthly from sheep sampled in Dahra, Senegal.

Figure 4. Adult ticks collected monthly from sheep sampled in Yonofere, Senegal.

Figure 5. Temporal pattern of detachment by larval H. truncatum while feeding on guinea pigs. The solid line represents number of larvae per day.

Figure 6. Temporal pattern of detachment by larval H. truncatum while feeding on laboratory rabbits. Data is for detaching engorged larvae (solid line), flat nymphs (lightly hatched) and engorged nymphs (darkly hatched).

Figure 7. Temporal pattern of detachment by larval H. truncatum while feeding on multimammate rat, slender gerbils and Nile rats. Stage and engorgement of ticks is as in Fig 4.

Figure 8. Temporal pattern of detachment by larval H. truncatum while feeding on hedgehogs. Stage and engorgement of ticks is as in Fig 4.

Figure 9. Temporal pattern of detachment by larval H. truncatum while feeding on hares. Stage and engorgement of ticks is as in Fig 4.

Figure 10. Temporal pattern of detachment by larval H. m. rufipes while feeding on sparrows and doves. Stage and engorgement of ticks is as in Fig 4.

Figure 11. Temporal pattern of detachment by larval H. m. rufipes while feeding on guinea fowl, canaries and diochs. Stage and engorgement of ticks is as in Fig 4.

Figure 12. Temporal pattern of detachment by larval H. m. rufipes while feeding on guinea pigs, hares and hedgehogs. Stage and engorgement of ticks is as in Fig 4.

Figure 13. Survival of adult H. truncatum under different temperatures and humidities.

Figure 14. Survival of adult H. truncatum under different temperatures and humidities.

Figure 15. Effect of CCHF virus inoculation route on survival of NB mice. Mice were inoculated (A) intraperitoneally, or (B) intracranially with a strain of CCHF virus. Each observation represents the mean survival per day of 3 groups of 10 mice each inoculated with undilute to 10^{-7} diluted virus.

Figure 16. Survival of NB mice inoculated with different routes and titers CCHF virus. Mean survival rates of NB mice inoculated either i.p. or i.c. with either high titered (undiluted to 10^{-4}) or low titered (10^{-5} to 10^{-7}) CCHF virus. Each observation represents the mean of 9 or 12 groups of 10 mice each, inoculated i.c. or i.p. with high or low virus titers.

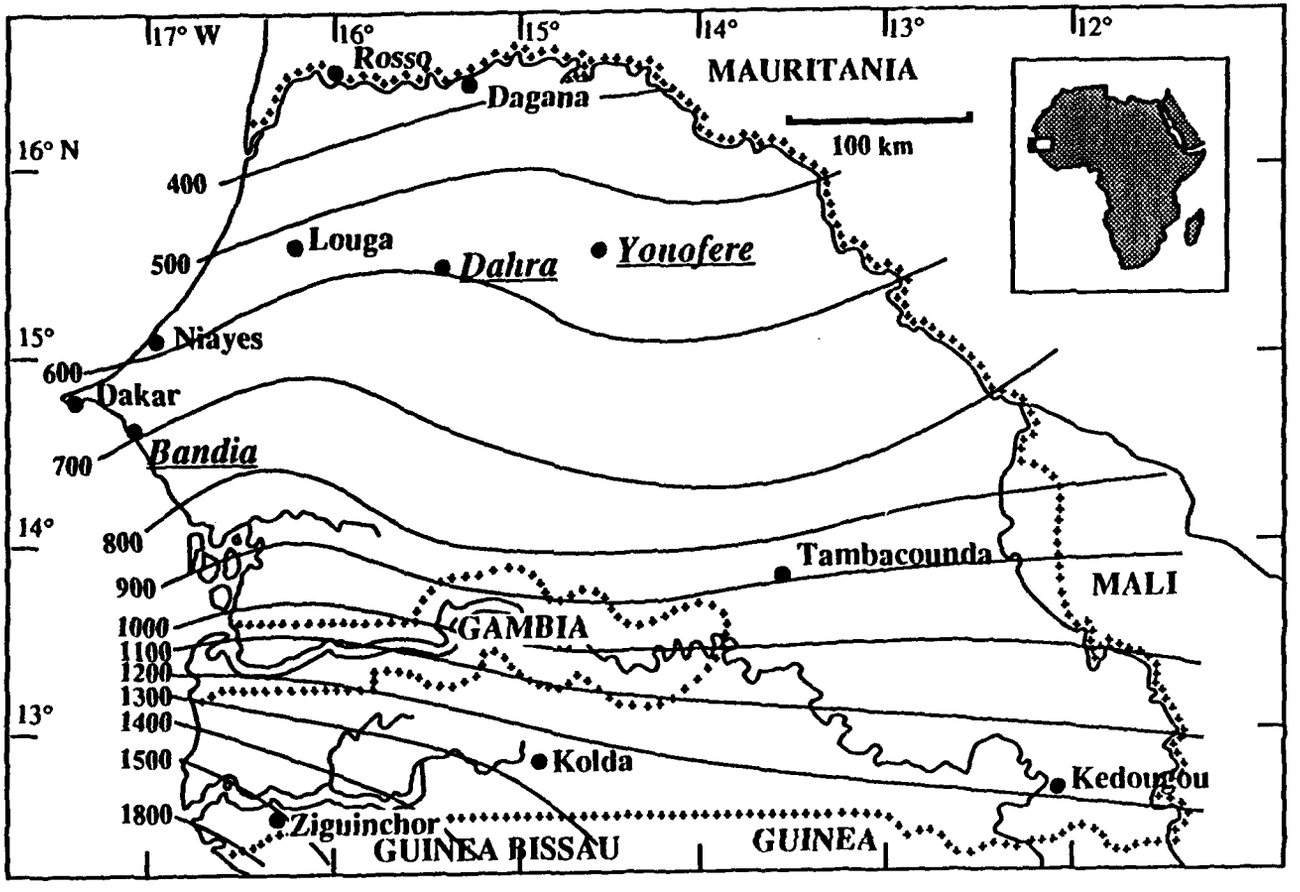
Figure 17. Age-related survival of young mice inoculated by CCHF virus. Effect of age when infected by CCHF virus on the survival of young mice inoculated i.p. with dilution 10^{-1} of virus. Each point represents the mean of 3 groups of 10 mice.

Figure 18. Age-related antibody-development of young mice inoculated with CCHF virus. Effect of age on antibody titer of young mice that survived i.p. inoculation with dilution 10^{-1} of CCHF virus. Each point represents the mean of 3 groups of 10 mice. Mice were tested on day 21 PI.

Figure 19. Effect of inoculation route of CCHF virus on IgG development in NB mice. IgG antibody responses in NB mice surviving i.p. or i.c. inoculation with dilutions 10^{-5} to 10^{-7} of CCHF virus. Mice were tested on day 21 PI.

Figure 20. IgG response in adult mice inoculated with different titers of CCHF virus. Development of IgG antibody responses in adult mice inoculated s.c. with a 0.25 ml solution of undiluted, 10^{-2}, 10^{-4}, and 10^{-6} diluted CCHF virus. Each point represents the average of 2 to 3 mice.

Figure 21. Infection date-dependent transfer of maternal anti-CCHF virus antibody to baby mice. Development of antibody response in NB mice born to mothers who had been inoculated s.c. with 0.3 ml of CCHF virus solution diluted 10^{-1} on (A) 1 d, (B) 3-4 d, or (C) 5-6 d prior to giving birth.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Bandia
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Tambacounda
MALI
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GAMBIA
1100
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Kolda
Kedougou
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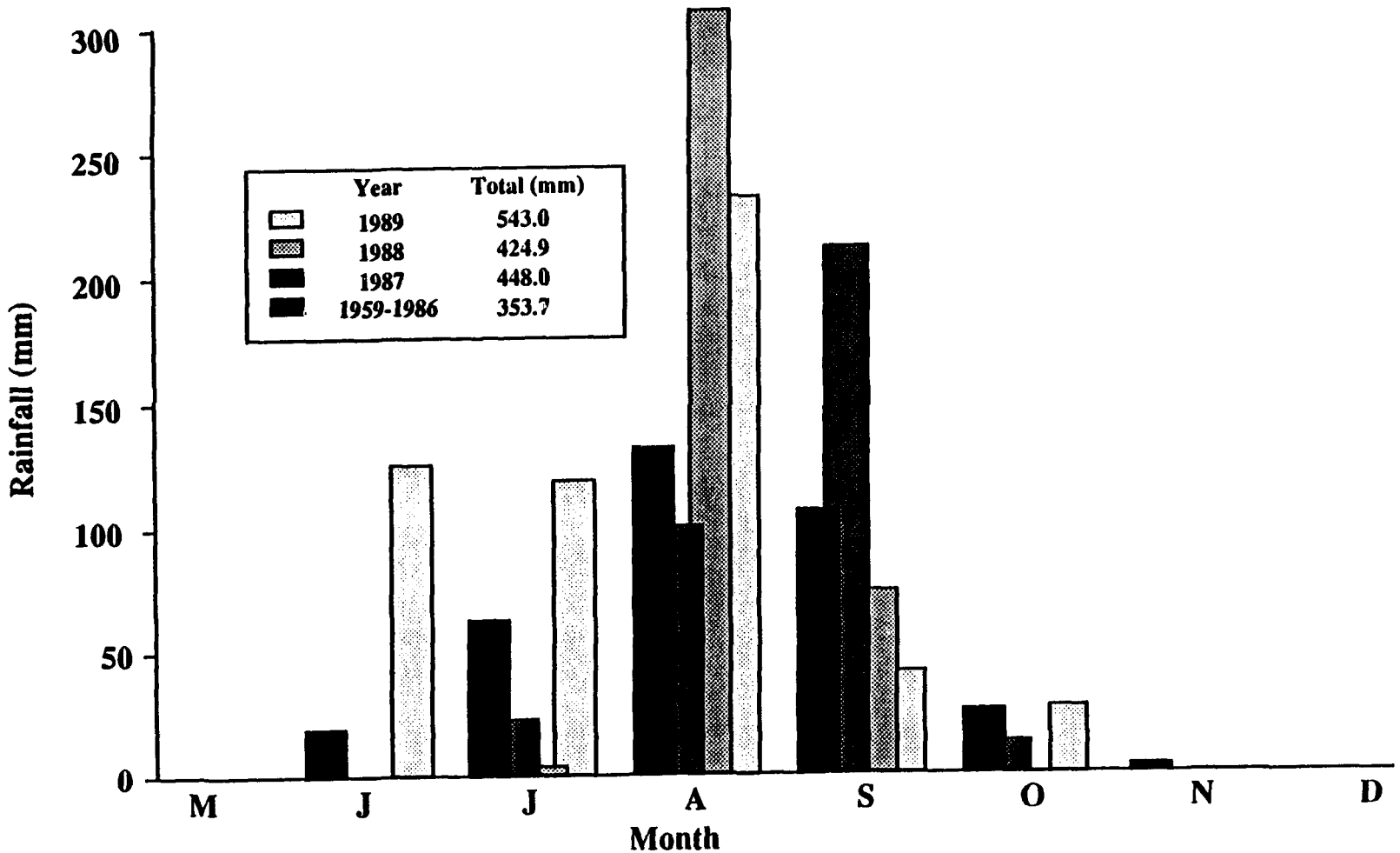
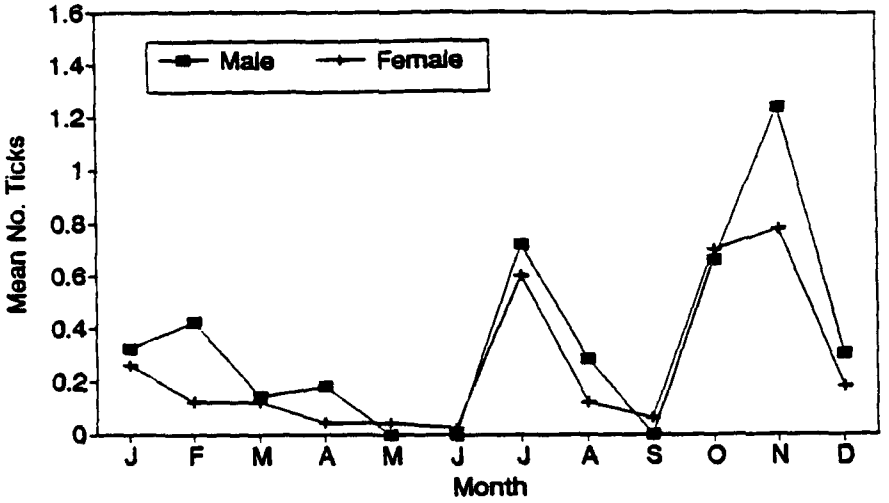
Ziguinchor
GUINEA BISSAU
GUINEA

Rainfall in Dahra during 1959-89



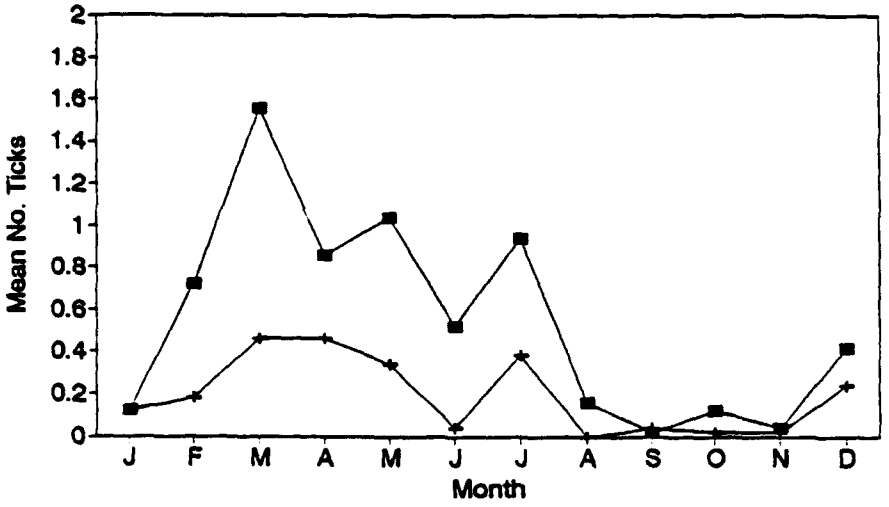
R. guilhoni - Yonofere

1990



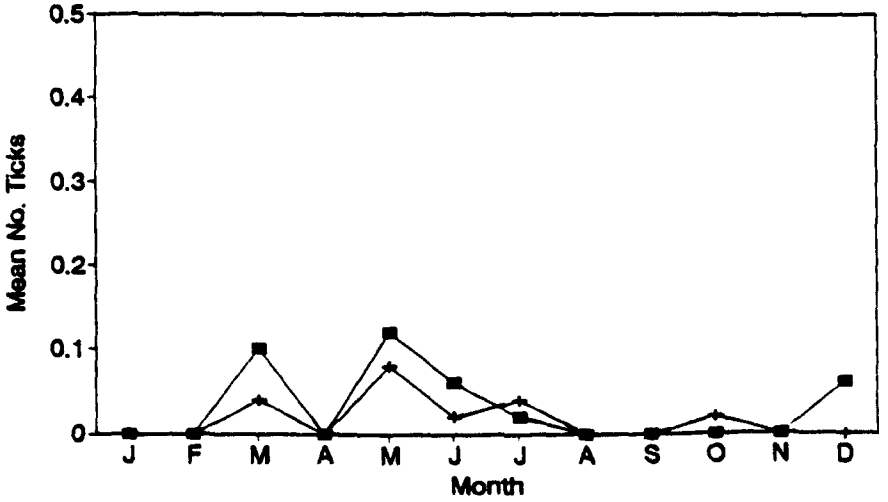
H. truncatum - Yonofere

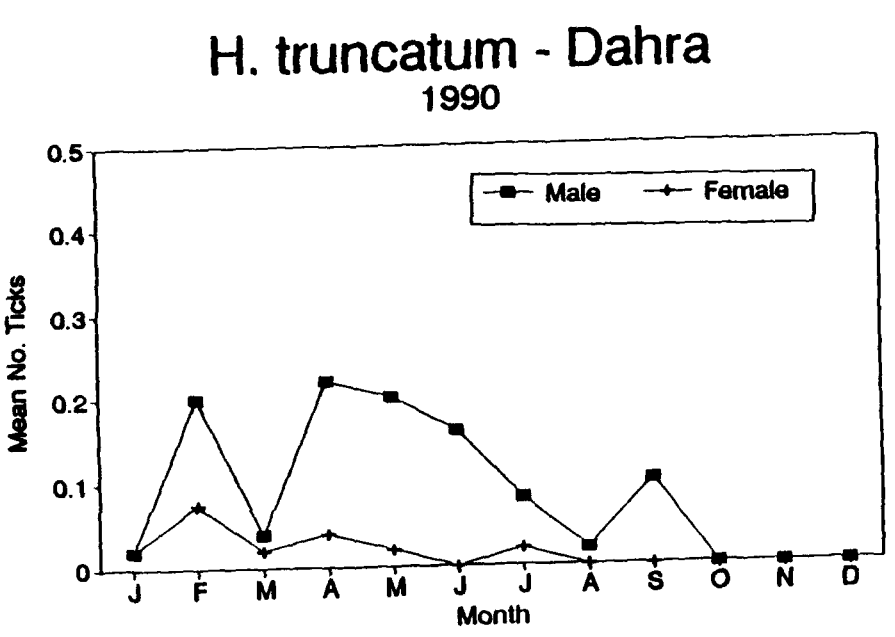
1990

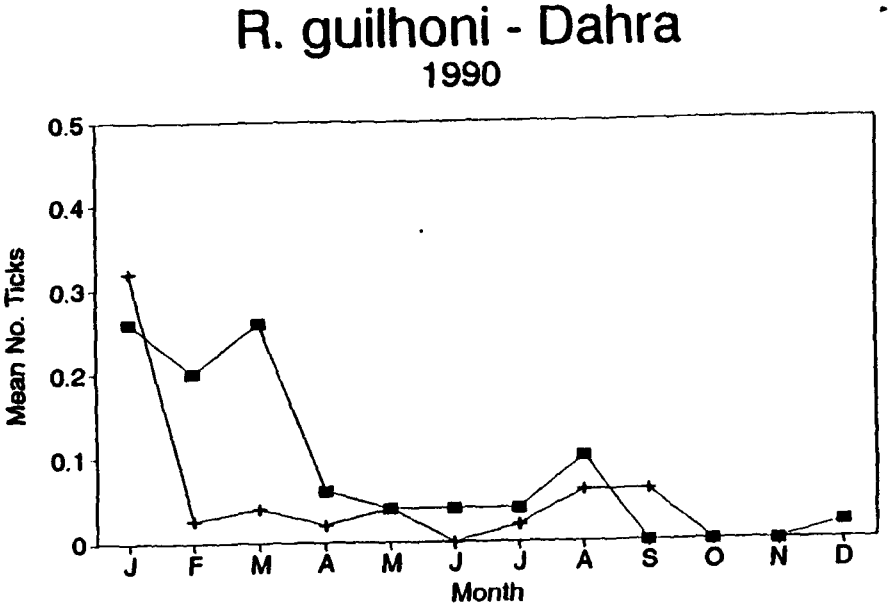


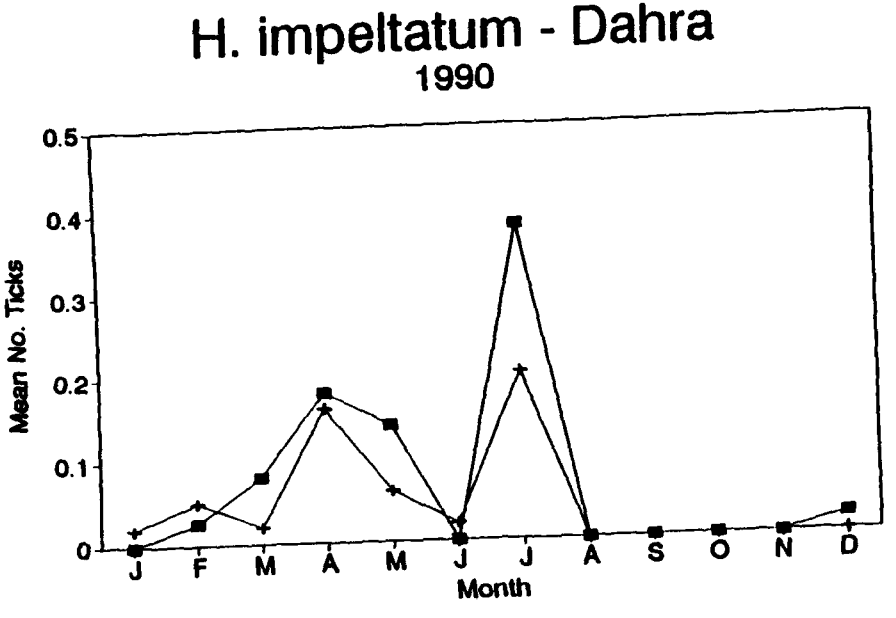
H. m. rufipes- Yonofere

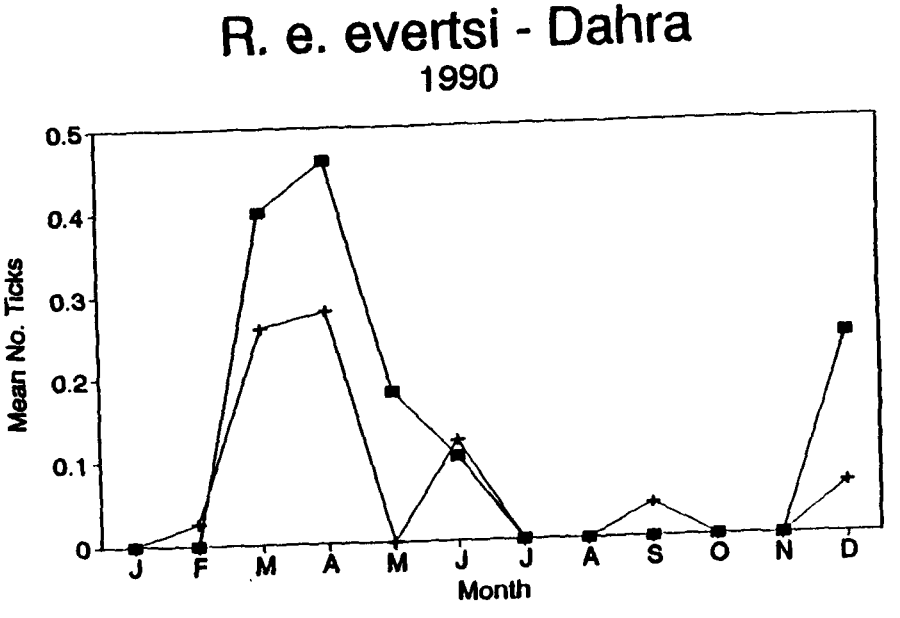
1990



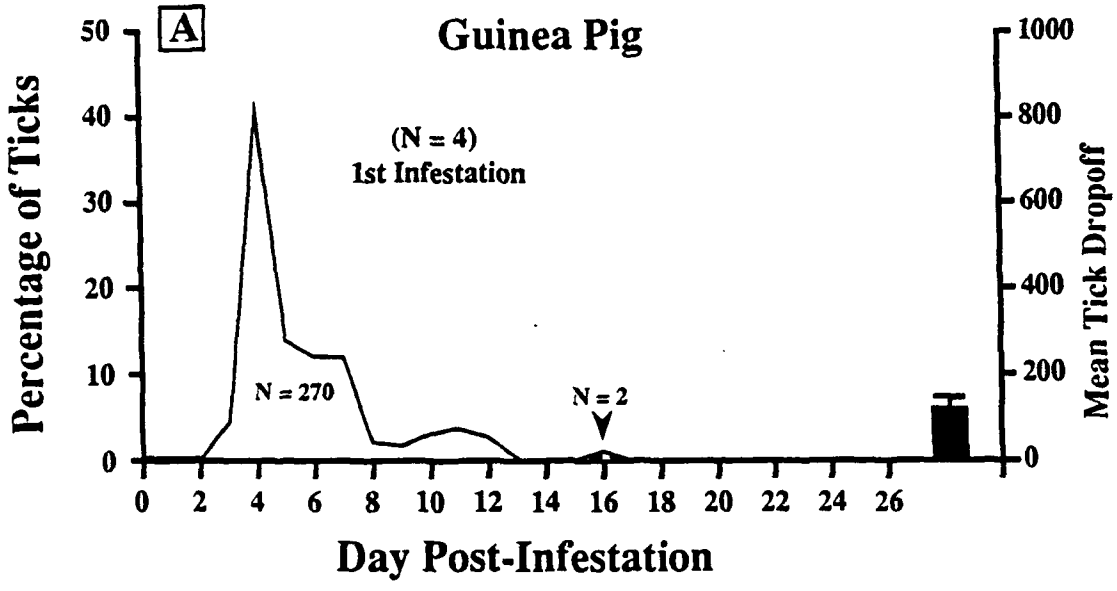

H. truncatum - Dahra
1990
Male
Female
Mean No. Ticks
0.5
0.4
0.3
0.2
0.1
0
J F M A M J J A S O N D
Month

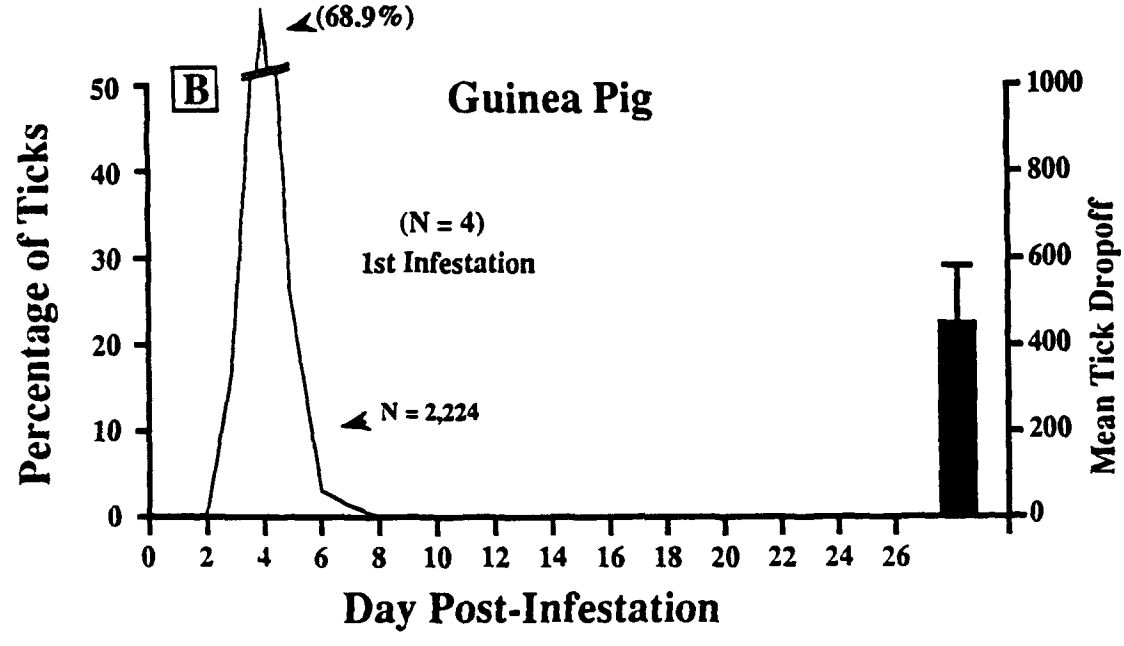

R. guilhoni - Dahra
1990
Mean No. Ticks
0.5
0.4
0.3
0.2
0.1
0
J F M A M J J A S O N D
Month


H. impeltatum - Dahra
1990
Mean No. Ticks
0.5
0.4
0.3
0.2
0.1
0
J F M A M J J A S O N D
Month

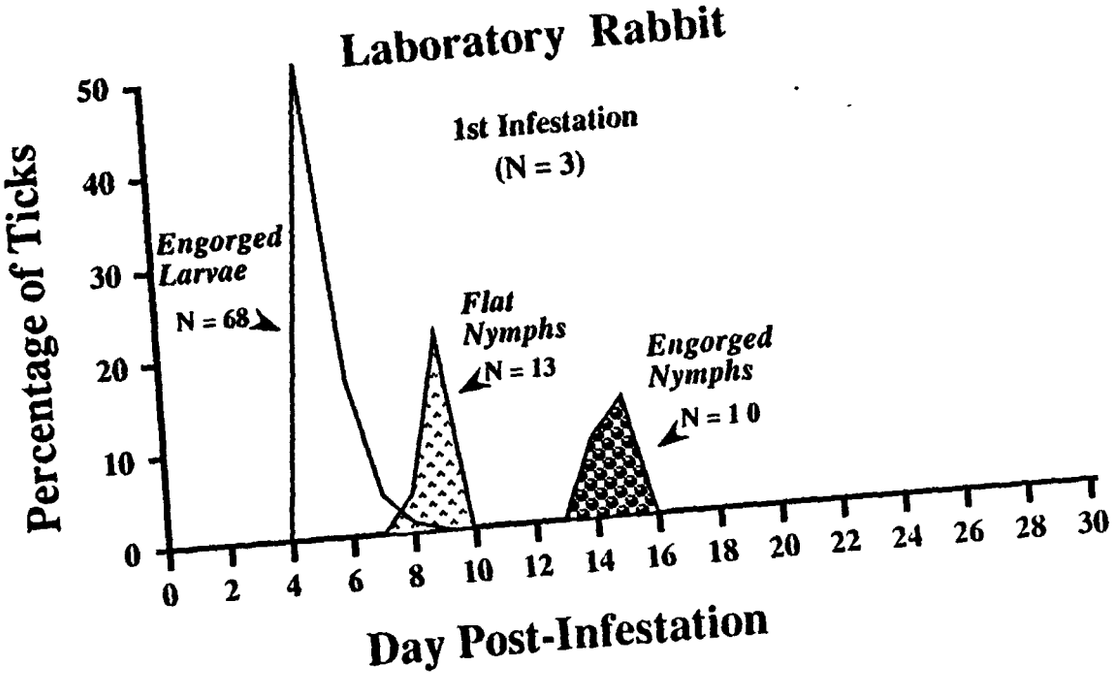

R. e. evertsi - Dahra
1990
Mean No. Ticks
0.5
0.4
0.3
0.2
0.1
0
J F M A M J J A S O N D
Month

Hyalomma truncatum

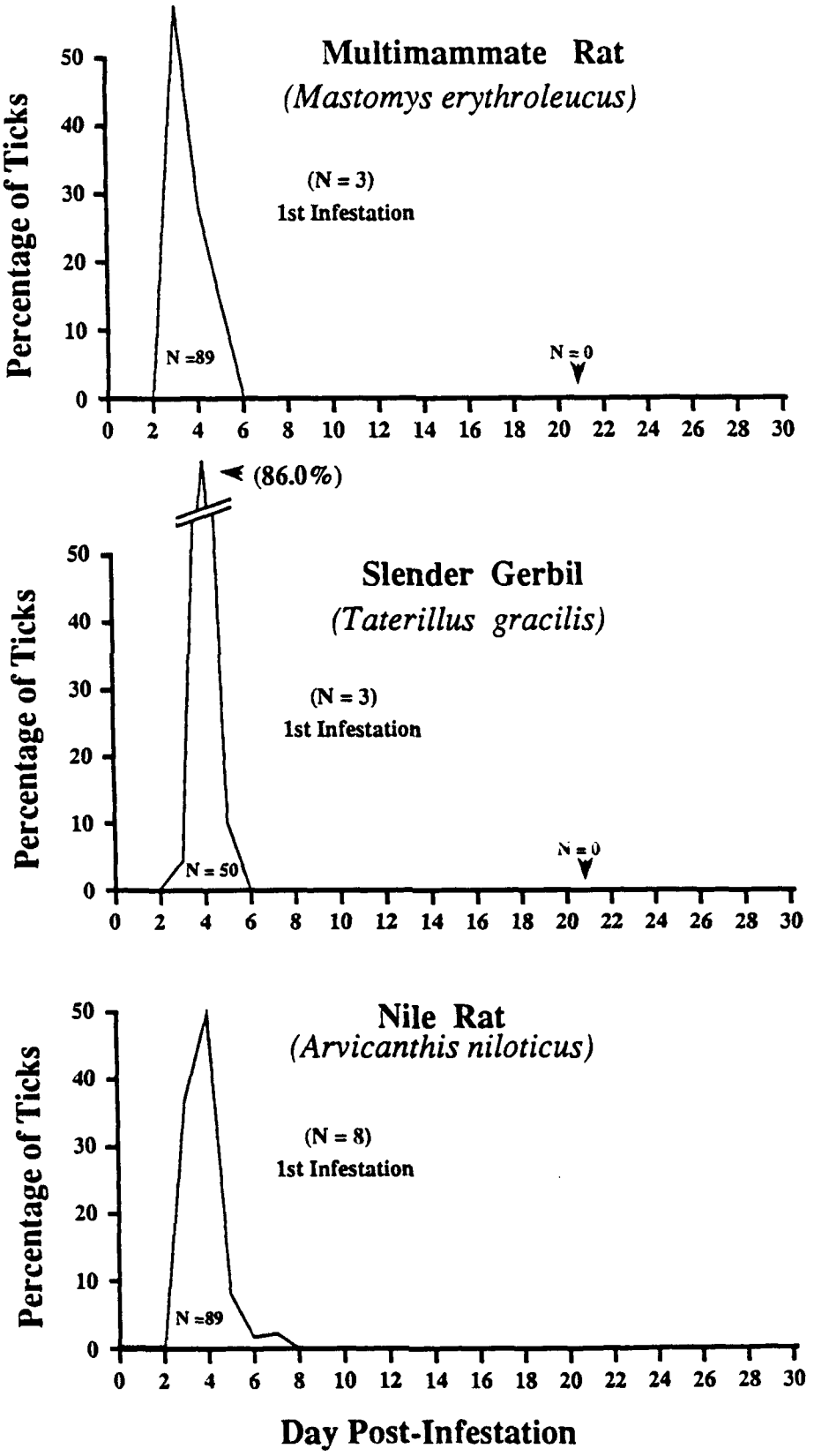


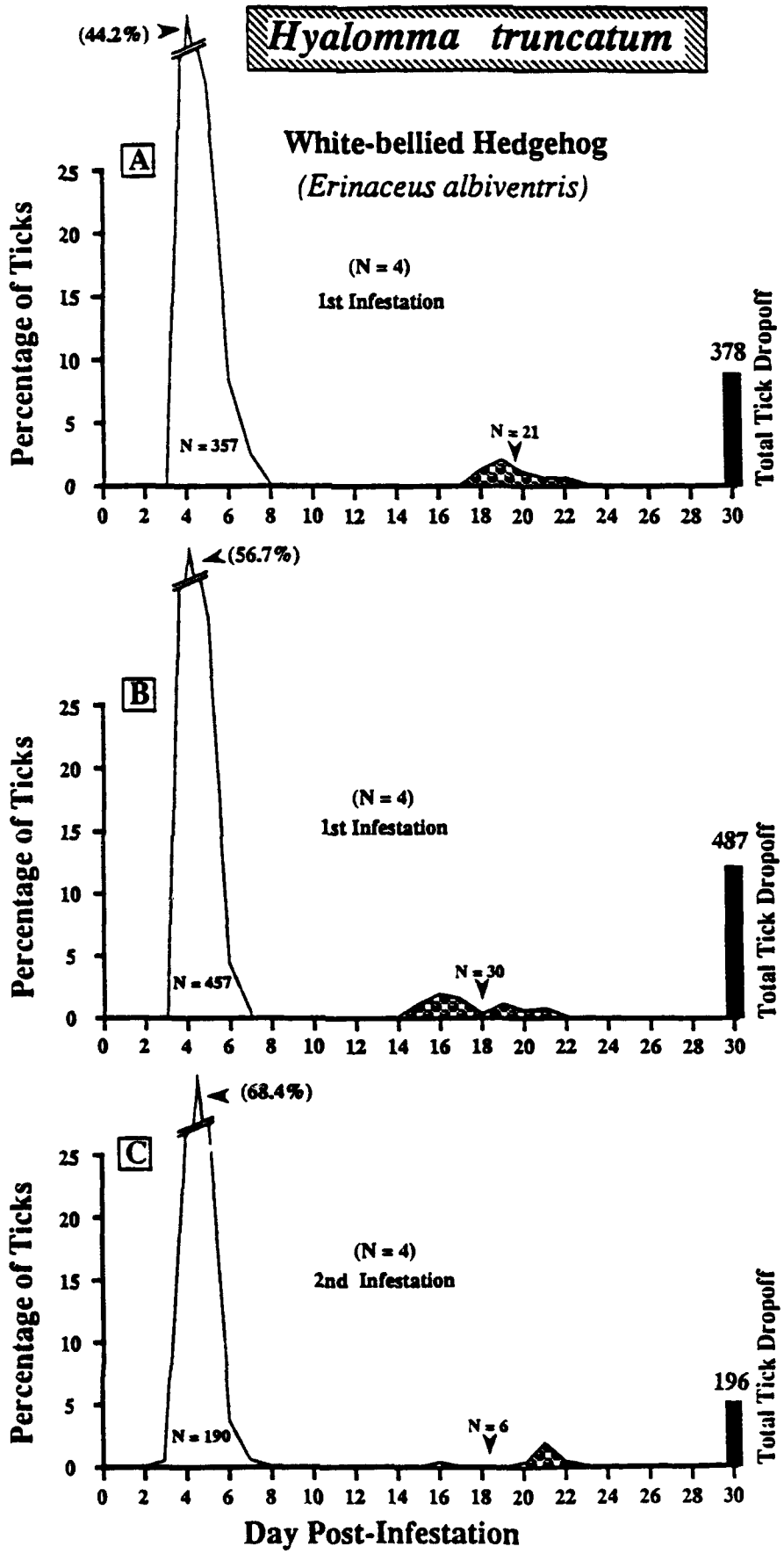


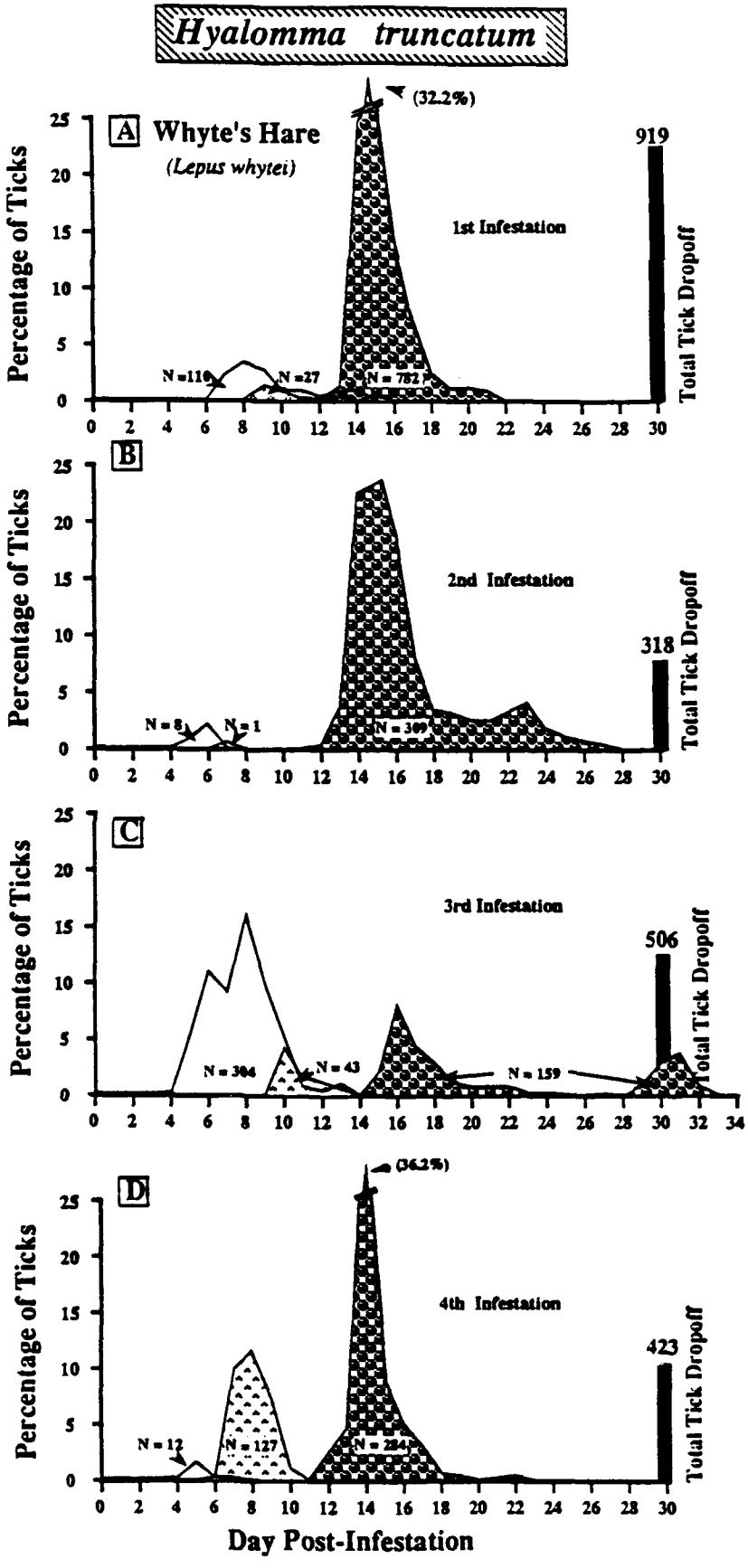
Hyalomma truncatum

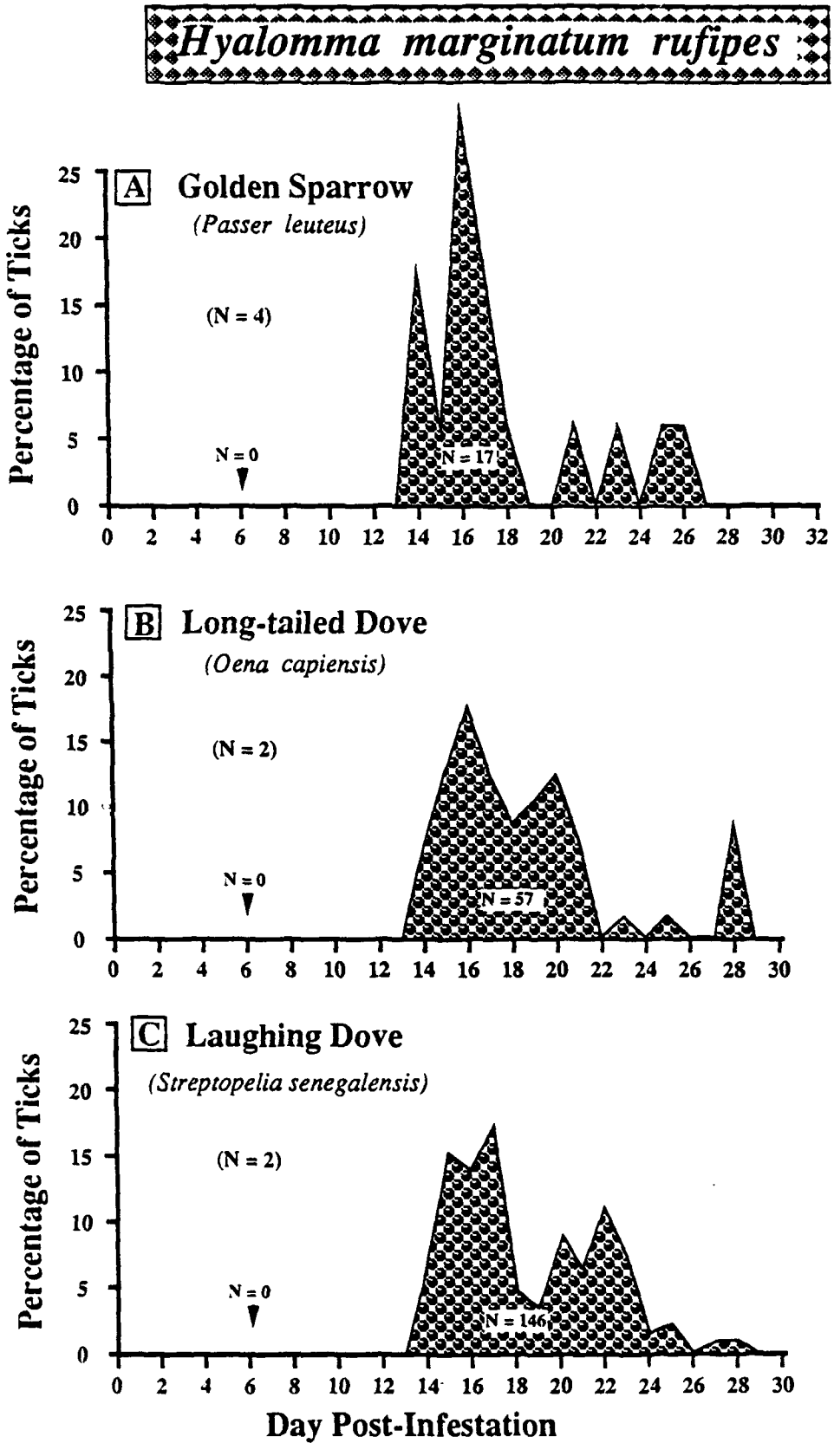


Hyalomma truncatum

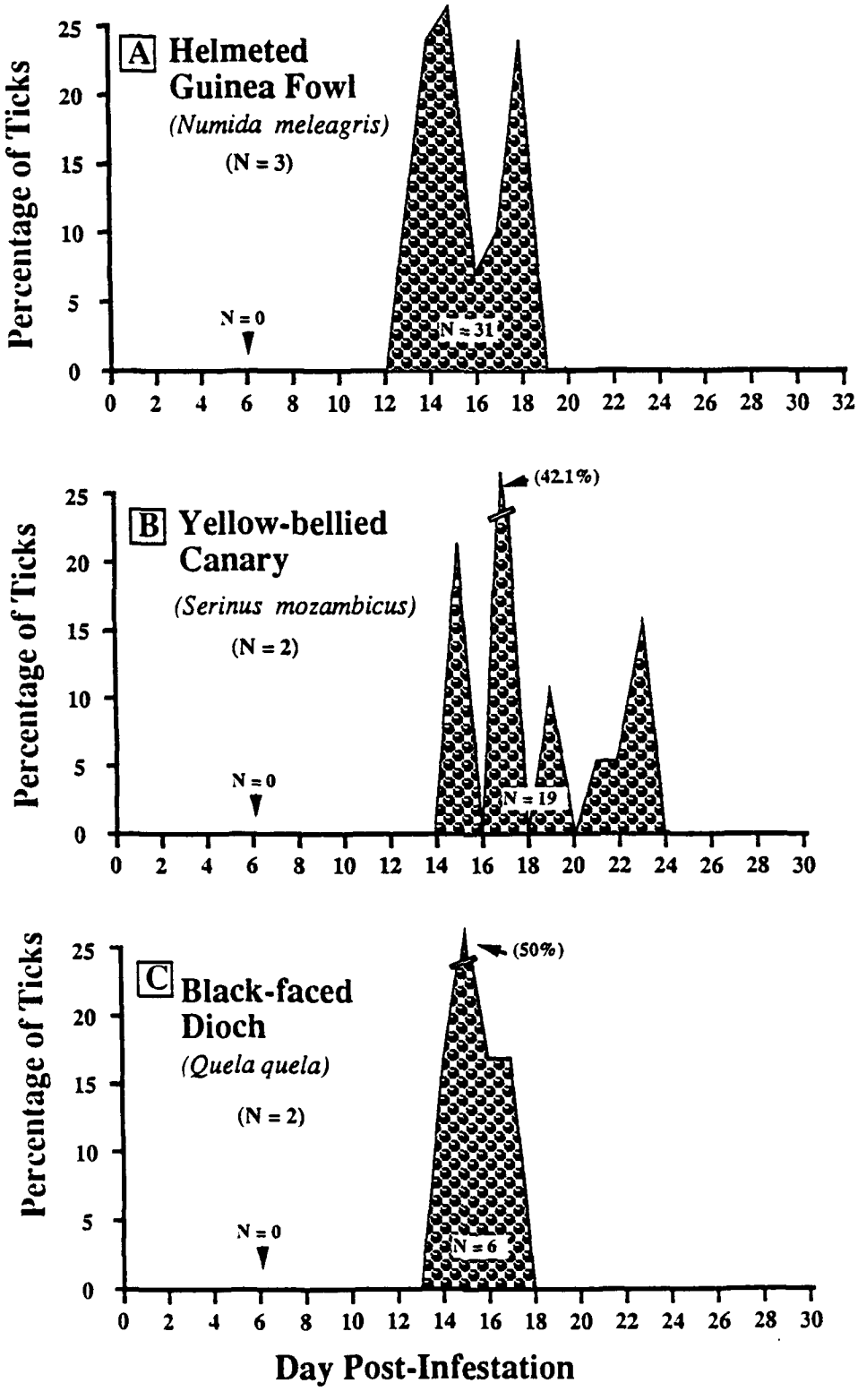



Hyalomma truncatum
White-bellied Hedgehog
(Erinaceus albiventris)
A
(44.2%)
(N = 4)
1st Infestation
N = 357
N = 21
378
Total Tick Dropoff
Percentage of Ticks
B
(56.7%)
(N = 4)
1st Infestation
N = 457
N = 30
487
Total Tick Dropoff
Percentage of Ticks
C
(68.4%)
(N = 4)
2nd Infestation
N = 190
N = 6
196
Total Tick Dropoff
Percentage of Ticks
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Day Post-Infestation

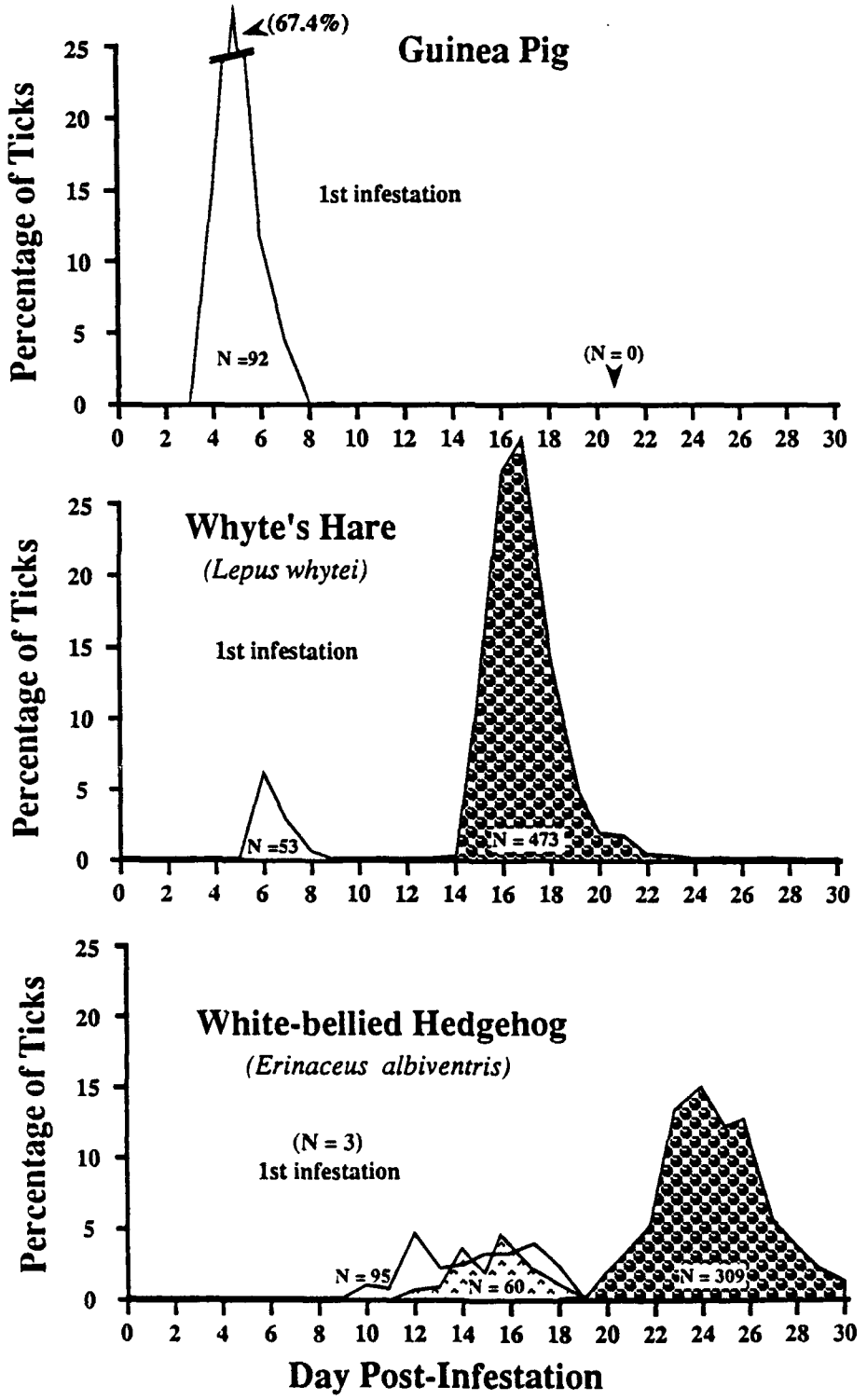

Hyalomma truncatum
A Whyte's Hare
(Lepus whytei)
(32.2%)
919
1st Infestation
N = 116
N = 27
N = 782
B
2nd Infestation
318
N = 8
N = 1
N = 309
C
3rd Infestation
506
N = 304
N = 43
N = 159
D
(36.2%)
4th Infestation
423
N = 12
N = 127
N = 284
Percentage of Ticks
Total Tick Dropoff
Day Post-Infestation


Hyalomma marginatum rufipes
A Golden Sparrow
(Passer leuteus)
(N = 4)
N = 0
N = 17
B Long-tailed Dove
(Oena capiensis)
(N = 2)
N = 0
N = 57
C Laughing Dove
(Streptopelia senegalensis)
(N = 2)
N = 0
N = 146
Percentage of Ticks
Day Post-Infestation

Hyalomma marginatum rufipes

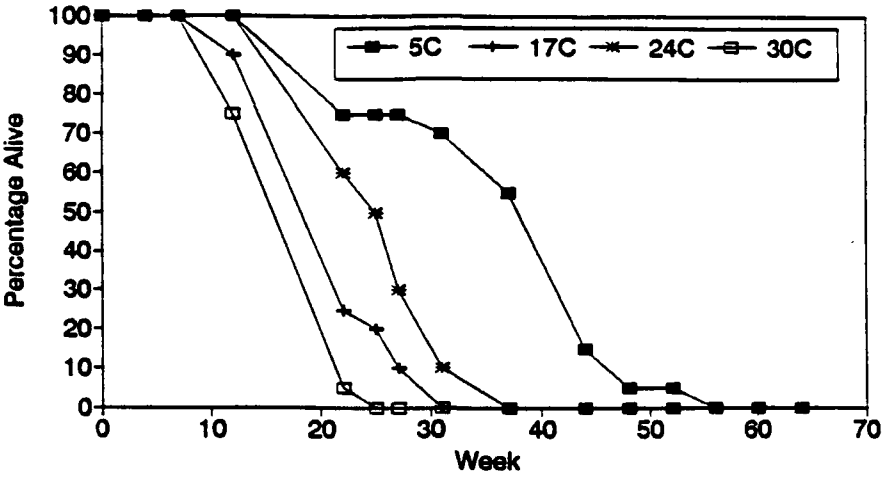


Hyalomma marginatum rufipes

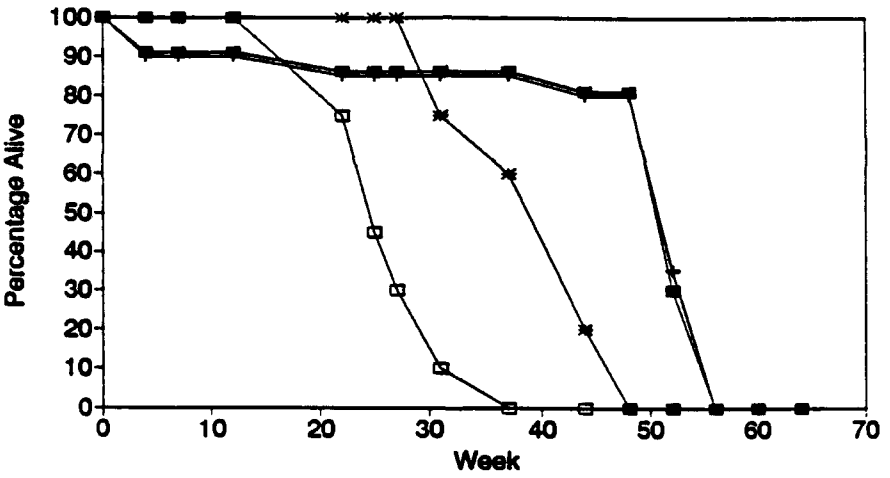


H. truncatum

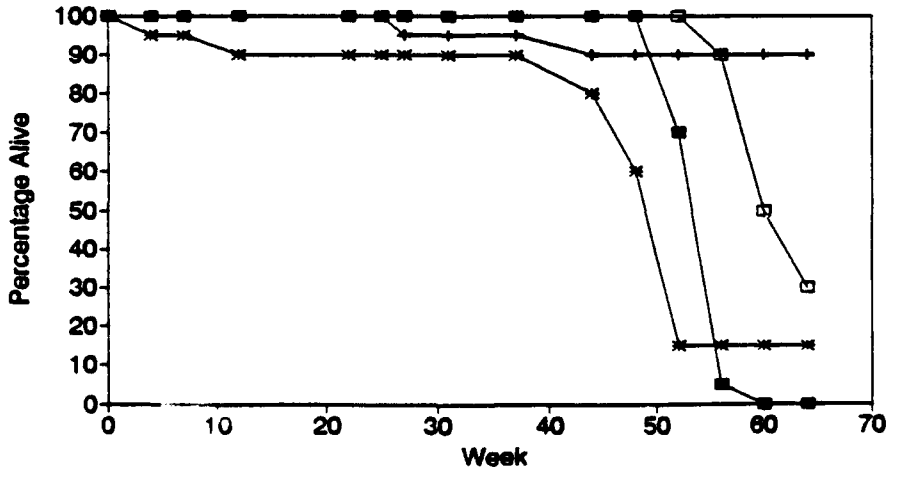
10% Relative Humidity

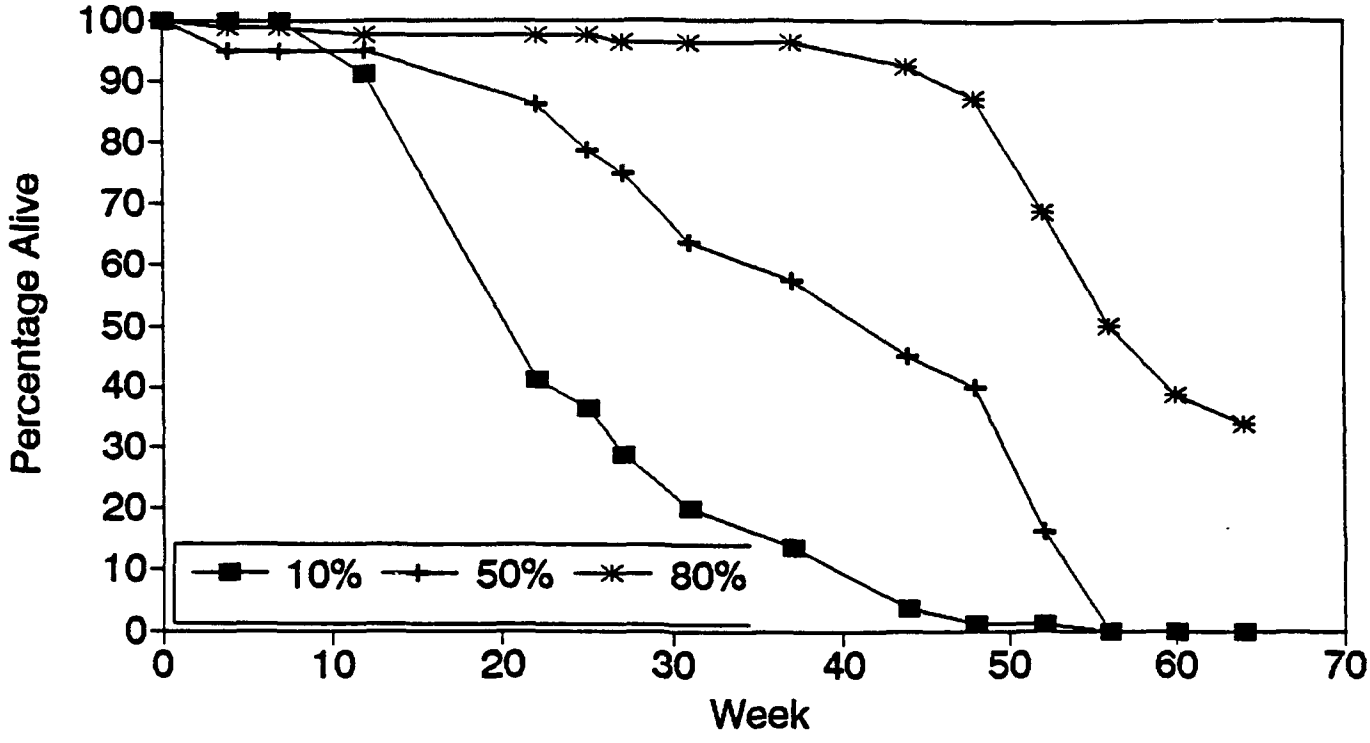


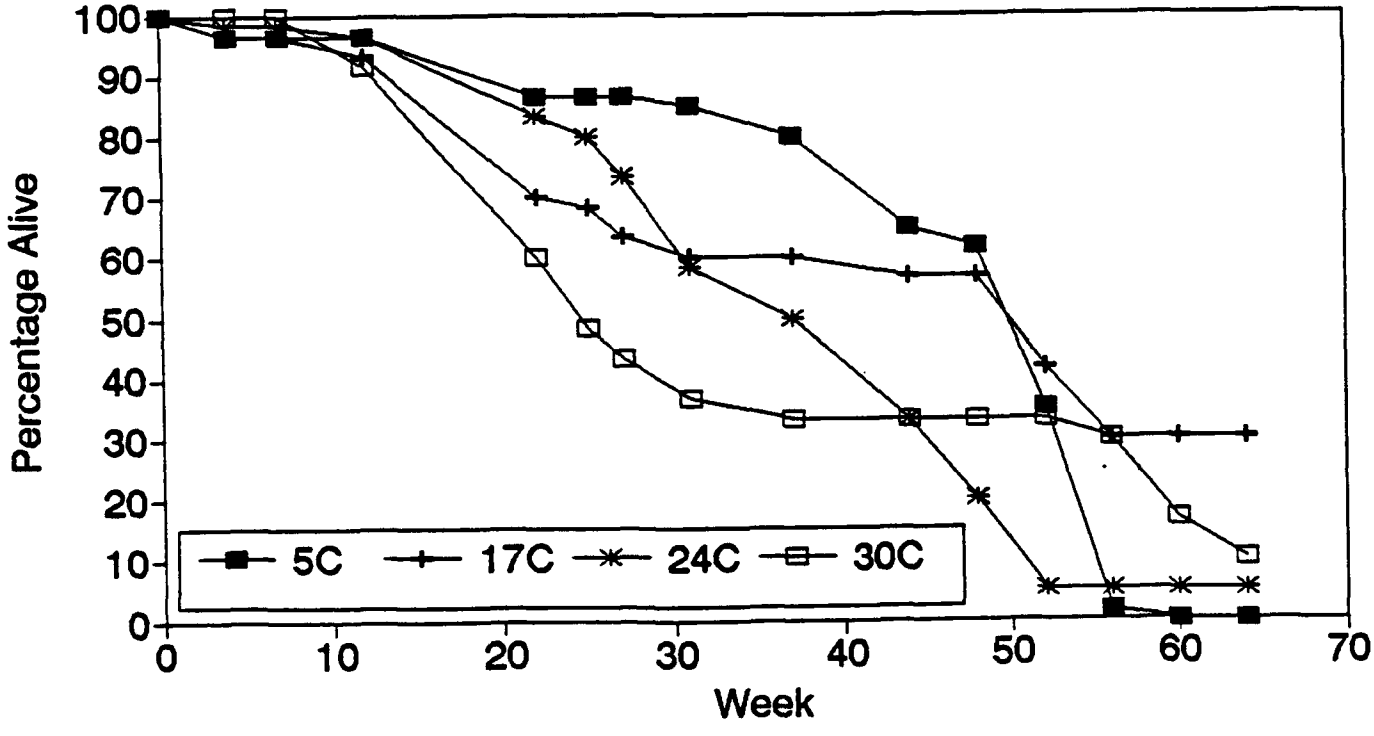
50% Relative Humidity

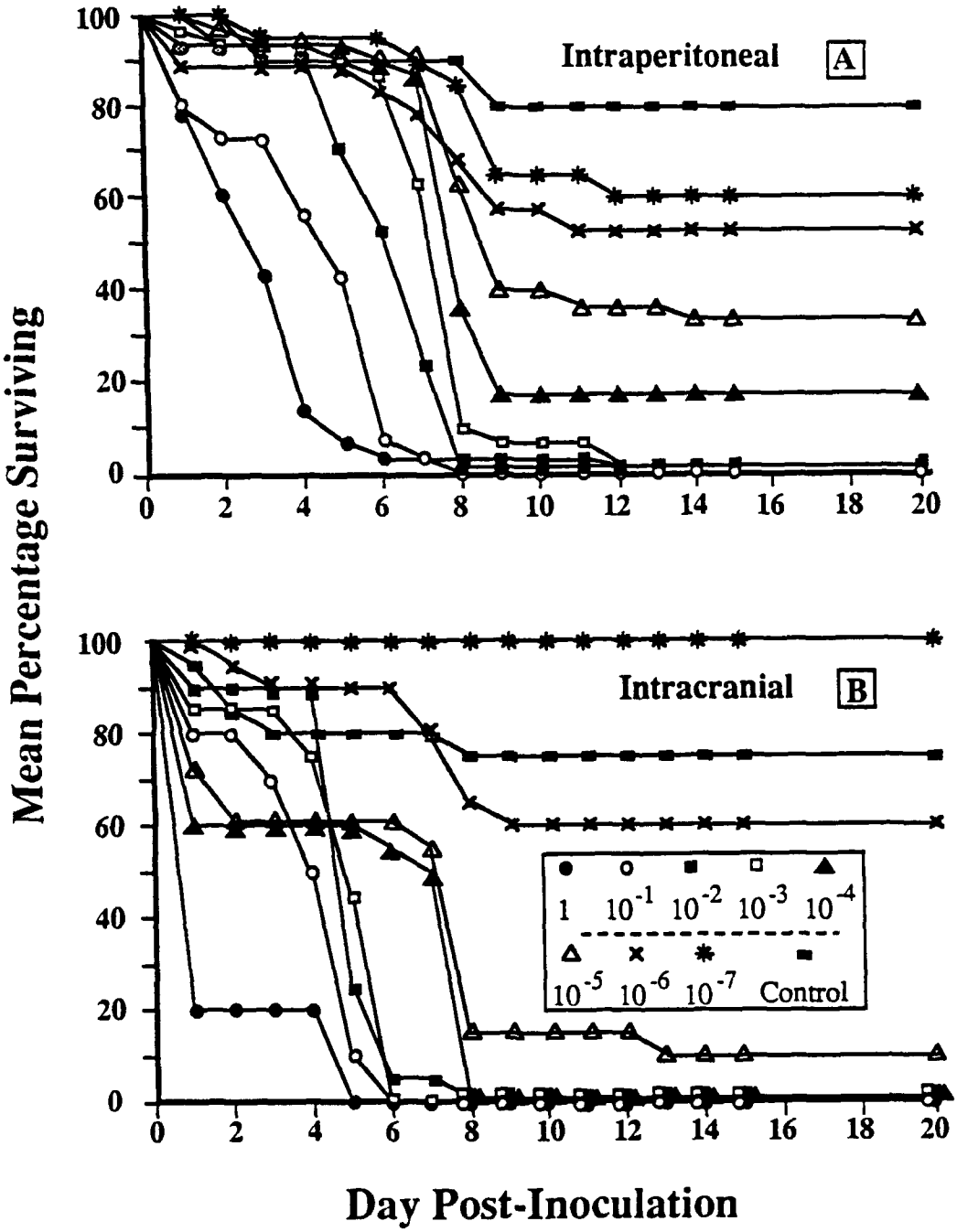


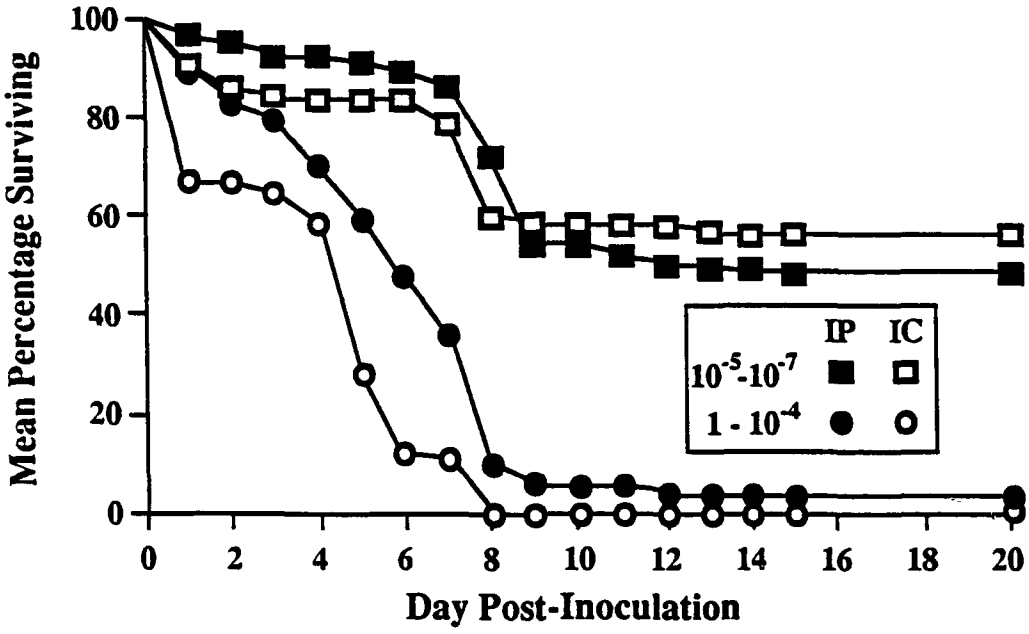
80% Relative Humidity

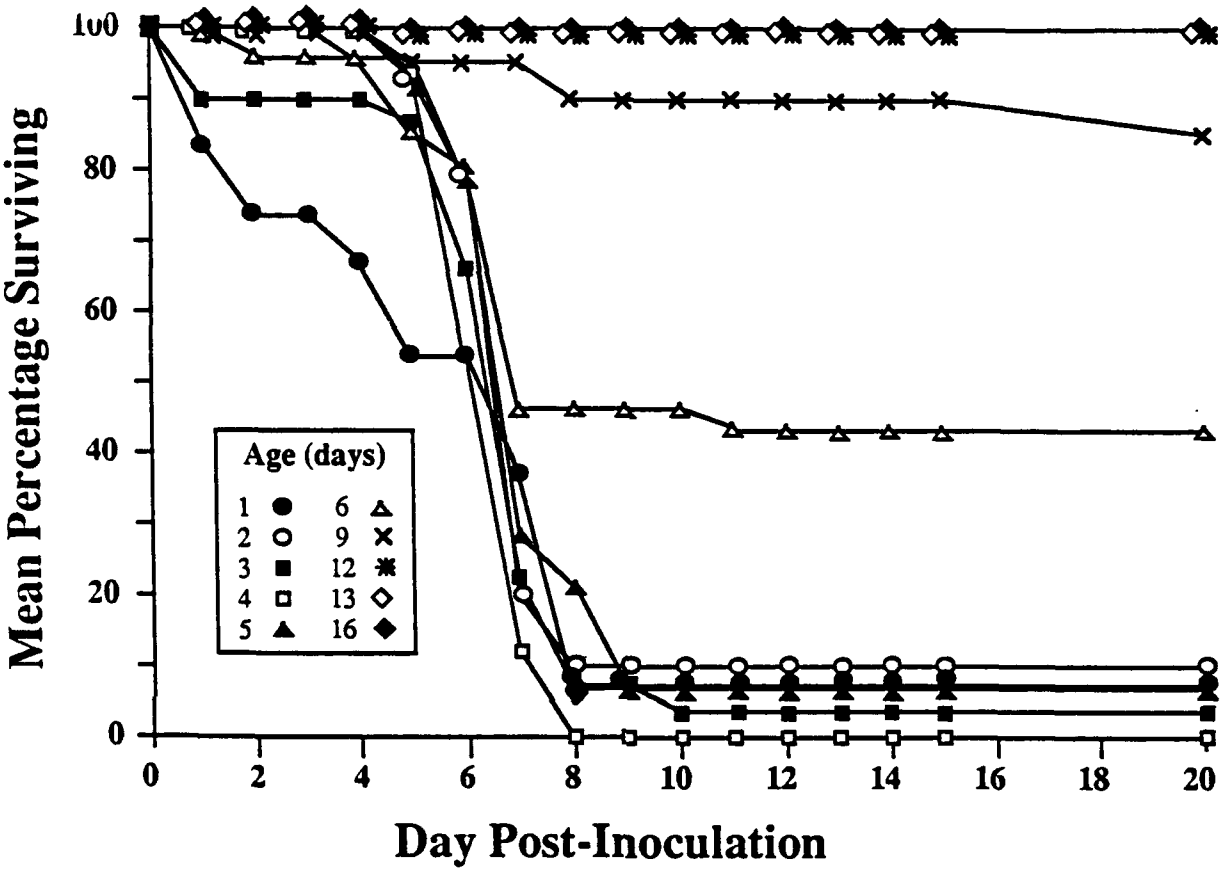


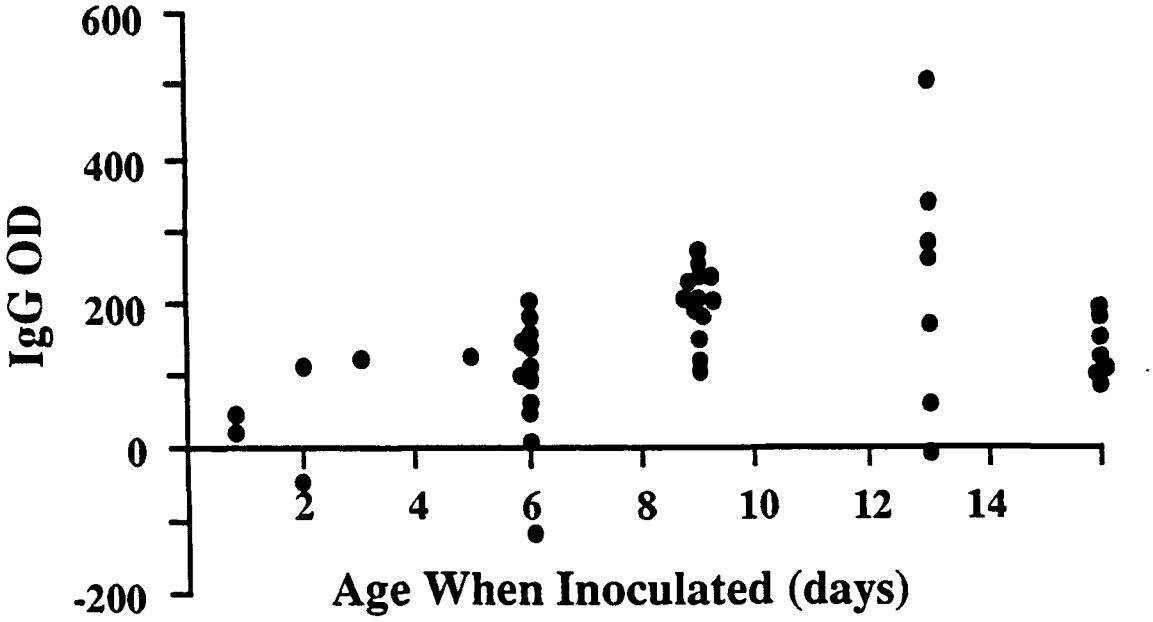

Percentage Alive
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
Week
10%
50%
80%

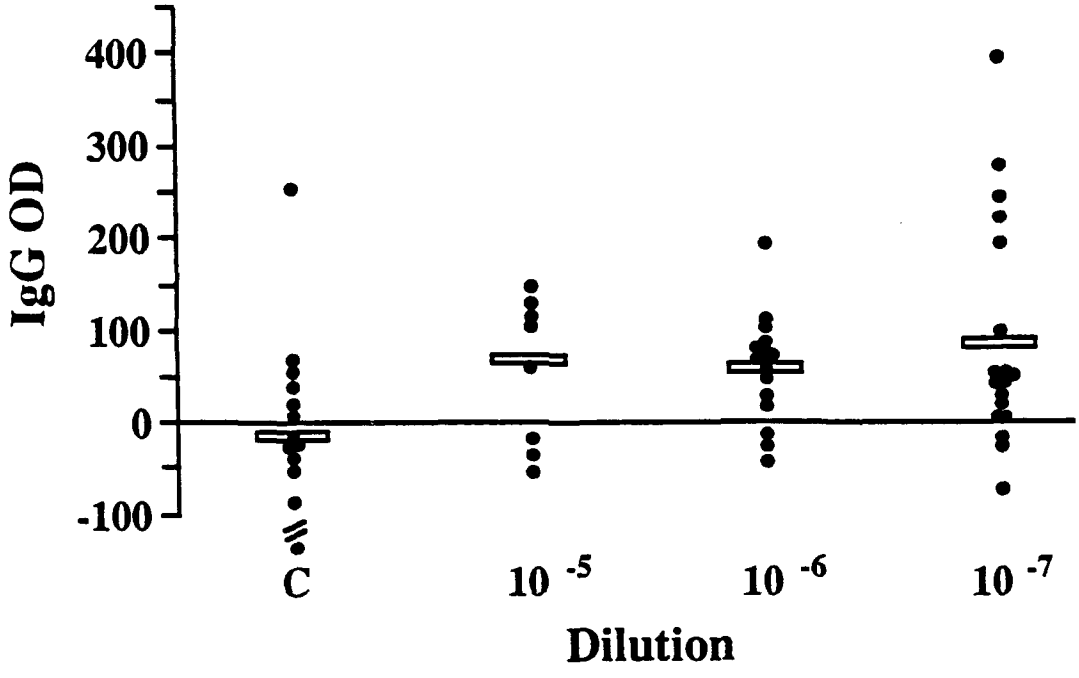

Percentage Alive
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
Week
5C
17C
24C
30C

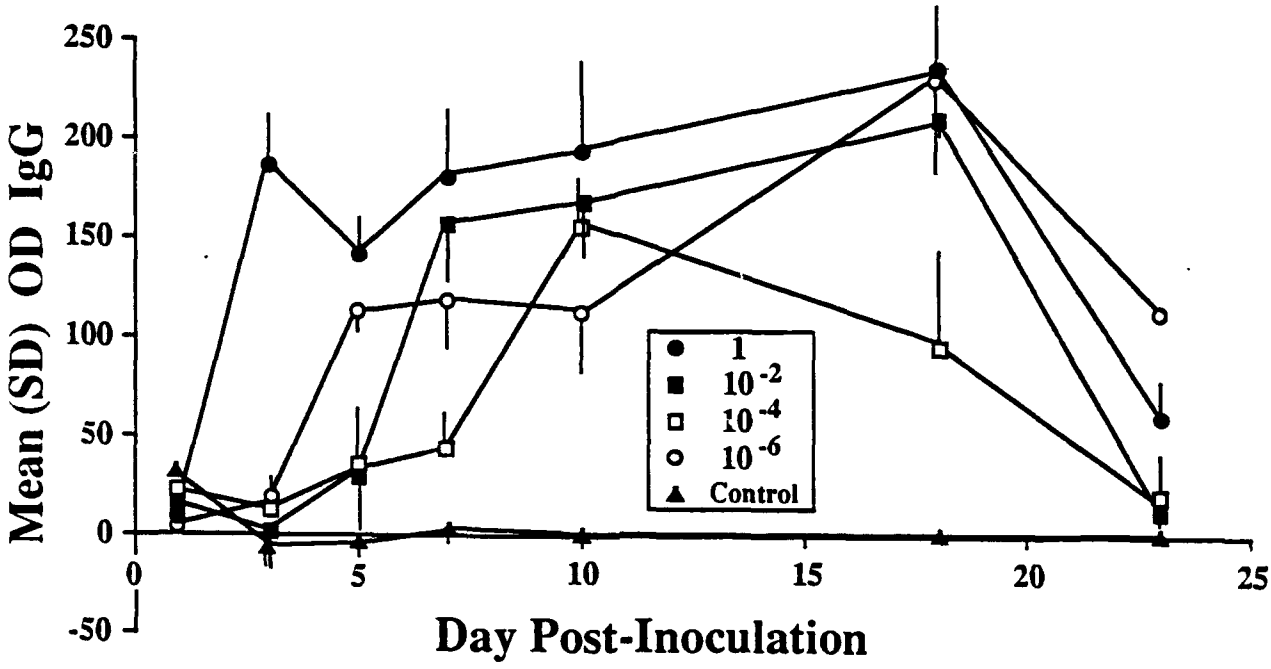

Intraperitoneal
A
Intracranial
B
Mean Percentage Surviving
Day Post-Inoculation
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
1
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
Control


Mean Percentage Surviving
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
Day Post-Inoculation
IP
IC
10^{-5}-10^{-7}
1 - 10^{-4}


Mean Percentage Surviving
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
Day Post-Inoculation
Age (days)
1
2
3
4
5
6
9
12
13
16


600
400
200
0
-200
IgG OD
2
4
6
8
10
12
14
Age When Inoculated (days)


400
300
200
100
0
-100
IgG OD
C
10 -5
10 -6
10 -7
Dilution


Mean (SD) OD IgG
250
200
150
100
50
0
-50
0
5
10
15
20
25
Day Post-Inoculation
1
10 -2
10 -4
10 -6
Control

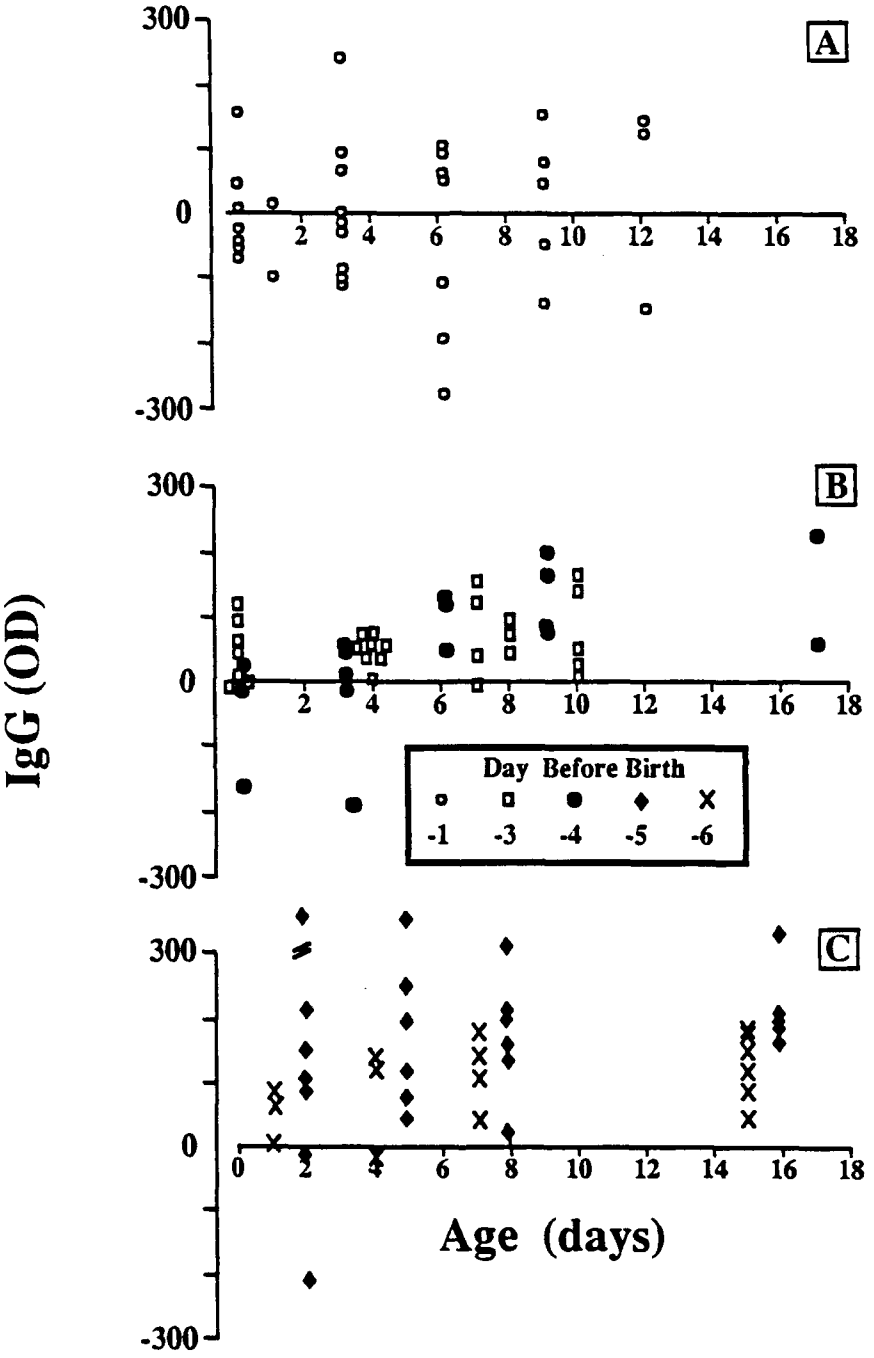

A
B
C
300
0
-300
2
4
6
8
10
12
14
16
18
0
IgG (OD)
Day Before Birth
-1
-3
-4
-5
-6
Age (days)

Table 1. Additional personnel who have participated in the studies presented in this report.

Camicas, Jean-Louis Cornet, Jean-Paul	Laboratoire ORSTOM de Zoologie medicale, Institut Pasteur de Dakar
Gonzalez, Jean-Paul Zeller, Herve LeGuenno, Bernard Diop, Aisha Ndiaye, Magueye Samb, Ibraham Sylla, Rougy	Institut Pasteur, Laboratoire d'Ecologie Virale, Laboratoire d'Epidemiologie des Arboviroses, et Laboratoire de Virologie
Calvo, Marie-Armande Mondo, Mireille	Institut Pasteur, O.M.S Centre de Reference et de Recherche sur les Arbovirus
Adam, Francois Ba, Kalilou Duplantier, Jean-Marc	ORSTOM, Laboratoire de Zoologie
Diop, Mamadou Diouf, Abdoulaye Gueye, Arona Sarr, Antoine Sow, Racine	ISRA, Laboratoire National de l'Elevage et de Recherches Veterinaires
Dykstra, Elizabeth A. Schmidt, Elizabeth A.	Institut Pasteur/U.S. Peace Corps, Senegal
Keyes, Linda E.	Yale University School of Medicine

Table 2. Presentations, reports and publications that have resulted from research under the grant.

PRESENTATIONS and ABSTRACTS - CONGRESSES

Camicas, J.L., Gonzalez, J.P., LeGuenno, B., Cornet, J.P., Digoutte, J.P., Calvo, M.A., Adam, F. and Wilson, M.L. 1989. Recherches menees conjointement par l'USAMRIID, l'IPD et l'ORSTOM sur l'epidemiology de la fievre hemorragique de Crimee-Congo au Senegal et en Mauritanie. *5eme Journee Dakaroise de Parasitologie de la Societe Ouest Africane de Parasitologie*, Dakar, 22 June, 1989.

Camicas, J.L., Cornet, J.P., Gonzalez, J.P., Calvo, M.A., Digoutte, J.P. and Wilson, M.L. 1989. Ecological data on the potential vectors of the CCHF virus in Senegambia and Mauritania and epidemiological implications. *Second Symp. Arboviruses in the Mediterranean Countries*, 24-29 September, Dubrovnik, Yugoslovia.

Chapman, L.E., Wilson, M.L., LeGuenno, B. and Fisher-Hoch, S.P. 1989. Risk factors for Crimean-Congo hemorrhagic fever in rural northern Senegal. *38th Ann. Meet. Am. Soc. Trop. Med. Hygiene*, 10-14 December, Honolulu, p 102.

Gonzalez, J.P., LeGuenno, B., Guillaud, M., Cornet, J.P., Camicas, J.P., Digoutte, J.P and Wilson, M.L. 1989. Virological and serological results from natural and experimental infections by Crimean-Congo Hemorrhagic Fever virus in West Africa. *38th Ann. Meet. Am. Soc. Trop. Med. Hygiene*, 10-14 December, Honolulu, p 235.

Gonzalez, J.P., Wilson, M.L., Cornet, J.P., Zeller, H.G., and Camicas, J.P., 1990. Transmission experimentale du virus de la fievre de Crimee-Congo par les tiques aux moutons touabir et peuhl-peuhl. *6eme Journee Dakaroise de Parasitologie de la Societe Ouest Africane de Parasitologie*, Dakar, 19 June, 1990.

Gonzalez, J.P., Wilson, M.L., Cornet, F. Adam, LeGuenno, B., J.P., Zeller, H.G., and Camicas, J.P., 1990. Developing experimental models of Crimean-Congo Hemorrhagic Fever virus using strains and hosts from West Africa. *VIIIth Interna. Cong. Virol.*, 26-31 August, Berlin.

Gonzalez, J.P., Wilson, M.L., Cornet, J.P., Zeller, H.G., and Camicas, J.P., 1990. Experimental transmission of Crimean-Congo Hemorrhagic Fever virus between ticks and West African vertebrates. *39th Ann. Meet. Am. Soc. Trop. Med. Hygiene*, 4-8 November, New Orleans, p 192.

Ksiazek, T.G., Jouan, A., Meegan, J.M., LeGuenno, B., Wilson, M.L., Peters, C.J., Digoutte, J.P., Guillaud, M., Merzoug, N.O. and Touray, O.I. 1988. Rift Valley Fever among domestic animals in the recent West African outbreak. *37th Ann. Meet. Am. Soc. Trop. Med. Hygiene*, 4-8 December, Washington, D.C., p 162.

Continued.....

Table 2. Continued.

LeGuenno, B., Gonzalez, J.P., Ksiazek, T.G., Camicas, J.L., Cornet, J.P., and Wilson, M.L. 1989. Development of antibody titres in CCHF virus infection: application of immuno-enzymatic techniques to epidemiologic and experimental studies. *Second Symp. Arboviruses in the Mediterranean Countries*, 24-29 September, Dubrovnik, Yugoslovia.

Wilson, M.L., Cornet, J.P., Gonzalez, J.P., LeGuenno, B., Digoutte, J.P., Calvo, M.A. and Camicas, J.L. 1988. Etat des recherches sur l'ecologyie du virus CHF-Congo au Senegal et dans les pays limitrophes. *Group d'Etude Virologie, Institut Pasteur*. 22-23 June, Dakar, Senegal.

Wilson, M.L., Camicas, J.L., Cornet, J.P. 1988. Host associations and seasonal activity of tick vectors of Crimean-Congo Hemorrhagic Fever virus in Senegal. *Proc. XVIII Intern. Cong. Entomol.* Vancouver, B.C., p 282.

Wilson, M.L., Dykstra, E.A., Cornet, J.P., Schmidt, E., Camicas, J.P. and Gonzalez, J.P. 1990. Host-dependent feeding patterns of vector ticks: implications for transmission of Crimean-Congo Hemorrhagic Fever virus in West Africa. *39th Ann. Meet. Am. Soc. Trop. Med. Hyg.* 4-8 November, New Orleans, p 193.

Wilson, M.L., LeGuenno, B., Camicas, J.L., Cornet, J.P., Saluzzo, J.F., Gonzalez, J.P. and Digoutte, J.P. 1988. Crimean-Congo Hemorrhagic Fever in Senegal: Infection rates and epidemiologic associations of tick vectors and vertebrate hosts. *37th Ann. Meet. Am. Soc. Trop. Med. Hyg.* 4-8 December, Washington, D.C., p 77.

Wilson, M.L., LeGuenno, B., Gonzalez, J.P., Camicas, J.P., Cornet, J.P., Guillaud, M., Calvo, M.A., and Digoutte, J.P. 1989. Epidemiology of Crimean-Congo Hemorrhagic Fever in Senegal: temporal and spatial patterns. *2nd Symp. Arbovir Mediter. Count.* 24-29 September, Dubrovnik, Yugoslovia.

Wilson, M.L., LeGuenno, B., Gonzalez, J.P., Cornet, J.P. and Camicas, J.L. 1989. Crimean-Congo Hemorrhagic Fever in Senegal: temporal and spatial patterns of infection related to vector biology. *38th Ann. Meet. Am. Soc. Trop. Med. Hygiene*, 10-14 December, Honolulu, p 174.

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Camicas, J.L., Hervy, J.P., Herve, J.P., Ferrara, L., Hebrard, G., Cornet, J.P. and Wilson, M.L. 1987. Activities du laboratoire ORSTOM de Zoologie medicale. pp. 118-131 IN Digoutte, J.P. (ed) *Rapp. sur la fonct. tech. de l'Institut Pasteur de Dakar*. pp 118-131.

Continued.....

Table 2. Continued

Camicas, J.L., Hervy, J.P., Herve, J.P., Ferrara, L., Cornet, J.P., Hebrard, G., Adam, F., Wilson, M.L. and Gordon, S.W. 1988. Activities du laboratoire ORSTOM de Zoologie medicale. IN Digoutte, J.P. (ed) *Rapp. sur la fonct. tech. de l'Institut Pasteur de Dakar.* pp 122-136.

Camicas, J.L., Hervy, J.P., Ferrara, L., Cornet, Traore-Lamizana, M., Adam, F., Wilson, M.L. and Dykstra, E. 1989. Activities du laboratoire ORSTOM de Zoologie medicale. IN Digoutte, J.P. (ed) *Rapp. sur la fonct. tech. de l'Institut Pasteur de Dakar.* pp 138-159.

Gonzalez, J.P., Guillaud, M. and Wilson, M.L. 1988. Activities du Laboratoire d'Ecology viral. IN Digoutte, J.P. (ed) *Rapp. sur la fonct. tech. de l'Institut Pasteur de Dakar.* pp 100-118.

Gonzalez, J.P., Wilson, M.L., Diop, A. and Fortez, C. 1989. Activities du Laboratoire d'Virology Experimentale. IN Digoutte, J.P. (ed) *Rapp. sur la fonct. tech. de l'Institut Pasteur de Dakar.* pp 111-131.

Gonzalez, J.P., Ndiaye, M., Diop, A. and Wilson, M.L. C. 1989. Activities du Laboratoire d'Ecologie Virale. IN Digoutte, J.P. (ed) *Rapp. sur la fonct. tech. de l'Institut Pasteur de Dakar.* pp 100-110.

Leguenno, B. 1988. Activities du Laboratoire du Virologie medicale. IN Digoutte, J.P. (ed) *Rapp. sur la fonct. tech. de l'Institut Pasteur de Dakar.*

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Table 2. Continued

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Table 3. Birds examined monthly at Yonofere, Senegal during January through November, 1990 and immature Hyalomma m. rufipes found parasitizing them.

Bird (Species) or Tick	Month J	F	M	A	M	J	J	N	J-N
Double-spurred Francolin *Francolinus bicalcaratus*	1	1	3						5
Stone-Partridge *Ptilopachus petrosus*	3								3
Grey-Breasted Helmet Guinea Fowl *Numida meleagris*	4				3				7
Senegal Bustard *Eupodotis senegalensis*			3			2			5
Black-bellied Bustard *Eupodotis melanogaster*	2								2
Laughing Dove *Streptopelia senegalensis*	9	3	1	2	2	2		3	22
Vinaceous Dove *Streptopelia vinacea*						2		1	3
Long-tailed Dove *Onea capensis*								3	3
Chestnut-bellied Sand-grouse *Pterocles exustus*	2	6		15	2				25
Red-beaked Hornbill *Tockus erythrorhynchus*					1	3			4
Chestnut-bellied Starling *Spreo pulcher*	9			5					14
Yellow-fronted Canary *Serinus mozambicus*				1					1
Unidentified Weavers *Ploceus spp.*	2	2			1				5
Scaly-fronted Weaver *Sporopipes frontalis*	3						1	5	9
Grey-headed Sparrow *Passer griseus*	5	3		5	10	2		19	44
Golden Sparrow *Passer luteus*			34					22	56
Cut-throat Weaver *Amandina fasciata*	1							1	2
Warbling Silverbill *Lonchura malabarica*	3	18							21
Senegal Fire-Finch *Lagonosticta senegala*	12	1		6		4	1		24
TOTAL BIRDS EXAMINED	57	37	41	34	20	15	2	54	260
No. Birds Parasitized	3	0	0	0	0	0	0	3	6
No. H. m. rufipes Larva	1	0	0	0	0	0	0	3	4
Nymph	6	0	0	0	0	0	0	3	9

Table 4. Monthly observations of immature Ixodid ticks found on small mammals that were examined during 1990 in Yonofere, Senegal.

Mammal Species[1]	Tick Sp.[2]	Stage	Mean No. ticks on (N) mammals during: F	M	A	M	J	J	A	S	O	N	1990
Mastomys sp.	H. trun.	L	-	-	-	-	-	-	0	-	0	-	0
		N	-	-	-	-	-	-	0	-	0	-	0
			(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(2)	(0)	(3)
Taterillus sp.[3]	H. trun.	L	0	0	0	0	-	-	-	0	-	0	0
		N	0	0	0	0	-	-	-	0	-	0	0
			(1)	(1)	(2)	(1)	(0)	(0)	(0)	(1)	(0)	(2)	(8)
Arvicanthus, sp.	H. trun.	L	0	-	-	0	-	0	-	-	0	0	0
		N	0	-	-	0	-	0	-	-	0	0	0
			(3)	(0)	(0)	(9)	(0)	(6)	(0)	(0)	(5)	(5)	(28)
Erinaceus albiventris	H. trun.	L	-	0	0	0	0	0	-	-	-	-	0
		N	-	1.0	0	0	0	0.2	-	-	-	-	1.2
	H. ruf.	L	-	0	0	0	0	0	-	-	-	-	0
		N	-	0	0	0	0	0	-	-	-	-	0
	R. guil.	A	-	0	0	0	0	0.2	-	-	-	-	0.2
			(0)	(3)	(6)	(3)	(3)	(5)	(0)	(0)	(0)	(0)	(21)
Lepus whytei	H. trun.	L	0	0	-	-	-	-	-	-	-	-	0
		N	1.5	52.0	-	-	-	-	-	-	-	-	26.8
	H. ruf.	L	0	0	-	-	-	-	-	-	-	-	0
		N	0	0	-	-	-	-	-	-	-	-	0
	R. guil.	A	0	0	-	-	-	-	-	-	-	-	0
			(2)	(2)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(4)
TOTAL	H. trun.	L	0	0	0	0	0	0	-	-	0	0	0
		N	0.5	17.3	0	0	0	0.1	-	-	0	0	1.2
	H. ruf.	L	0	0	0	0	0	0	-	-	0	0	0
		N	0	0	0	0	0	0	-	-	0	0	0
	R. guil.	A	0	0	0	0	0	0.1	-	-	0	0	<0.1
			(6)	(6)	(8)	(15)	(3)	(11)	(1)	(1)	(7)	(7)	(88)

1. In addition to those species listed the following were also examined: January, 1 Canus adustus parasitized by many Ripicephalus sanguineus; May, 1 Xerus erythropus unparasitized; March, 1 Erinaceus albiventris parasitized by Haemaphysalis spinosa.
2. Tick species are Hyalomma truncatum, H. marginatum rufipes and Ripicephalus guilhoni.
3. The species within the genus Taterillus are visually indistinguishable. Both T. pygargus and T. gracilus are encountered at this site.

Table 5. Number of ticks pooled and tested for the presence of CCHF virus from 3 sites in Senegal during 1990.

Tick Species[1]	No. ticks (pools) tested from: Yonofere		Dahra		Bandia		TOTAL	
H. truncatum	3551	(216)	251	(30)	120	(32)	3922	(278)
H. m. rufipes	258	(58)	17	(7)	183	(61)	348	(126)
H. impeltatum	11	(2)	67	(17)	0	-	78	(19)
H. impressum	0	-	0	-	1	(1)	1	(1)
R. guilhoni	2379	(154)	195	(24)	507	(123)	3081	(301)
R. e. evertsi	399	(36)	131	(16)	127	(25)	657	(77)
R. sanguineus	0	-	0	-	47	(19)	47	(19)
A. variegatum	0	-	0	-	1058	(178)	1058	(178)
B. decoloratus	0	-	0	-	192	(75)	192	(75)
Total	6598	(466)	661	(94)	2235	(514)	9494	(1074)

1. Genera are Hyalomma, Ripicephalus, Amblyomma, Boopholus.

Table 6. Transmission of CCHF virus between adult Hyalomma truncatum males that were hypostomectomised and females with gonopores occluded.

Type of Transmission	Tick Treatment: Hypostomectomy (Male)	Tick Treatment: Gonopore occlusion (Female)	No. virus isolates (No. tested): Males	Females	F1 Eggs[a]	F1 Larvae[a]	F1 Nymphs[b]	Rabbit IgG from Male Pre-feeding	Rabbit IgG from Male+Female Feeding
Preinfected male									
Mating	Yes	No	2 (3)[c]	4 (6)	2 (4)[d] 0 (2)[e]	1 (6)[d] 0 (4)[e]	0 (15)[d] none	+	+
Cofeeding	No	Yes	3 (3)	3 (3)	-	-	-	+	−
Control	Yes	Yes	3 (3)	0 (3)	-	-	-	+	−
Preinfected female									
Control	Yes	Yes	0 (3)	3 (3)	-	-	-	−	+
Uninfected Ticks									
Control	No	Yes	0 (3)	0 (3)	-	-	-	−	−

a. Each observation pooled from the eggs of one female.
b. Nymphs from positive pools of larvae.
c. A fourth tick that was dead tested negative.
d. Pools from previous stage that tested positive.
e. Pools from previous stage that tested negative.