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

Annual Report

Mark L. Wilson and Jean-Pierre Digoutte

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SUMMARY

The factors that influence the spatial and temporal distribution of Crimean-Congo Hemorrhagic Fever (CCHF), a life-threatening tick-borne viral zoonosis, remain speculative. Human disease or enzootic transmission occur in 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. Various species of wild mammals exhibit CCHF virus antibodies, yet the role of such vertebrates in horizontal transmission or amplification of the virus remains undefined.

During the third year of a project to study the factors that contribute to transmission of CCHF virus in Senegal, numerous observations and experiments were undertaken on the biology of vector ticks, their capacity to transmit the virus, and vertebrate responses to infection. We continued to monitor changes in the densities of the most prominent vertebrates and ticks indigenous to our prospective study sites in northern Senegal. Prospective longitudinal analysis of the tick and vertebrate fauna at Dahra, Yonofere, and Bandia continued throughout the year. Numerous tick species are under study, most notably *H. truncatum*, *H. marginatum rufipes* and *H. impeltatum*, all potentially important vectors of CCHF virus. Immature ticks and serum samples are being taken from birds, rodents (e.g. *Mastomys*, *Arvicanthis* spp.) and other small mammals (e.g. hedgehogs and hares) all of which are considered candidate reservoirs. Domestic ungulates are being sampled regularly in studies of adult tick seasonal activity, density and host associations; more than 2,500 cattle and sheep have been examined during 1989.

Research on infection in vertebrates is designed to estimate risk, define temporal and spatial distribution of transmission, and clarify their role in the natural cycle of CCHF virus transmission. Field studies of the incidence of infection among individually identified ungulates demonstrated epizootic during March-July 1988 that corresponded temporally with increased abundance of *Hyalomma* ticks (Wilson et al. 1990a). We continued to monitor that site during this year. To complement these field observations, laboratory studies of infection in sheep, rodents, and certain birds were begun. The development of antibodies and viremia following inoculation with CCHF virus showed that numerous vertebrates can be infected. Studies with adult ticks feeding on infected sheep demonstrated horizontal transmission. Similarly, ticks inoculated intracoeleomically infected sheep on which they fed.

Studies of human infection included a questionnaire-based serological study of risk factors associated with past infection of CCHF virus. Overall, 13.0% of 284 persons studied showed IgG, with no difference between males and females. Prevalence increased with age. Risk of infection in men was associated with the frequency of reported bites by male *H. truncatum* and with treating sick livestock.

Virus isolation attempts from more than 11,000 ticks sampled from domestic animals at our permanent study sites has yielded 2 more strains of CCHF virus from pools of *H. truncatum*.

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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INTRODUCTION

The zoonosis known as Crimean-Congo Hemorrhagic Fever (CCHF), is one of a group of arthropod-borne viral diseases producing acute, sometimes fatal febrile and hemorrhagic symptoms. Initially involving the nervous system, disease may in severe cases progress to vascular disorders such as profuse diapedetic hemorrhages, brain edema, general malaise, and ultimately cardiac arrest. Human disease was first recognized from 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). Crimean-Congo Hemorrhagic Fever virus, family Bunyaviridae, genus Nairovirus, was later found to be identical to that of "Congo virus" from Africa (Casals, 1969).

The recognized distribution of CCHF includes 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. 1985(a), Gonzalez et al. 1990). An initial serosurvey by Chunikhin et al. (1969(a)) first demonstrated evidence of infection in domestic animal of Senegal. Later, ticks from a Dakar, Senegal abattoir were studied and numerous strains of CCHF virus were isolated (Robin & Le Gonidec 1972, Robin 1972, 1973, 1974, 1975). Similar recent observations of human and domestic animal sera identified other foci of transmission in various regions of Senegal and along the border with Mauritania. (Saluzzo et al. 1984, 1985(a), 1985(b), 1986, Camicas et al. 1986). Thus, CCHF virus transmission has existed in this region for at least 2 decades; whether these foci persist with a low level of enzootic transmission, periodically erupt in epizootics, or are regularly reestablished by migrating reservoirs remains unknown.

Of the more than 30 species of Ixodid ticks have been shown to be capable of supporting infection by CCHF virus (Camicas et al. 1990) it seems likely that only a few species are important in maintaining the transmission cycle in nature. Many epidemiological reports implicate certain *Hyalomma* species that vary according to geographic region. In particular, *H. m. rufipes* is often associated with intense transmission in western Africa (Hoogstraal 1979). This association, however, is based upon sparse evidence of naturally acquired tick infection, combined with information on ecological associations among adult ticks. CCHF virus has also been isolated from *H. truncatum*, and *H. impeltatum* two species equally abundant in our region. While the hosts and population dynamics of these ticks have been studied in Senegal (e.g. Camicas et al. 1986, Gueye et al. 1986, 1990), surprisingly little is known about the ecology, population dynamics and host-associations of the immature stages of any

of the *Hyalomma* species. The vertebrates that might serve as maintenance reservoirs of the virus are unknown, although circumstantial evidence suggests certain species. The importance of transovarial transmission of the virus between tick generations in nature, relative to that of horizontal transmission, is also unknown. Thus, the manner in which the numerous individual components of the natural cycle of CCHF virus interact is poorly understood.

The long-term objectives of our project, in general, remained essentially unchanged during the third year of the project: to investigate those variables that are ill-defined and probably important in the transmission cycle of CCHF virus, and to integrate these new observations with existing knowledge in an attempt to develop a complex model capable of describing the enzootic cycle and the epidemiology of human disease. Particular studies, notably those involving experimental transmission, have become increasingly important in this endeavor. The report summarizes accomplishments during this third year of study.

Objectives

The principal objectives for 1989 included:

1. Continuation of longitudinal field observations of tick-host associations and seasonal patterns of tick abundance.
2. Systematic serosurveillance of human and 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 and laboratory observations of infection using natural hosts.
4. Analysis of feeding patterns of various suspected vector ticks on natural and laboratory hosts.

The studies described in this document are being undertaken by a team of investigators comprised of scientists and technicians from numerous institutions. The effort is one of collaboration, involving ecologists, entomologists, immunologists, epidemiologists, and virologists from a variety of different organizations (Table 1). Numerous presentations at scientific congresses, reports, and publications have resulted from research under the grant. The most significant of such products during 1988-1989 are noted in Table 2.

Study Sites

Field sites for prospective studies were designated from information of previous research undertaken at the Institut Pasteur (Camicas et al. 1986, Saluzzo et al. 1985(a) and unpublished data). The sites have remain unchanged. Details of the three 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, a dry "thorn-brush" habitat dominated by grasses and widely dispersed trees, particularly *Acacia* spp. (Barral 1982). Rainfall occurs principally during July through September and may vary considerably from year to year (Fig. 2), averaging about 400 mm. annually (Fig. 2). 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 (designated Dahra-village).

Yonofere is a small village of roughly 900 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. 1970) 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, 1989 through 31 December, 1989. Certain data is still being analyzed, and these results are summarized rather than presented in a final form. Results are organized by topical questions divided into three sections: I. Tick Ecology and Behavior, II. Vertebrate-Virus Interactions, and III. Virus Transmission.

1. TICK ECOLOGY and BEHAVIOR

Prospective observations were continued to determine which species of tick(s) and vertebrate(s) are important in CCHF virus transmission. The agent of CCHF is unusual among zoonotic arboviruses: the number and ecological diversity of potential vectors and vertebrate hosts with which it is associated is enormous. In addition it is found in a variety of faunal regions throughout the world. Previous studies have implicated various 2-host or 3-host African ticks, either by inference or evidence of infection (reviewed by Hoogstraal 1979). In Senegal, at least 8 such ticks are found (Camicas et al., 1990), including 5 species of the genus *Hyalomma*, two species of *Rhipicephalus* and *Amblyomma variegatum*. Each of these ticks are being studied at our 3 permanent sites. In addition, laboratory studies of the feeding behavior of immature ticks have been undertaken.

Adult Tick Seasonal Activity

The temporal, spatial and ecological patterns of activity and population density of these ticks and their hosts, is being characterized by observations at the 3 major study sites. This work is being undertaken in collaboration with Drs. Jean-Paul Cornet and Jean-Louis Camicas.

Random selection of Sheep. A herd of sheep is chosen by chance encounter, in Yonofere and Dahra-village, 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 to the present. More than 3,200 sheep have been examined thus far. From these animals more than 15,000 adult *H. truncatum*, *H. impeltatum*, *H. marginatum rufipes*, *H. dromedarii*, *Rhipicephalus evertsi evertsi*, and *R. guilhoni* have been sampled. Three species dominate: *H. truncatum*, *H. impeltatum*, and *R. guilhoni*.

The abundance of all adult ticks during 1989 was extremely low as compared with previous years. The results for *H. truncatum* and *H. impeltatum* on Dahra-CRZ sheep (Fig. 3) are similar to those obtained in Yonofere. A seasonal pattern, therefor, was indiscernable for these two species. The abundance of *Rhipicephalus guilhoni* was also relatively low, though a distinct temporal pattern similar to previous years appeared: maximal activity of adults occurs during and just after the rainy season. These results correspond with those from other sites, and are consistent with a well-defined and limited period of adult activity for other species of the genus. Although the role of *R. guilhoni* as a potential vector of CCHF virus has not been investigated, we isolated a strain

in 1988 from specimens of this tick taken from sheep in Yonofere (Camicas et al. 1990).

Sentinel Animals. Additional observations are being gathered on adult tick abundance from privately-owned sheep and cattle in Yonofere and Bandia. These animals have been tagged but are maintained as part of their original herd. About 40 sheep, 12 goats and 2 cattle in Bandia and nearly 200 sheep in Yonofere are being studied at regular intervals. Such observations permit comparisons with a somewhat different ecological regions (Fig. 1). Repeated observations of individual animals allow us to consider differences in infestation rates. Here again, however, adult tick abundance was unusually low, making analyses of seasonal patterns of activity difficult. Nevertheless, such observations of differences among years contribute to our understanding of the long-term temporal dynamics of CCHF virus transmission (see Section III) and to the focal nature of epizootics.

Larval and Nymphal Population Ecology

Studies of activity patterns and hosts associations of immature stages of most ixodid ticks focus on vertebrates different than those for adults. 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. Investigations of the host associations and population dynamics of the immature stages of these potential vectors attached to birds and small mammals at have been continued during 1989 at our long-term field sites.

Immature ticks on birds. In order to determine the role of birds as hosts to larval and nymphal ticks, as well as to characterize seasonal activity and densities of the ectoparasite, we have continued monthly samples of birds at Yonofere. Birds are shot and are trapped using Japanese mist-nets, a locally constructed ground net. Each bird is carefully examined by blowing air through a tube to separate the feathers and view the skin. Attached larvae and nymphs are placed into live vials until they have molted (thereby facilitating identification). Organs and blood of killed birds are stored at -70°C. for later study.

A total of 543 birds were examined at Yonofere during 1989, of which 42 (7.7%) harbored 126 ticks (Table 3). Most ticks were immature *H. m. rufipes* (47 larvae, 51 nymphs), and there were 8 larval *H. truncatum*. The majority of ticks were recovered during a few months at the end of and following the rainy season; variation in number and species of birds examined throughout the year prevents us from making statement about seasonality. A total of 8 species of birds were found infested on at least one occasion including: 4/28 Red-beaked Hornbills (*Tockus erythrorhynchus*), 1/3 Vitelline Masked Weavers (*Ploceus velatus*), 1/1 Village Weavers (*Ploceus*

cucullatus) and 7/62 Grey-headed Sparrows (*Passer griseus*) 1/128 Golden Sparrows (*Passer luteus*). The majority of ticks removed from these birds were immature *H. marginatum rufipes*. In addition, 1 *Tockus erythrorhynchus* was by an adult *Rhipicephalus guilhoni*, 1 *Francolinus albobularis* was found with 7 larval *Argus streptopelia* and 4 *Passer luteus* were parasitized by 17 immature *A. arboreus*. As before, the temporal pattern of these observations makes statements about the importance of particular species difficult. However, nearly all observations of immature *H. marginatum rufipes* occurred at the end of the rainy season. In general, ground feeding birds appear to be more often infested.

Immature ticks on small mammals. The importance of small mammals as hosts to immature *Hyalomma* ticks is becoming increasingly evident. Studies that complement those on birds suggest that individual hosts generally are more heavily infested. At Yonofere and Bandia, modified Manufrance live-capture traps, baited with peanutbutter, are placed 10 meters apart in lines located near suitable habitat. About 120 and 140 trapnights per month produce rodents at Yonofere and Bandia, respectively. Small mammals are carefully inspected for ectoparasites by blowing against the fur to view the skin surface. Attached ticks are removed with fine forceps and kept alive as described for birds; alternatively, small mammals are held for 7 days in cages over water where engorged ticks that detach are recovered.

Rodent abundance at Yonofere during 1990 was very low (Table 4), making any estimates of tick attachment rates difficult. Conversely, numerous hares (*Lepus whytei*) and hedgehogs (*Erinaceus albiventris*) were examined: these hosts were often parasitized and occasionally with many immature ticks, especially *H. truncatum*. In addition, *H. marginatum rufipes* immatures and *Rhipicephalus guilhoni* adults were captured. No evidence of seasonal variation was discernable.

A similar trapping effort at Bandia produced numerous observations of *Mastomys erythroleucus* and *Arvicanthis niloticus*, however few immature *H. truncatum* were recovered (Table 5). Numerous larval and nymphal *R. guilhoni* were found on these rodents, and a predominance of activity at the end of the rainy season were evident.

Feeding Patterns of Immature Ticks

The response of immature ticks feeding on their vertebrate hosts may play an important role in horizontal transmission of CCHF virus. Successful engorgement is necessary for refeeding during the next stage which is required for the maintenance of transmission if such is not entirely transovarial. Specifically, the diurnal dropoff rhythms, extent of engorgement, and stage of detachment each will influence how many potentially infectious blood meals may

occur. In extreme cases as with most *Boophilus* ticks, larvae, nymphs and adults feed on the same individual animal, thereby preventing horizontal transmission. We have undertaken a series of laboratory observations on natural and artificial hosts designed to address these questions.

Results from our studies during 1989 indicate that the drop-off rhythms of larval *Hyalomma truncatum* and *H. impeltatum* feeding on various hosts (including rabbits, chickens, guinea pigs and wild *Mastomys* spp.) occurs mainly during daylight, principally between 48 and 96 hours after attachment. Thus, nocturnal vertebrates would be in burrows or nests during peak detachment of engorged larvae, suggesting that newly molted adult ticks might emerge from such protected sites. (However, the excavation of more than 100 rodent burrows during 1987-88 produced only a few Ixodid ticks). Preliminary studies of other hosts to immature stages are underway. Drop-off of adult *H. truncatum* from sheep, however, showed no such clear pattern.

Other tick feeding studies have produced preliminary results suggesting that the feeding pattern of *H. truncatum* larvae is determined by the vertebrate from which it takes its blood meal. More than 95% of larval *H. truncatum* detach as engorged larvae (typical "3-host" pattern) when fed on certain hosts, about half as engorged larvae and half as engorged nymphs from other hosts, and nearly all larvae detach as engorged nymphs ("2-host" pattern) on still different vertebrate hosts. The physiological or behavioral mechanisms by which the host species influences the engorgement pattern of immature *H. truncatum* remains unknown.

Our studies suggest that both "2-host" and "3-host" patterns exist in nature, although this varies with tick and host species (unpublished). Similarly, potential vector ticks feed predominately on certain host species, a pattern that varies with tick stage, as well as with tick species and, with host availability. Preliminary results also suggest that the proportion of larval *H. truncatum* responding as 2- vs. 3-host feeders changes with the history of previous infestation of the host. Thus, there appears to be a complex interplay between tick and host that influences feeding associations, duration and success. The impact on vectorial capacity in enzootic horizontal transmission, and for zoonotic transmission to humans is potentially enormous.

II. VERTEBRATE-VIRUS INTERACTIONS

The variables comprising vertebrate responses to CCHF virus infection contribute to the transmission dynamics of this virus in a complex manner. Experimental studies in this domain have represented an increasingly important component of our research program. Although numerous other variables, such as the population densities of hosts, influence the significance of a given level of tick infestation, host inhibition of vector feeding or pathogenic effects on host and vector demography also might influence the interactions that ultimately determine the incidence of transmission. Similarly, tick infestations of vertebrates that do not develop high viremias may be of little consequence to the maintenance of CCHF virus transmission. Thus, various studies of CCHF virus infection in certain vertebrates considered likely natural reservoirs of the virus have been undertaken.

Responses to Experimental Inoculation

Under the direction of Dr. Jean-Paul Gonzalez, experimental infections of vertebrates have been undertaken to study the virological and serological responses of these animals. Our studies have focused principally on vertebrate species that we believe likely to be reservoirs in nature. Thus, rodents, hedgehogs, birds and sheep have been infected in an attempt to describe their serological and virological responses. In addition, infection of laboratory animals has helped us to interpret these observations.

Antibody and Antigen Detection and Evaluation. Sera have been 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 (strains IbAr 10200 from Sokoto, Nigeria and the recently isolated Dak H49119 from a human in Rosso, Mauritania) 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, Compiègne, 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 Laboratories, Irvine, Scotland) coupled to a microcomputer. By iterations of the distribution of OD values, we determined the mean of the population of negatives. Sera were considered positive if the OD was greater than three standard deviations above the mean of negatives.

IgM antibodies were detected by immunocapture ELISA (Saluzzo and LeGuanno, 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 LeGuanno, 1987) was also 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. IgM antibodies were detected by immunocapture ELISA (Saluzzo and LeGuanno, 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.

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.

Numerous native and domestic vertebrates have been tested, including the rodents *Mastomys erythroleucus* and *Arvicanthis niloticus*, the hare *Lepus whytei*, domestic chickens and wild helmeted guinea fowls *Numida meleagris*. Serologic and virologic responses of most vertebrates were similar: antibodies were detected during the first week post-inoculation and IgG remained elevated for months or years. Viremia was detected during a brief period, usually at 4-8 days post-inoculation. Laboratory mice and rabbits exhibited this response (Fig. 4) as did sheep (Fig. 5). In addition, the body temperature of sheep rose and slowly and descended in concert with viremia (Fig. 5). IgM titers in sheep rose rapidly after primary infection and remained detectable up to 2 months later (Fig. 6a). In addition, the ELISA DO value of these antibodies corresponded to the calculated titer (Fig. 6b). Responses of chickens was less clear, though a detectable antibody signal emerged early after inoculation. A similar response was found for Guinea Fowl (Fig. 7).

Natural Prevalence of Infection in Vertebrates

Prospective studies of the natural prevalence of CCHF virus infection in domestic animals have been continued. These observations are designed to examine the temporal distribution of transmission as well as the vertebrate species that are likely to be important in the transmission cycle. Blood and tissue samples are being obtained at all three main study sites in northern Senegal. Blood samples are taken from domestic ungulates (primarily sheep, goats and cattle) as well as from birds, small mammals, dogs and other vertebrates. Blood is allowed to clot and then held at +5-10°C for 1-4 days after which the serum is removed and stored at -70°C.

Prevalence of Infection among Sheep and Goats

Individually-identified, privately-owned sheep in Yonofere, as well as sheep and goats at the Dahra-CRZ are periodically bled to determine the prevalence of IgG antibodies. Numbered eartags have been placed on nearly 200 sheep in 4 herds from Yonofere that are sampled every 2 months. In addition, we are monitoring the antibody status of more than 300 sheep and goats every three months at the Dahra CRZ. Domestic animals are being bled to estimate the local prevalence of infection and to correlate this with variation in tick abundance and other variables. During 1989, samples from 215 cattle and 1,408 sheep or goats from Dahra were tested. Among 20 - 30 cattle sampled monthly, IgG antibody prevalence remained in the range of from 4% - 15% , depending on the group sampled; very little IgM was apparent. These results are similar to those we 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 and Digoutte, 1989); slight differences were observed among the sample periods, with prevalence varying between 29.5% and 41.0% as births and deaths changed the sample population. Few new infections were observed. The prevalence of antibody among individually-identified sheep at Yonofere has remained similarly low during 1989.

To describe the frequency of new CCHF virus infection acquired under natural conditions, and ultimately relate this to the seasonal dynamics of various *Hyalomma* species, we have continued to monitor these animals and to identify individual seroconversions. At Dahra, where an epizootic was followed during 1988, very few new seroconversions were found during 1989. There, the prevalence of IgM among sheep remained at less than 4% (Fig. 3) an observations that is consistent with the generally diminished density of hard ticks found on these animals. Although a higher prevalence of new infections among sheep was seen in Bandia, small sample sizes prevent extensive

speculation. Only 2 seroconversions (<1% of sheep sampled) sero-converted in Yonofere during the 1989. This two is consistent with generally diminished densities of adult ticks on these ungulates.

Risk Factors in Human Infection

The variables that contribute to human risk of infection are equivocal. Although nosocomial infections from fulminate cases are well documented (e.g. Burney, et al. 1980), the "natural" frequency of direct transmission, whether from animals or other humans, is not well understood. Most enzootic transmission is believed to be vector-borne (Swanepoel et al. 1987), and human infection associated with tick-bite has been documented in certain circumstances (reviewed by Watt et. al, 1988). The relative importance of indirect, vector-mediated infections of humans has yet to be thoroughly studied.

In West Africa, CCHF virus transmission to humans has been demonstrated from virus isolation and/or antibodies (Watts et al. 1988), and we recently documented at least one fatal case of CCHF in southern Mauritania (Gonzalez et al. 1990). The factors that influence human risk of infection in this area have not been previously studied. In collaboration with Dr. Louisa Chapman, an EIS officer at the CDC, we undertook a questionnaire-based epidemiological investigation of factors associated with human infection of CCHF virus in northern Senegal.

Study site and methods. The study was based around the village of Yonofere, Senegal where other prospective studies on CCHF virus transmission are ongoing. Humans living there are semi-nomadic, periodically moving with their animal herds and with changes in agriculture. Animal herding activities vary seasonally. During and shortly after the rainy season, ?? local are kept near their camps. As the dry season progresses, these animals are grazed over increasingly greater distances, often 10-30 km daily; typically, they return for the night to obtain water. Under extreme conditions, young men temporarily displace the herds some hundreds of kilometers for periods of a few months. In addition to these permanent residents, the large government-owned well at the center of the village annually attracts migrating herdspeople that move tens of thousands of domestic ungulates through the region seeking forage. These Maur nomads drive their cattle, camels, sheep and goats southward during the dry season and again northward each year when the rains recommence. Because of the well, Yonofere abounds with resident and migratory domestic and wild vertebrates. During two-thirds of the year when no rain falls and standing water is unavailable (October-June), the well serves as the principle source of water for resident people and animals within a 15-20 km. radius.

The study population consisted of all persons 5 years of age or older who lived within a 10 km of the Yonofere well during the hot-dry season of 1989. People inhabit small grass huts that are typically constructed in clusters of 3 to 8. Each cluster of huts, termed a Compound, is used by a family unit that is headed by an elder male who is considered the Compound chief. Enrollment of Compounds in the study depended upon the consent of the Compound chief. All persons at least 5 years old were then bled and questioned. Two types of questionnaires were administered: the "Head of Family" to each Compound chief asked general questions, and the "Individual" to all participants asked questions specific to that person. Head of Family questionnaires asked for demographic, kinship, and residency information of all Compound inhabitants, as well as animal husbandry and nomadic practices of certain residents. Individual questionnaires were administered to understand personal activities involving putative exposure risks and illness within the past 2 years. A card with preserved, mounted ticks was used to explore tick exposure.

Questions were conceived in English then translated to Peular from discussions in French with team members and other American and French researchers who had undertaken similar studies of these people. Three team members administered the questionnaires; 2 Senegalese (Peul) men and one American woman. The Head of Family questionnaire was asked by the men (always of a man) and the Individual questionnaire was asked by all three questioners. Usually men questioned men and boys, and the woman similarly questioned women and girls.

A complementary serological study of sheep owned by members of participating Compounds was undertaken to compare sero-prevalence with that of the humans. Compounds were also mapped to examine the spatial pattern of infection, proximity to animal bedding areas, and other possible methods of transmission.

Human and animal blood samples were held at ambient temperature for from 1-7 days, centrifuged, and sera was stored at -20°C until tested for the presence of IgG and IgM as described above.

Questionnaire responses and serological results. From a total of 722 persons occupying 83 compounds in the study area, 304 were questioned; 284 of these also provided a usable blood sample (Fig. 8). The age and sex distribution, and all other demographic variables considered, did not differ between the respondents and those residents who did not participate.

Seroprevalence of IgG among the 284 people sampled was 13.0% with no difference between males (13.6%) and females (12.7%). Two persons (0.7%), one male and one female, also tested positive for IgM. The prevalence of IgG was correlated with age: older people had been infected more often (Fig. 9).

Spatial clustering of infection was suggested by the distribution among Compounds of the 37 IgG-positive people (Table 6). Seropositives from these 19 Compounds housed 63% of the study population. We divided compounds into those having 0, 1, 2, or 3 or more seropositives, and compared this observed to an expected that would occur by chance alone (Table 6). If the seropositives were randomly distributed among the 284 inhabitants of the 36 Compounds, chance pairings of seropositives within a Compound would be expected to occur on 21 occasions. However, 32 pairs of seropositives were observed, again indicating clustering of infections. Other evidence of spatial clustering was not found: of 10 Compounds with at least 2 seropositives, a sleeping hut was shared by seropositives in only 3 Compounds. Of the 51 hutmates of seropositives, only 6% were also seropositive, suggesting that person-to-person transmission between hutmates was unlikely.

Of 16 deaths that were reported by Compound chiefs within the past 2 years, nearly all occurred among the very young or very old. Twelve deaths were associated with fever and 3 with hemorrhage. Only one death was associated with both, suggesting CCHF as a possible cause. One of two IgM-positive participants reported a febrile disease associated with nosebleed, but no statistical association between recent febrile/hemorrhagic disease and seropositivity was found. Neither of the two IgM positive individuals lived in a compound in which we received reports of deaths within the past 2 years that were associated with hemorrhagic symptoms.

A subset of 20 Compounds with a total of approximately 1100 sheep agreed to have samples of their sheep tested. From 371 (33.7%) of sheep tested, 9.4% were seropositive (range 0% to 28.6%). Among the 213 inhabitants of these Compounds that were part of our study, 198 (93%) were tested, and 14.7% were seropositive (range 0-37.5%). Among Compounds, seropositivity of sheep was negatively correlated with that of humans ($p=0.01$).

The risk factors associated with seropositivity for men included those involving contact with ticks and treating sick animals (Table 7). Specifically, men who indicated having been bitten by the sample specimen that was a male *H. truncatum*, increasing frequency of tick bites and having been bitten by ticks during the cold-dry season all were significantly associated with seropositivity. Consistent with the specificity of this risk was the finding that non-specific tick exposure was not significantly associated with IgG (Table 8). Additionally, men who reported treating sick animals or taking them for veterinary care showed an elevated prevalence of past infection. Curiously, no clear risk factors emerged for women (Table 7). In addition, numerous activities that might have placed people in contact with infectious tissue were found not to be risk factors (Table 8).

III. VIRUS TRANSMISSION

Natural Prevalence of Virus in Ticks

The intensity of infection in populations of ticks is an important component of their capacity as vectors of CCHF virus. Studies of ticks collected from vertebrates continue to be systematically analyzed by suckling mouse inoculation and cell culture. Under the direction of Dr. Camicas, who also collects material from Bandia, ticks from Yonofere and Dahra are identified and pooled by him and tested for virus. Virus isolation efforts are coordinated by Dr. Zeller. Species-specific pools from individual animals or herds are held at -70°C until testing, at which time they are ground for virus isolation. In collaboration with Ms. Calvo, the identity of viruses from ticks or eggs was confirmed using a CF test following mouse passage.

Three types of tick collections are being undertaken: "en masse" from cattle and sheep, from randomly selected sheep being studied for evidence of tick activity patterns and from individually identified sheep, cattle and goats being bled for evidence of antibodies and virus. Approximately 500 - 1,000 ticks were collected monthly for virus isolation by herdspeople from Yonofere and surrounding villages. Cooperants are given tubes into which they place ticks that they have removed from their animals. The ticks collected from 100 sheep sampled in Yonofere and Dahra each month for determination of tick seasonality also are tested for the presence of virus. In addition, sheep and cattle at the Dahra-CRZ, sheep in Yonofere, and goats and cattle in Bandia which are under study for the incidence of new CCHF infection are deticked during bleeding; these ticks also are being tested for virus.

During 1989, a total of 11,206 ticks were captured from Yonofere, Dahra and Bandia and tested in 916 pools that were inoculated. The principal species tested were *Hyalomma truncatum*, *H. impeltatum*, *H. marginatum rufipes*, *R. guilhoni*, and *Amblyomma variegatum*. Of 603 thus far tested, 2 strains of CCHF virus have been isolated: one from each of two pools of *H. truncatum* from Bandia. Other viruses that have been isolated from these ticks include Dugbe, Bandia, and numerous strains of a virus closely related to Wad Medani virus.

Experimental Transmission Using Sheep

Despite strong evidence that ixodid ticks are vectors of CCHF virus in nature (Hoogstraal, 1979) the importance of particular species in the maintenance of transmission remains poorly understood. Under the direction of Dr. Jean-Paul Gonzalez, experimental observations of infected *H. truncatum* and *H. marginatum rufipes* feeding on sheep have been

undertaken to study the virological and serological responses of these ungulates as a model for tick-transmitted infection.

As with inoculation of CCHF virus to sheep, transmission by feeding ticks produces detectable viremia lasting from day 4 to day 8 post-infestation, followed by rapid increase in IgM titer which then descends during the following few weeks (Fig. 10). IgG remains elevated for months. Naive adult ticks that are co-feeding with these infected adults become infected. Studies on horizontal transmission are continuing.

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APPENDICES

Figure Legends

Figure 1. Map of Senegal indicating rainfall isoyhets, the principal cities, and three major study sites discussed in this report.

Figure 2. Average monthly rainfall for the periods 1959-1986, 1987, 1988, and 1989 at the Dahra-CRZ Research Station.

Figure 3. Mean number of adult *Hyalomma truncatum* (A) and *H. impeltatum* (B) and prevalence of IgM against CCHF virus among sheep in during May 1987 through December 1988.

Figure 4. Serological response of laboratory mice (A) and rabbits (B) inoculated with CCHF virus.

Figure 5. Changes in temperature (A) and evidence of viremia (B) of sheep inoculated with CCHF virus.

Figure 6. Serological response to primary or secondary infection of sheep (A) and comparison of OD by ELISA and titer of IgG and IgM (B).

Figure 7. Serological response of domestic chickens (A) and helmeted Guinea Fowl (B) inoculated with CCHF virus.

Figure 8. Summary of population questioned and surveyed for antibodies against CCHF virus in Yonofere, Senegal and overall results.

Figure 9. Age-specific prevalence of IgG against CCHF virus in Yonofere, Senegal.

Figure 10. Viremia and IgM response of sheep infected by CCHF virus from feeding adult *Hyalomma truncatum* (A) and comparison of their IgM and IgG responses (B).

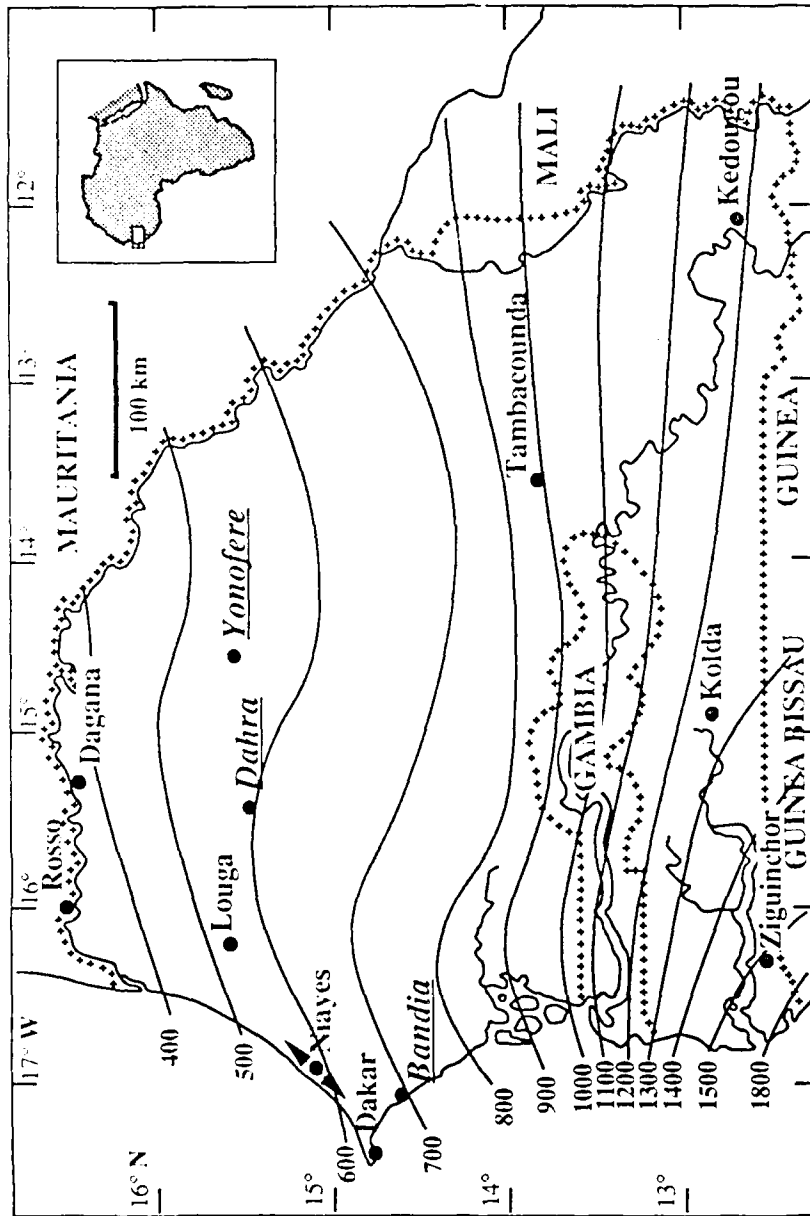


Figure 1. Map of Senegal indicating rainfall isoyhets, the principal cities, and three major study sites discussed in this report.

Rainfall in Dahra during 1959-89

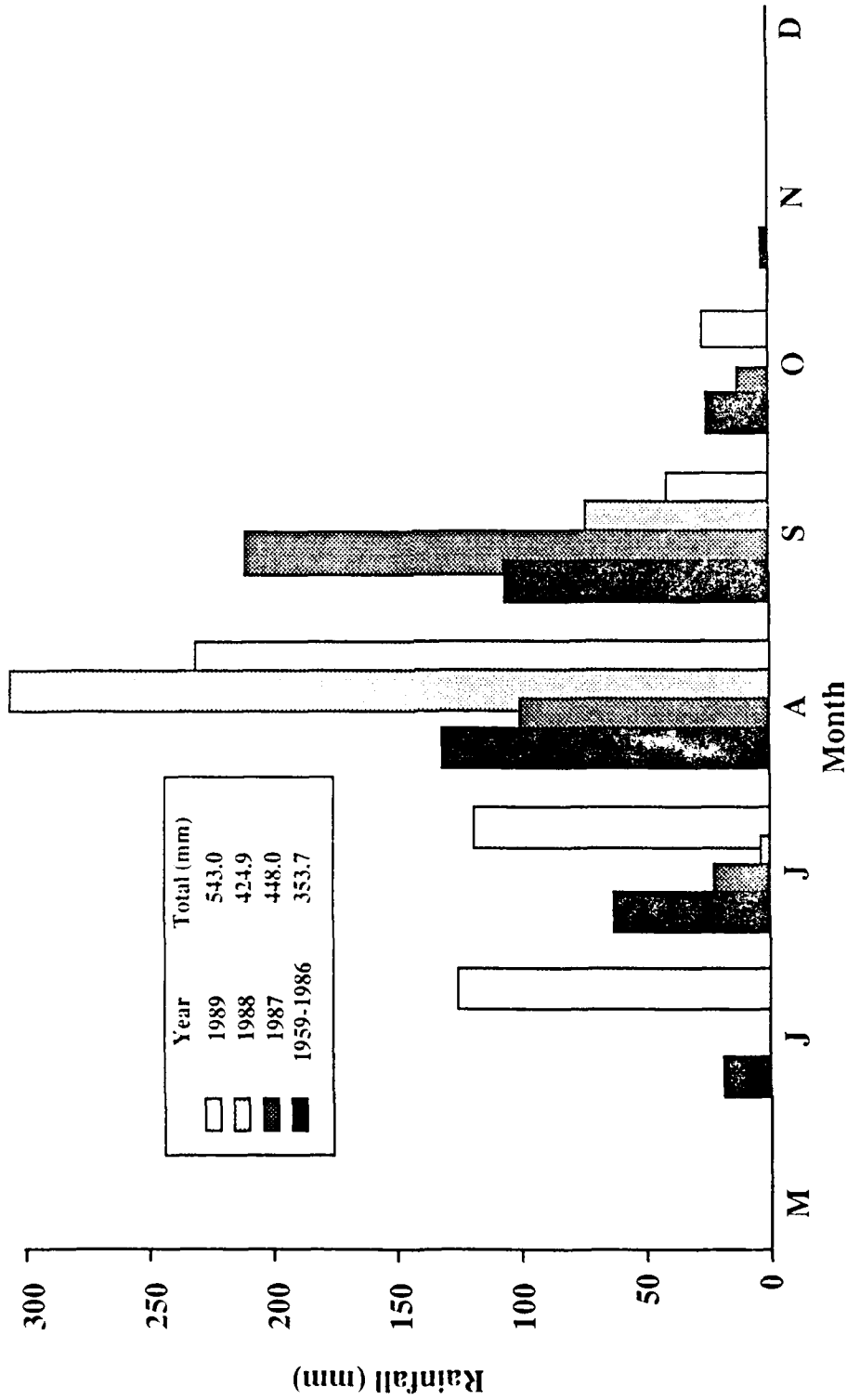


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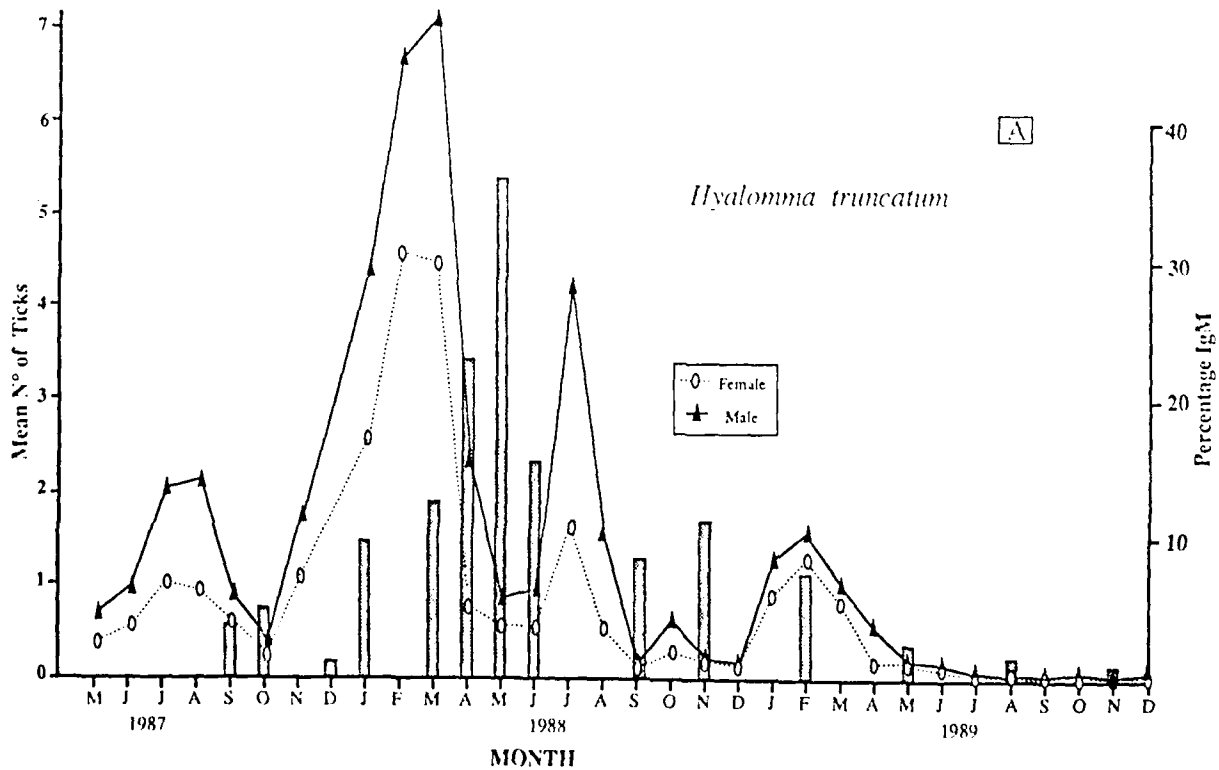
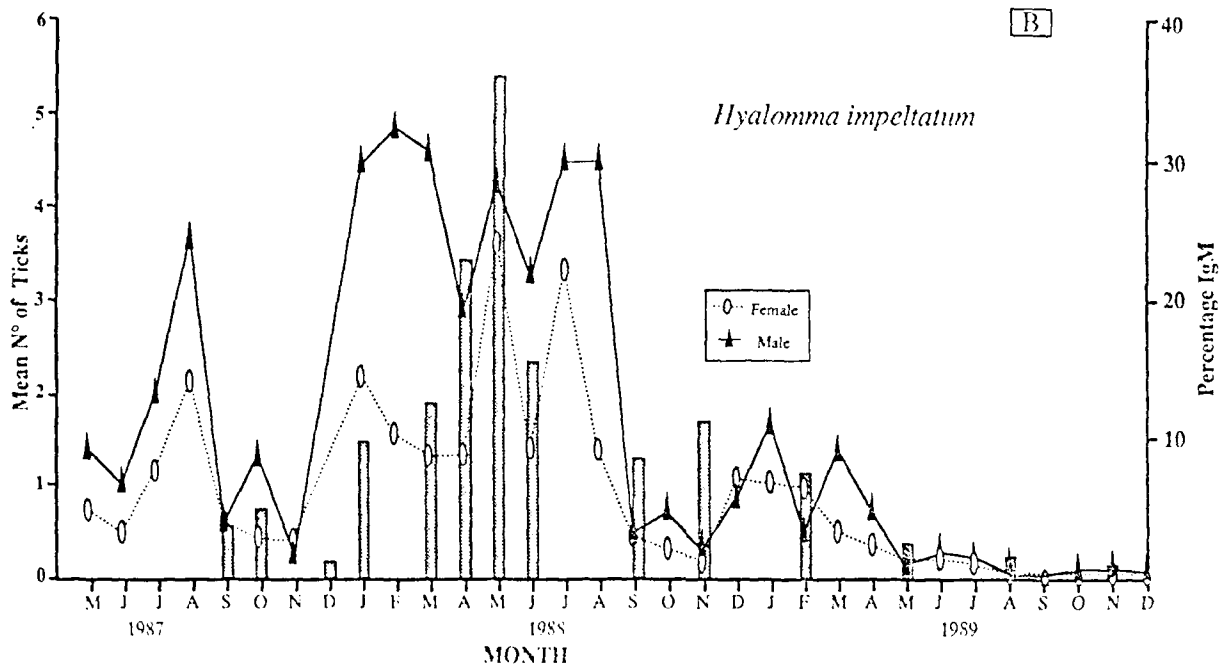


Figure 3. Mean number of adult *Hyalomma truncatum* (A) and *H. impeltatum* (B) and prevalence of IgM against CCHF virus among sheep in during May 1987 through December 1988.

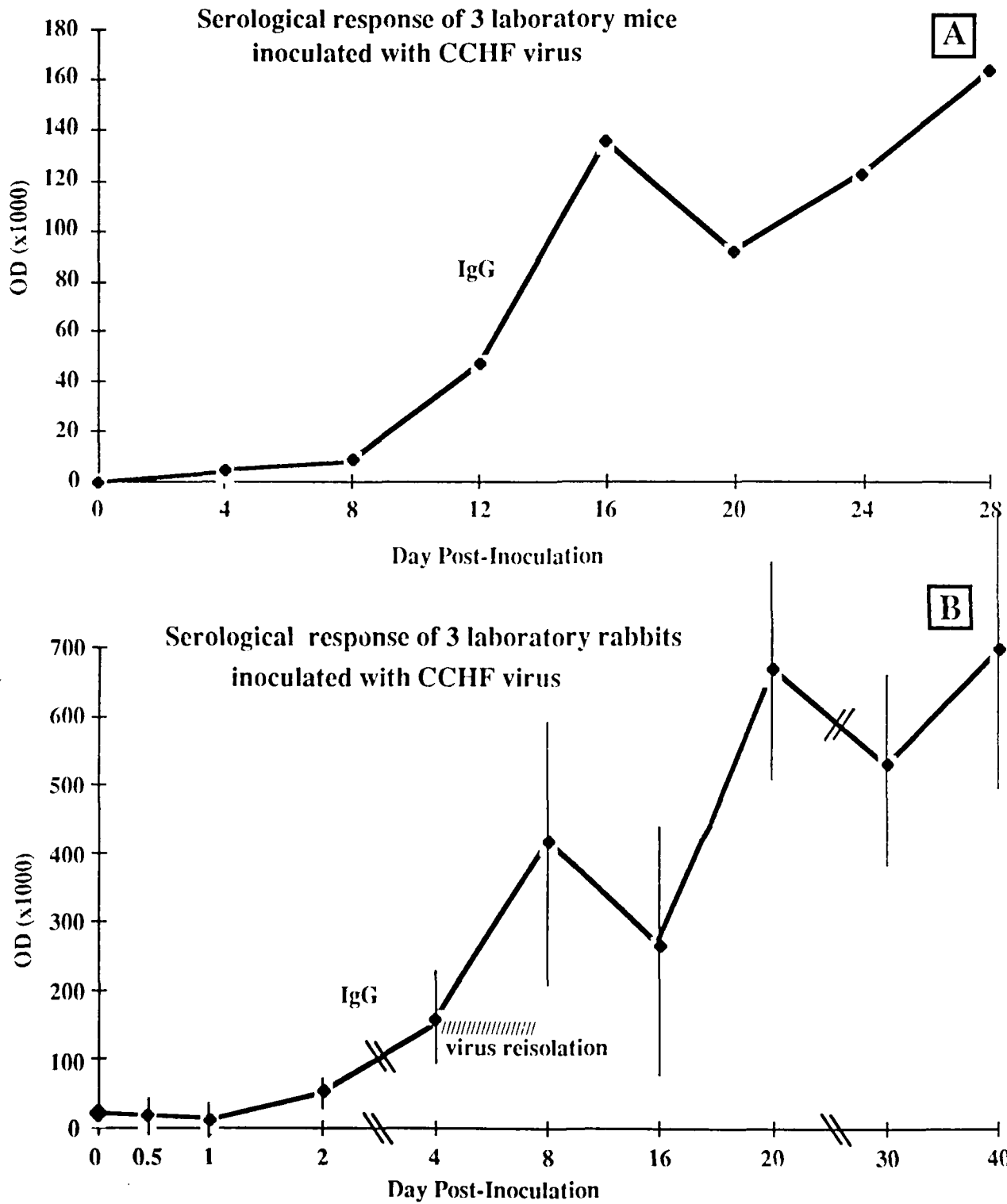


Figure 4. Serological response of laboratory mice (A) and rabbits (B) inoculated with CCHF virus.

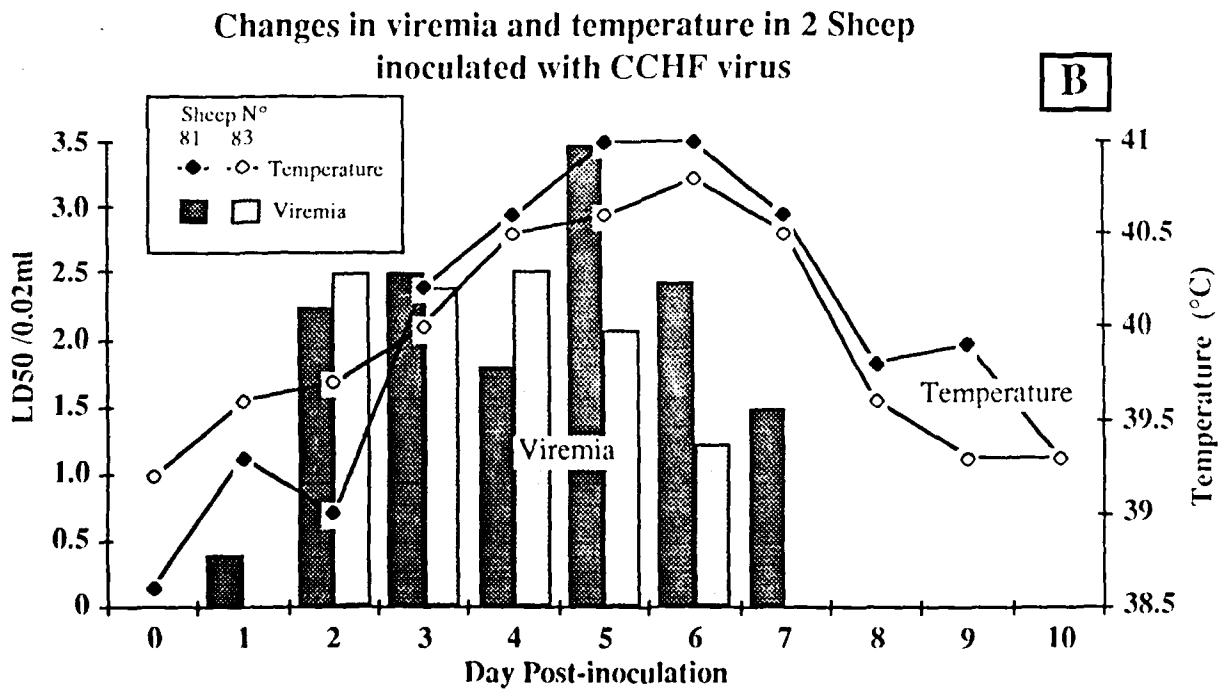
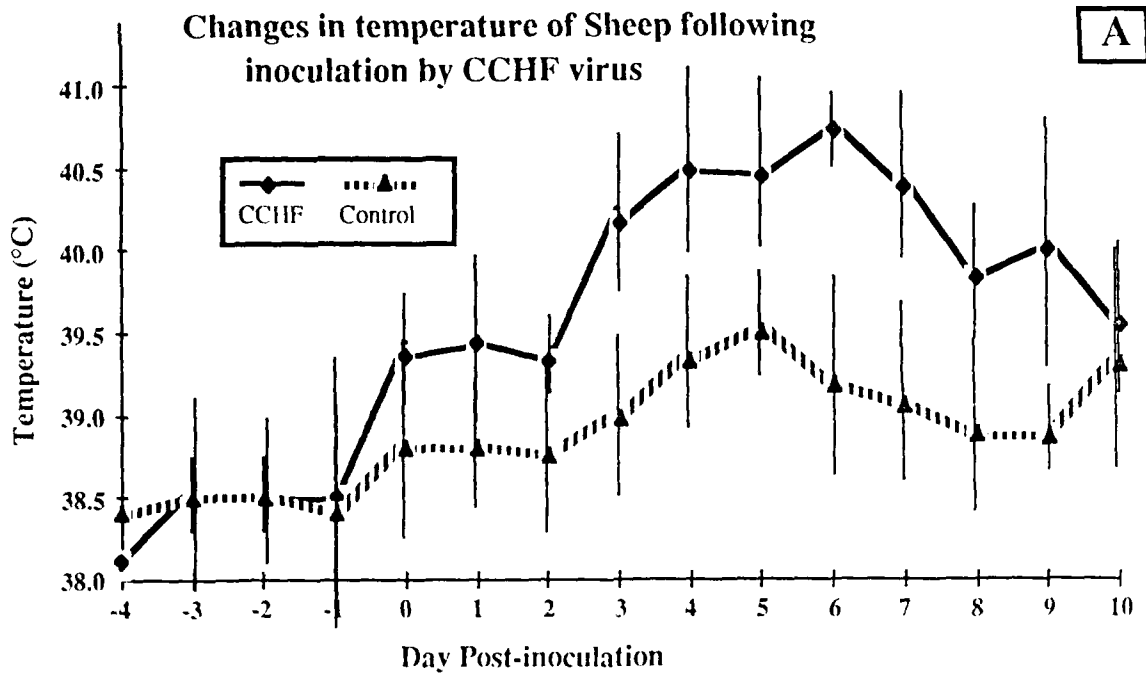


Figure 5. Changes in temperature (A) and evidence of viremia (B) of sheep inoculated with CCHF virus.

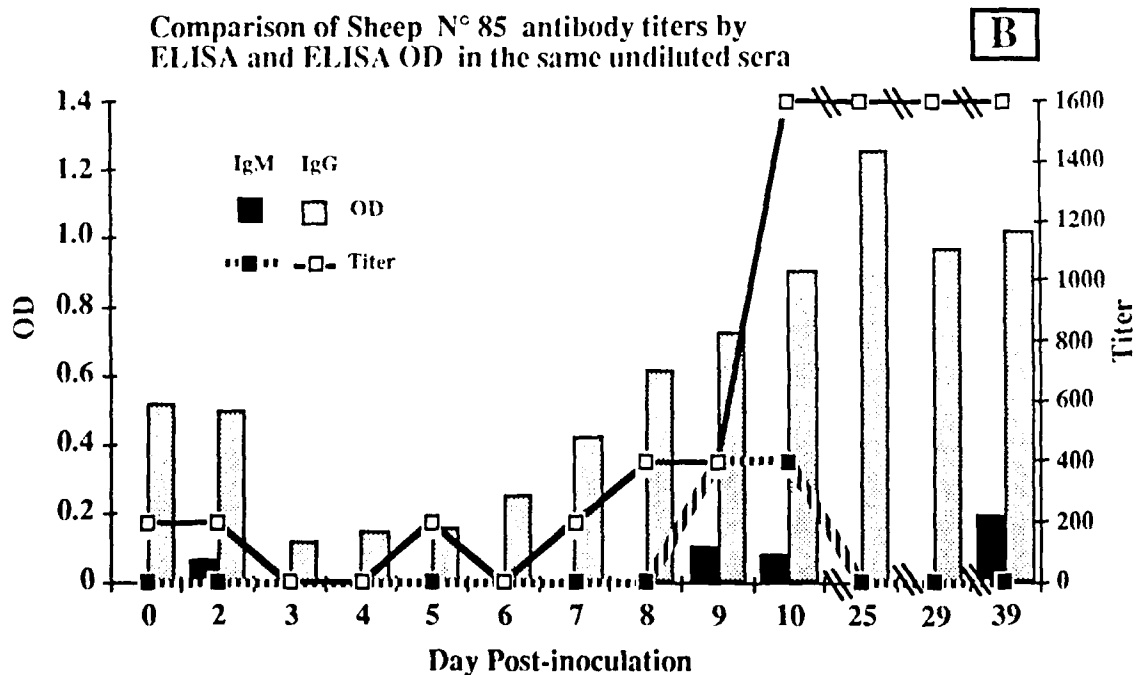
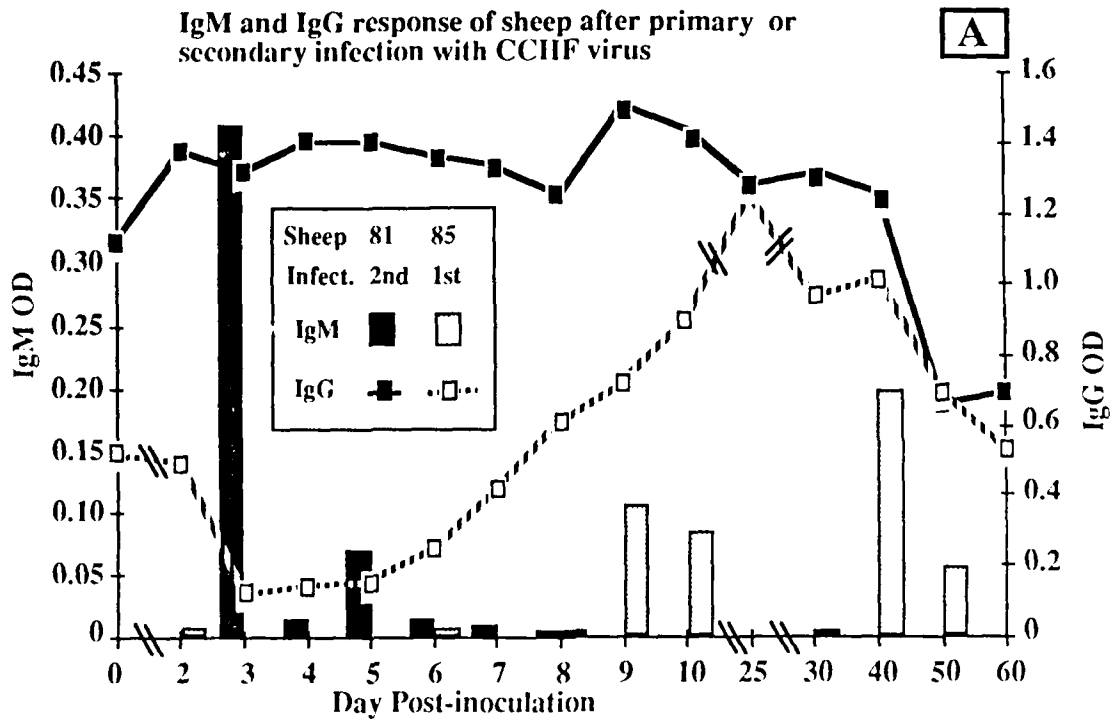
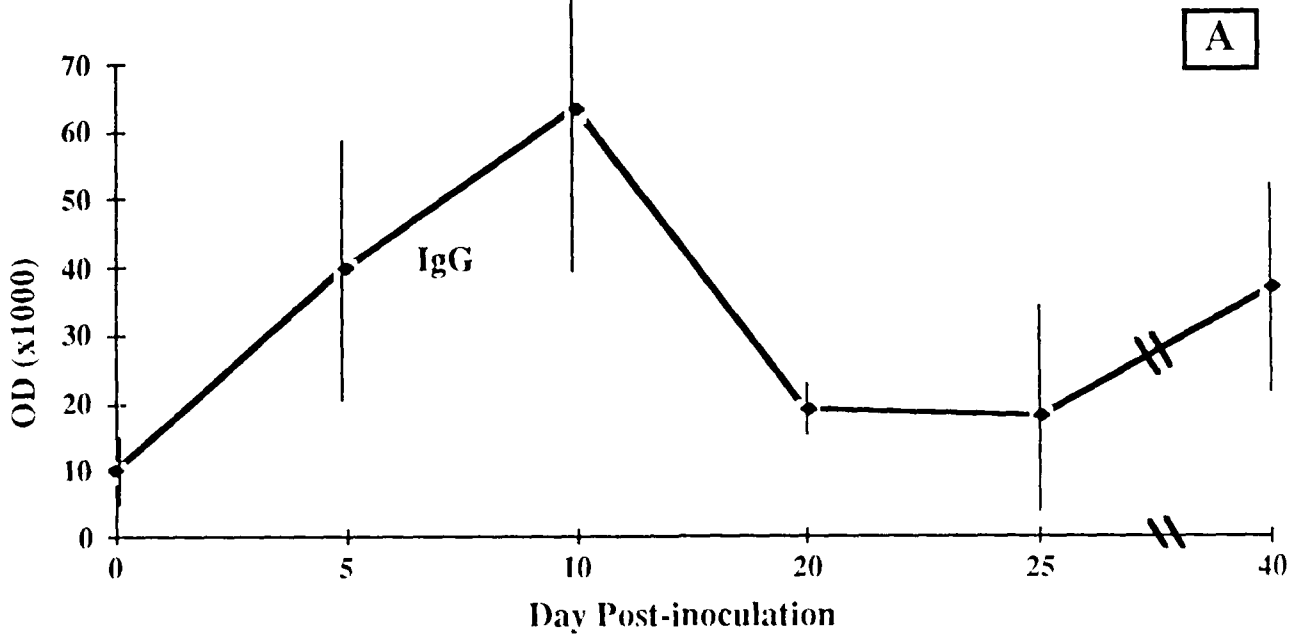


Figure 6. Serological response to primary or secondary infection of sheep (A) and comparison of OD by ELISA and titer of IgG and IgM (B).

Serological responses of domestic chickens inoculated with CCHF virus



Serological responses of 3 Guinea Fowl inoculated with CCHF virus

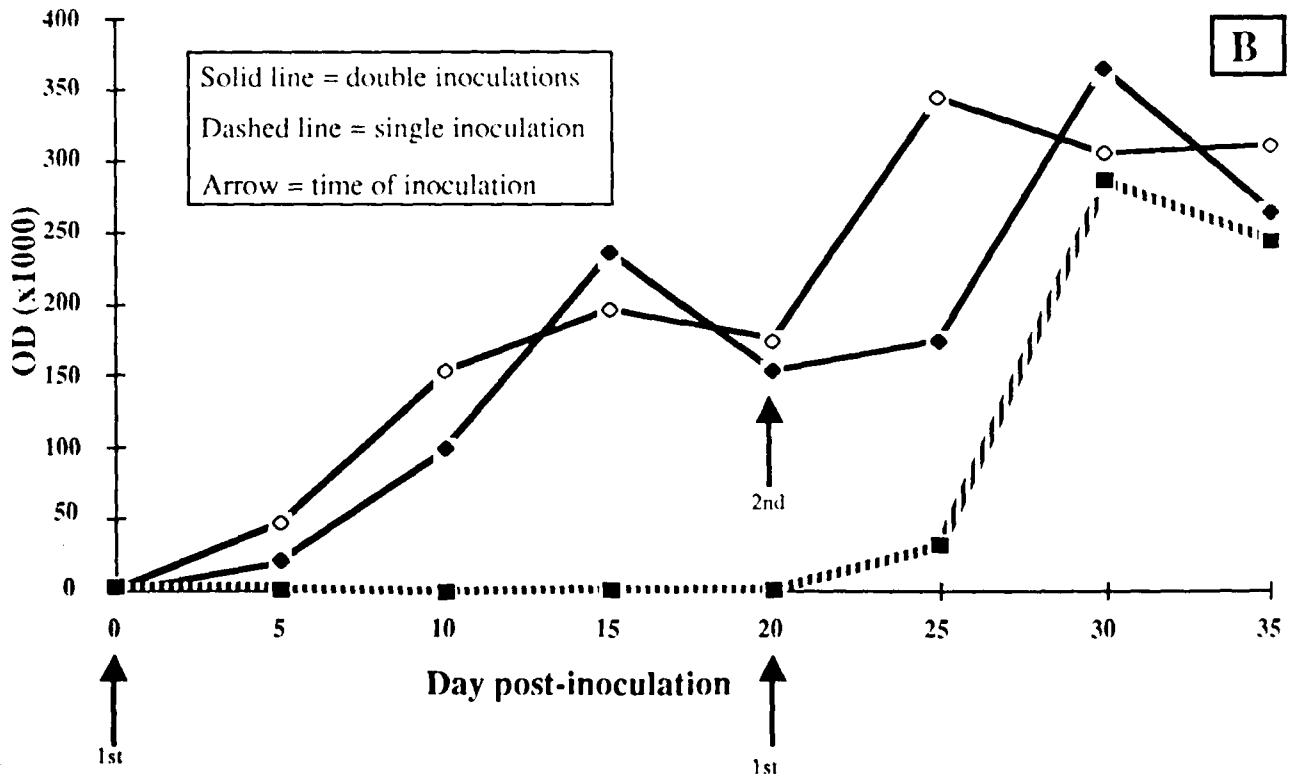


Figure 7. Serological response of domestic chickens (A) and helmeted Guinea Fowl (B) inoculated with CCHF virus.

Resident Population

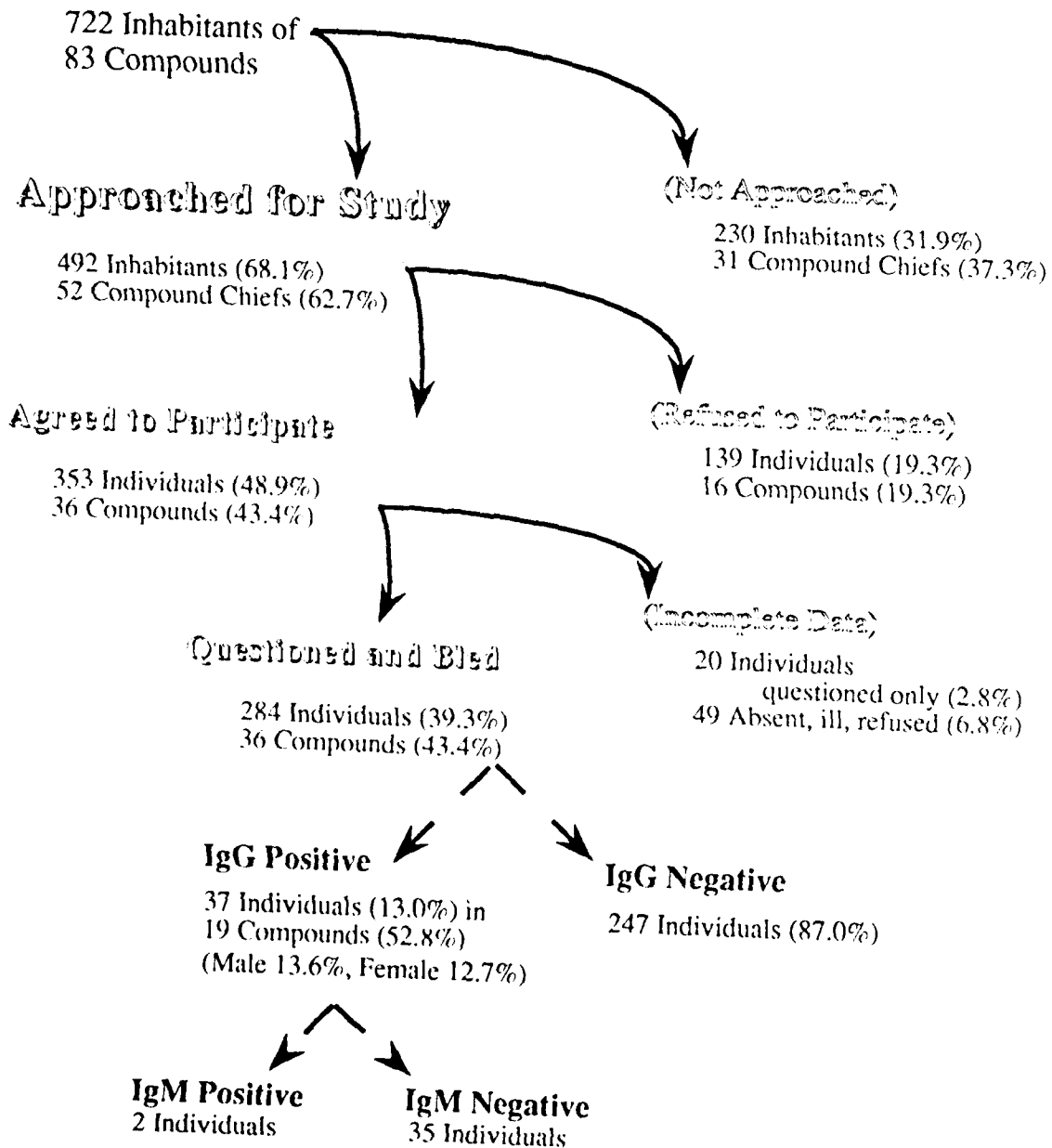


Figure 8. Summary of population questioned and surveyed for antibodies against CCHF virus in Yonofere, Senegal and overall results.

Prevalence of IgG against CCHF virus in Yonofere, Senegal by age-class

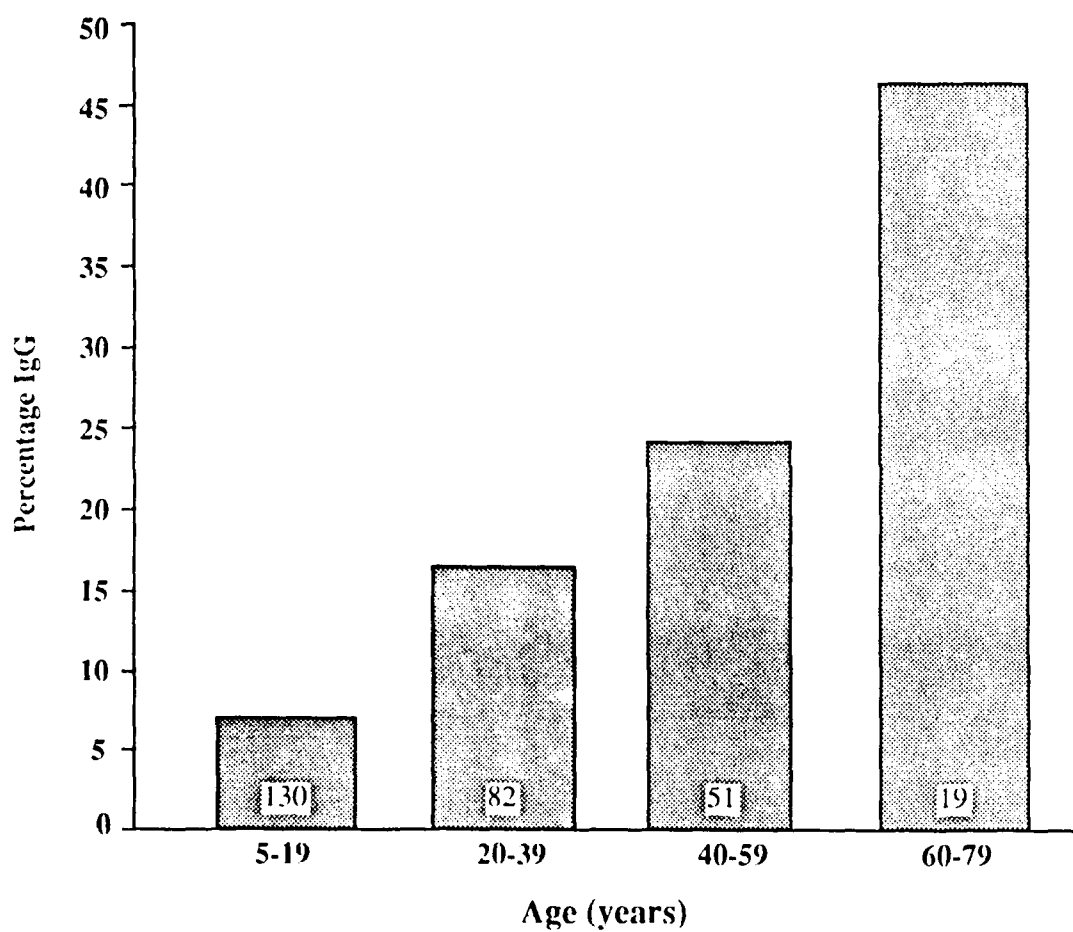


Figure 9. Age-specific prevalence of IgG against CCHF virus in Yonofere, Senegal.

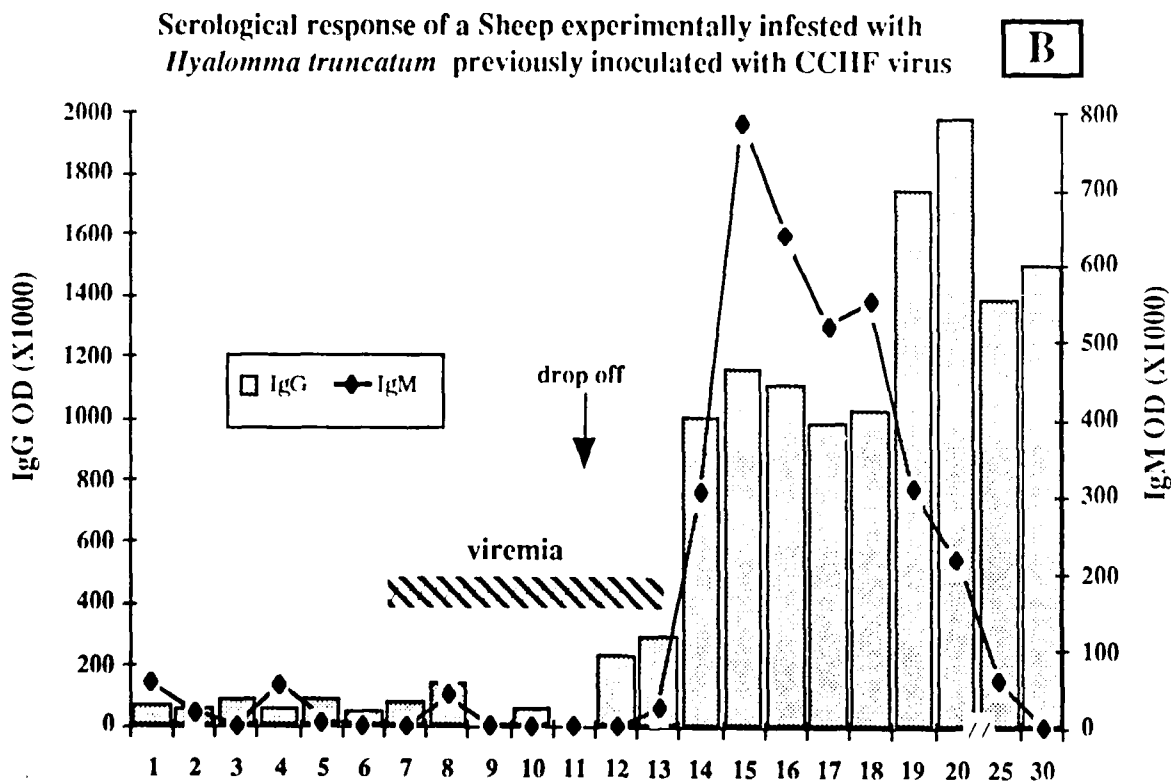
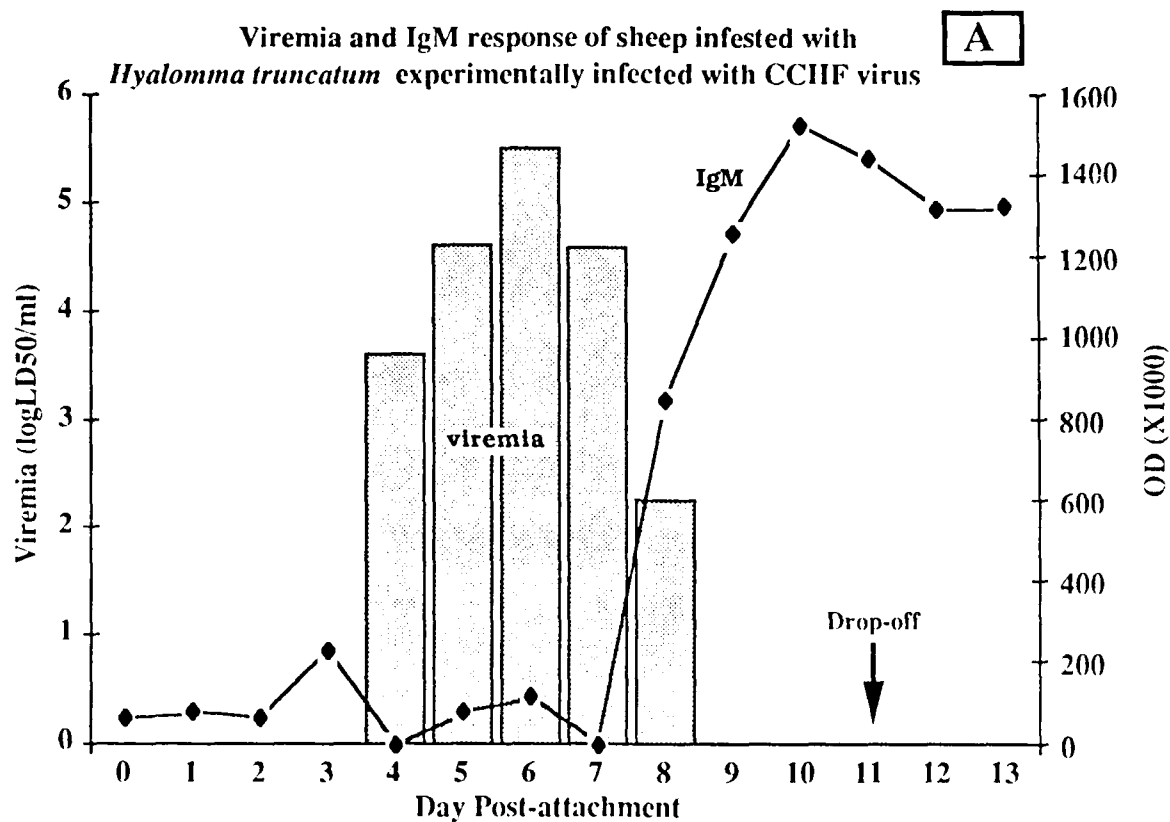


Figure 10. Viremia and IgM response of sheep infected by CCHF virus from feeding adult *Hyalomma truncatum* (A) and comparison of their IgM and IgG responses (B).

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, Ibrahama 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
Chapman, Louisa E. Fisher-Hoch, S. P.	Epidemiology Office/DVD/CID/CDC, Atlanta
Dykstra, Elizabeth A.	Institut Pasteur/U.S. Peace Corps, Senegal

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

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Table 3. Birds examined monthly at Yonofere, Senegal during January through December, 1989, and immature ticks (*Hyalomma spp.*) found parasitizing them.

Bird (Species) ¹ or Tick	Month												J-D
	J	F	M	A	M	J	J	A	S	O	N	D	
Double-spurred Francolin <i>Fringilla bicalcaratus</i>												2	2
White-throated Francolin <i>Fringilla albogularis</i>			1	3			3	2	2	1	1		13
Stone-Partridge <i>Ptilopachus petrosus</i>						2			2	2	2		8
Grey-Breasted Helmet Guinea Fowl <i>Numida meleagris</i>	3						1	1	2	3		3	13
Senegal Bustard <i>Eupodotis senegalensis</i>		2	2				1			2	2		9
Black-bellied Bustard <i>Eupodotis melanogaster</i>			2										2
Laughing Dove <i>Streptopelia senegalensis</i>	3	8	2	7	10	10	4	5			1		50
Red-eyed Dove <i>Streptopelia semitorquata</i>		1											1
Vinaceous Dove <i>Streptopelia vinacea</i>										3	2		5
Long-tailed Dove <i>Oena capensis</i>		2	1									2	5
Chestnut-bellied Sand-grouse <i>Pterocles exustus</i>			4	6									10
Abyssinian Roller <i>Coracias abyssinica</i>										2	2		4
Red-beaked Hornbill <i>Tockus erythrorhynchus</i>	8	1	1		1					2	3	12	28
Crag Chestnut-winged Starling <i>Onychognathus morio</i>												9	9
Purple-headed Glossy Starling <i>Lamprotornis purpureiceps</i>										1			1
Black-headed Plover <i>Vanellus tectus</i>				2									2
African Golden Oriole <i>Oriolus auratus</i>	3			2								1	6
Yellow-fronted Canary <i>Serinus mozambicus</i>	3						1						4
Senegal Coucal <i>Centropus senegalensis</i>											1		1
Unidentified Weavers <i>Ploceus spp.</i>			5		5							2	12

CONTINUED.....

Table 3. Continued

Bird (Species) ¹ or Tick	Month												J-D
	J	F	M	A	M	J	J	A	S	O	N	D	
Golden Sparrow <i>Passer luteus</i>						29					50	49	128
Vitelline Masked Weaver <i>Ploceus velatus</i>	1				2								3
Village Weaver <i>Ploceus cucullatus</i>							1						1
Orange Weaver <i>Ploceus aurantius</i>		6											6
Scaly-fronted Weaver <i>Sporopipes frontalis</i>								1					1
Buffalo Weaver <i>Bulbalornis albirostris</i>							2						2
Grey-headed Sparrow <i>Passer griseus</i>	25	14	1	1	6	8	3				4		62
Cut-throat Weaver <i>Amandina fasciata</i>							5	5	2				12
Warbling Silverbill <i>Lonchura malabarica</i>					34		29	3			2	44	112
Senegal Fire-Finch <i>Lagonosticta senegala</i>	4	15	3		3			3					28
Hoopoe <i>Upupa epops</i>											1		1
Broad-tailed Paradise Whydah <i>Vidua orientalis</i>								1					1
Red-cheeked Cordon-bleu <i>Estrilda bengala</i>		1											1
ALL SPECIES	50	50	22	21	61	49	50	21	8	16	73	122	543
No. Birds Parasitised	4	4	2	0	0	0	0	0	0	0	10	15	35
Total Ticks (Stage)													
<i>H. rufipes</i> (Larva)	11	8	0	0	0	0	0	0	0	0	8	20	47
<i>H. rufipes</i> (Nymph)	6	5	1	0	0	0	0	0	0	0	3	36	51
<i>H. truncatum</i> (Larva)	0	0	0	0	0	0	0	0	0	0	8	0	8
<i>H. truncatum</i> (Nymph)	0	0	0	0	0	0	0	0	0	0	0	0	0

1. In addition to those species listed the following were also examined: in February, 1 *Tockus erythrorhynchus* parasitized by *Ripicephalus guilhoni* (1 A); March, 1 *Francolinus albogularis* parasitized by *Argus streptopelia* (7 L); November, 1 *Tockus erythrorhynchus* parasitized by *Argus* sp. (1 L), 4 *Passer luteus* parasitized by *A. arboreus* (17).

Table 4. Monthly observations of immature Ixodid ticks found on small mammals

examined during 1989 in Yonofere, Senegal.

Mammal Species ¹	Tick		Mean No. ticks on (N) mammals during:												
	Sp. ²	Stage	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	1989
<i>Mastomys</i> sp. ³	<i>H. trunc.</i>	L	0	-	-	-	-	-	-	-	-	-	0	0	0
		M	0	-	-	-	-	-	-	-	-	-	0	0	0
			(19)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(5)	(2)	(26)
<i>Taterillus</i> sp. ³	<i>H. trunc.</i>	L	-	-	-	0	-	-	-	-	-	-	0	-	0
		M	-	-	-	0	-	-	-	-	-	-	0	-	0
			(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(2)
<i>Arvicantbus</i> sp.	<i>H. trunc.</i>	L	-	-	-	-	-	-	-	-	-	-	0	0	0
		M	-	-	-	-	-	-	-	-	-	-	0	0	0
			(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(4)	(7)	(11)
<i>Erinaceus albiventris</i>	<i>H. trunc.</i>	L	-	0	0.5	-	-	0	0	2.0	-	2.5	0	0	0.7
		M	-	1.0	0.5	-	-	0	0	2.2	-	0.5	1.0	12.0	1.3
	<i>H. ruf.</i>	L	-	0	0	-	-	0	0	0	-	1.0	0	0	0.1
		M	-	0	0	-	-	0	0	0	-	0	0	0	0
			(0)	(1)	(4)	(0)	(0)	(3)	(5)	(4)	(0)	(2)	(1)	(1)	(21)
<i>Lepus whytel</i>	<i>H. trunc.</i>	L	0	0	2.0	0	52.5	0	0	3.5	-	0	1	0	4.2
		M	0.3	9.8	2.5	4.5	9.0	1.2	2.5	0	-	1	12	11.5	5.4
	<i>H. ruf.</i>	L	0	1.0	0	0	0	0	0	0	-	0	0	0	0.2
		M	0	0.8	0	0	0	0	0	0	-	0	0	1.0	0.3
	<i>R. gull.</i>	A	0.3	0.4	0	0	0	0	0	2.5	-	0	0	0	0.3
			(3)	(5)	(2)	(2)	(2)	(4)	(2)	(2)	(0)	(1)	(1)	(4)	(28)
TOTAL	<i>H. trunc.</i>	L	0	0	1.0	0	52.5	0	0	2.5	-	1.7	0.1	0	2.1
		M	<<0.1	8.3	1.2	3.0	9.0	0.7	0.7	1.5	-	0.7	1.1	4.1	2.0
	<i>H. ruf.</i>	L	0	0.8	0	0	0	0	0	0	-	0.7	0	0	0.1
		M	0	0.7	0	0	0	0	0	0	-	0	0	0.3	0.1
	<i>R. gull.</i>	A	<<0.1	0.3	0	0	0	0	0	0.8	-	0	0	0	0.1
			(22)	(6)	(6)	(3)	(2)	(7)	(7)	(6)	(0)	(3)	(12)	(14)	(88)

1. In addition to those species listed the following were also examined: January, 1 *Canis adustus*, 2 *Canis pallidus* parasitized by *Ripicephalus sanguineus*, and 1 *Viverra civetta* parasitized by *R. guilhoi*; February, 1 *Xerus erythropus* parasitized by *Haemaphysalis kowyi*; March, 1 *Erinaceus albiventris* parasitized by *H. spinosa*; August, 1 *Phacochoerus aethiopicus* parasitized by *Hyalomma truncatum* and *Ripicephalus cuspidatus*.

2. Tick species are *Hyalomma truncatum*, *H. marginatum rufipes* and *Ripicephalus guilhoi*.

3. The species within the genus *Taterillus* are visually indistinguishable. *T. pygargus* and *T. gracilis* are both encountered at this site.

Table 5. Monthly observations of immature Ixodid ticks found on small mammals examined during 1989 in Bandia, Senegal.

Mammal Species	Tick		Mean No. ticks on (N) mammals during:												
	Sp. ¹	Stage	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	1989
<i>Mastomys erythroleucus</i>	<i>H. trunc.</i>	L	0	0	0	0	0	0	0	0	-	0	0	0	0
		N	0	0	0	0	0	0	0	0	-	0	0	0	0
	<i>R. gui.</i>	L	0	0	0	0	0	0	0	0	-	0	0.9	0	0.1
		N	<0.1	0	0	0	0	0	0	0	-	0	1.8	<0.1	0.2
			(38)	(14)	(7)	(5)	(4)	(10)	(15)	(12)	(0)	(26)	(11)	(15)	(157)
<i>Arvicanthis niloticus</i> ²	<i>H. trunc.</i>	L	0	0	0	0	0	0	0	0	-	0	0	0	0
		N	0	0	0	0	0	0	0	0	-	0	0.2	0	<<0.1
	<i>R. gui.</i>	L	0.1	0.1	0	0	0	0	0	0	-	1.4	0.2	0.4	0.2
		N	0.7	0.1	0.1	0.7	0	0	0	0	-	4.2	0.6	0.2	0.7
			(10)	(16)	(55)	(6)	(2)	(3)	(1)	(0)	(0)	(16)	(12)	(20)	(141)
TOTAL	<i>H. trunc.</i>	L	0	0	0.1	0.1	0	0	0	0	-	0	0	0	0
		N	0.3	0.2	0	<0.1	0	0	0	0	-	0	0.1	0	<<0.1
	<i>R. gui.</i>	L	0	<<0.1	<<0.1	0	0	0	0	0	-	0.4	0.6	0.2	0.1
		N	0.2	0	0	0	0	0	0	0	-	1.3	1.2	0.2	0.4
			(48)	(30)	(62)	(11)	(6)	(13)	(16)	(12)	(0)	(52)	(23)	(35)	(308)

1. Tick species are *Hyalomma truncatum* and *Rhipicephalus gullhoni*.
2. In addition to those ticks listed, 1 *Arvicanthus niloticus* parasitized by *Amblyomma variegatum* was examined in October

Table 6. Analysis of clustering of IgG against CCHF virus among humans inhabiting Compounds within a 10 km. radius of the Yonofere well during February-May 1989. Clustering significant (p=0.03) using Walters' test for aggregation.

No. Seropositives Per Compound	No. Compounds		Difference Obs.-Exp.
	Observed	Expected	
1	18	14.6	3.4
2	9	12.7	-3.7
3	5	6.2	-1.3
4	5	3.4	1.6

Table 7. Exposures associated with evidence of past infection by CCHF virus among humans in Yonofere, Senegal during February-May 1989.

Exposure	P-value of exposure for	
	Males	Females
Bite by male <i>H. truncatum</i>	0.033	0.58
Increasing number of tick bites	0.047	0.056
Tick bite during cold-dry season	0.001	0.18
<i>H. truncatum</i> bite during cold-dry season	0.012	no reports
Treating sick animals	0.002	0.56
Taking animals to the veterinarian	0.007	0.38

Table 8. Exposures found not to be associated with evidence of past infection by CCHF virus among humans in Yonofere, Senegal during February-May 1989.

Exposure	P-value
<u>Animal blood and secretions</u>	
Killing hares	0.61
Slaughtering animals	0.65
Plucking fowl	0.84
Preparing fresh meat	0.85
Branding animals	0.92
<u>Human blood and secretions</u>	
Nursing sick	0.16
Circumcising	0.38
Healing sick	0.58
Midwifery	0.68
Piercing ears	0.75
<u>Tick contact (non-specific)</u>	
Tick bite	0.86
Tick exposure	0.90
Deticking animals	0.89

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