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DISTRIBUTION OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRAL ANTIBODY IN SENEGAL: ENVIRONMENTAL AND VECTORIAL CORRELATES

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Abstract. The spatial pattern in Senegal of Crimean-Congo hemorrhagic fever (CCHF) virus IgG antibody prevalence in human and sheep was determined as was the relative abundance of potential tick vectors. A systematic, country-wide serological survey of sheep demonstrated that 10.4% of sheep exhibited IgG to CCHF virus. Sexes were infected equally. Antibody prevalence increased with age from 2.1% during the first year to 18.2% among sheep ≥ 3 years of age. IgG prevalence was highest in the northern, arid Sahelian zone, averaging 75.7% seropositivity, and decreased to zero in the southern, moister Sudano-Guinean and Guinean zones. Human IgG prevalence ranged from 21% to <1% among the 8 sites that were sampled throughout the country, being greatest in the arid north and least in the south. *Hyalomma* ssp. ticks predominated in those biotopes where antibody prevalence was highest. The abundance of *Hyalomma* ticks may be the proximal determinant of endemic transmission.

The worldwide distribution of Crimean-Congo hemorrhagic fever (CCHF) is astonishingly large for an arboviral zoonosis, including most of southern Eurasia, southern Europe, the Middle East, and Africa.¹ Active in the Palearctic, Oriental, and Ethiopian faunal regions, CCHF virus transmission occurs in ecologically diverse sites of ≥ 30 different countries.^{1,2} Although apparently limited by extremely cold temperatures or high humidity, evidence of this virus is found in a variety of habitats and climatologic conditions. The physical and biological variables that prevent, limit or enhance transmission of CCHF virus remain poorly understood.

Factors that influence transmission of CCHF virus include the density of competent vector ticks, particularly of the genus *Hyalomma*, and the abundance of vertebrates that serve both as hosts to these ticks and as possible reservoirs of the virus.¹ At least 30 species of ticks^{1,3} and >20 different vertebrate species² have been shown to be infected. Sustained transmission is found only where *Hyalomma* ticks are present, and epizootic or epidemic transmission is believed to occur during periods of increased abundance of these ticks.² However, the relationship between tick species diversity and the magnitude of CCHF virus transmission within a particular geographic region has not been studied systematically.

In sub-Saharan West Africa, circulation of CCHF virus has been demonstrated by the isolation of virus from ticks and the isolation of virus or the demonstration of antibodies from domestic and wild vertebrates or humans in Senegal,⁴⁻¹⁰ Mauritania,¹¹⁻¹³ Burkina Faso,^{14,15} Benin,¹⁴ and Nigeria.¹⁶⁻¹⁸ Initial observations in Senegal during the late 1960s demonstrated indirect evidence of domestic animal and tick infections.⁴ During the 1970s and early 1980s, researchers at the Pasteur Institute in Dakar isolated numerous strains of CCHF virus from ticks feeding on cattle and sheep at slaughterhouses in Senegal.⁵⁻⁹ Antibodies also were detected at various sites in the country, and a human case and antibodies in domestic animals were documented along the border in Mauritania.¹² More recently, we studied a fatal human illness due to CCHF virus infection in association with epizootic transmission during May 1988 near Rosso, Mauritania, on the Senegal River border.¹⁹ Despite 2 decades of recognized CCHF virus activity in Senegal, the spatial pattern of transmission and the prevalence of human infection throughout the country have not been studied systematically. We report here on research designed to investigate the spatial and temporal aspects of CCHF virus transmission within Senegal by evaluating the seroprevalence among sheep and humans

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compared with various bioclimatic variables and the relative abundance of ixodid ticks.

MATERIALS AND METHODS

Animal serology

Blood samples from sheep throughout Senegal were obtained as part of a project initiated by l'Institut Senegalais de Recherches Agricoles (ISRA). The country was divided into 80 sample districts of approximately equal areas that corresponded to 1 or more governmental administrative districts. Of these sample districts, 26 were randomly chosen for study. The number of animals sampled in each district was predetermined at 1:3,000 of the estimated resident sheep density, which had been calculated previously from a government census undertaken by personnel of ISRA. Villages from the interior of each district were chosen and flocks were selected randomly. In large flocks, 10–20 sheep were bled; in small flocks, all sheep were bled until the predetermined sample size for that district was achieved. Between November 1986 and January 1987, sheep from 26 sample districts in 9 of Senegal's 10 governmental regions were bled. The sex and age for each animal were recorded. Sheep

(1 hr, 60°C); 100 µl CCHF virus suspension was added followed by 100 µl of test sera, diluted 1:400 in ELISA buffer. Then 100 µl of test-species specific anti-gamma-chain immunoglobulin conjugated with horseradish peroxidase (Biosys, Compiègne, France) was added to detect the IgG. Finally, 100 µl of chromogenic substrate (orthotolidine, 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 (Titertek, 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 >3 SD above the mean of negatives.

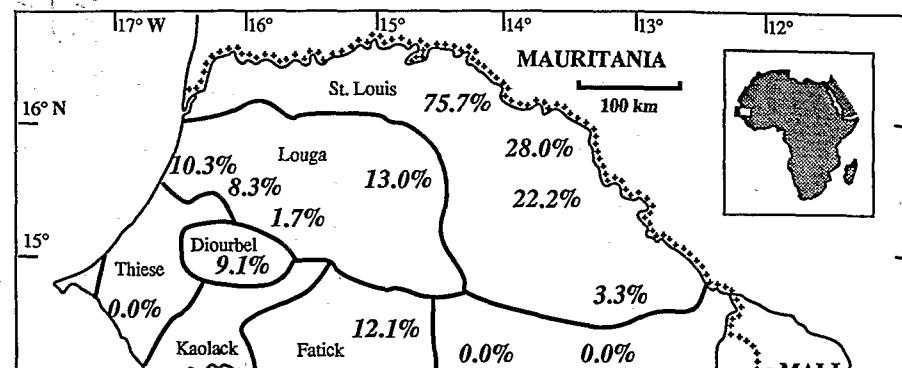
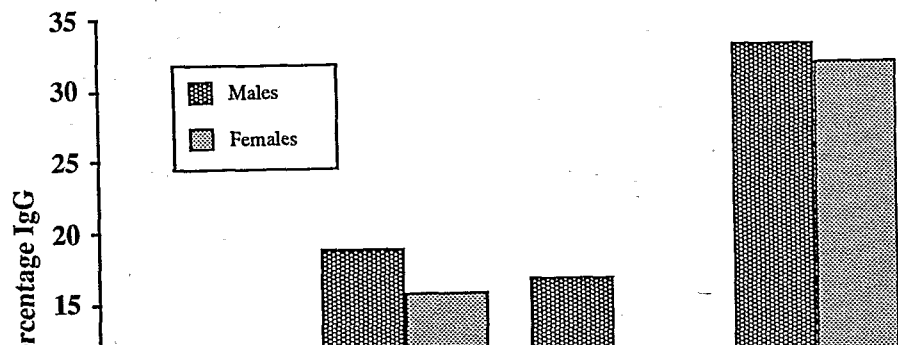
The specificity of this test was examined by using Dugbe, Bandia, and Bakel viruses as antigen, the 3 other major Nairoviruses that have been frequently isolated in Senegal and identified at the WHO Collaborating Center for Arbovirus Reference and Research. These viruses were tested against sera from a sheep inoculated with CCHF virus, a large sample of wild sheep that had tested positive against CCHF virus, and 3

samples of domestic ungulates³ combined with similar data from Gueye and others^{22–27} that were reanalyzed for this purpose. Ticks were detached from sheep, goats, or cattle monthly for ≥1 year in order to avoid bias due to seasonal variation in tick activity. We sampled 3 sites in northern Senegal: Yonofere, Dahra, and Bandia. In Yonofere, 5 flocks of sheep were chosen monthly by chance encounter from May 1987 through May 1989 and 10 randomly selected individuals from each herd were sampled. All ticks were removed with forceps and stored for later identification. The same methods were used in Dahra from May 1987 through August 1989. In Bandia, 12 sentinel goats and 2 cows were examined similarly 3 times each month from April 1987 through March 1989.

Published data from other sites in Senegal^{22–27} were reanalyzed to make them consistent with our methods. We calculated the mean number of adult ticks per host from monthly samples of 40 cattle, sheep, or goats made for ≥12 consecutive months during 1985–1989. Because these ticks differ somewhat in their propensity for feeding on the 3 hosts, we transformed the data from all 7 sites to qualitative values that represent the relative abundances of each tick species among the sites. Each tick was categorized as either very

TABLE 1
Prevalence of IgG antibodies against Crimean-Congo hemorrhagic fever virus among 942 sheep from 66 randomly selected villages from 9 regions in Senegal, November 1986–January 1987

Region	No. villages sampled	No. sheep tested	Ratio M:F	IgG prevalence (%)
St. Louis	2	37	1:8.3	75.7
	1	9	1:2	22.2
	1	16	1:1.7	28
Louga	3	30	1:3.3	3.3
	7	78	1:10.1	10.3
	8	192	1:11.8	13
	4	60	1:3.3	13.3
Thies	4	60	1:2.2	1.7
	3	48	1:2.4	0
Diourbel	3	44	1:3	9.1
Fatick	3	41	1:7.2	22
Kaolack	3	65	1:4.9	4.6
	3	33	1:7.3	12.1
Tambacounda	2	20	1:4	0
	2	21	1:20	0
	2	22	1:4.5	0
	1	12	1:3	0
Kolda	2	21	1:20	0
	4	43	1:6.5	0
	1	19	1:18	0
Ziguinchor	4	34	1:5.8	0
	3	37	1:2.1	0
Totals	66	942	1:4.7	10.4



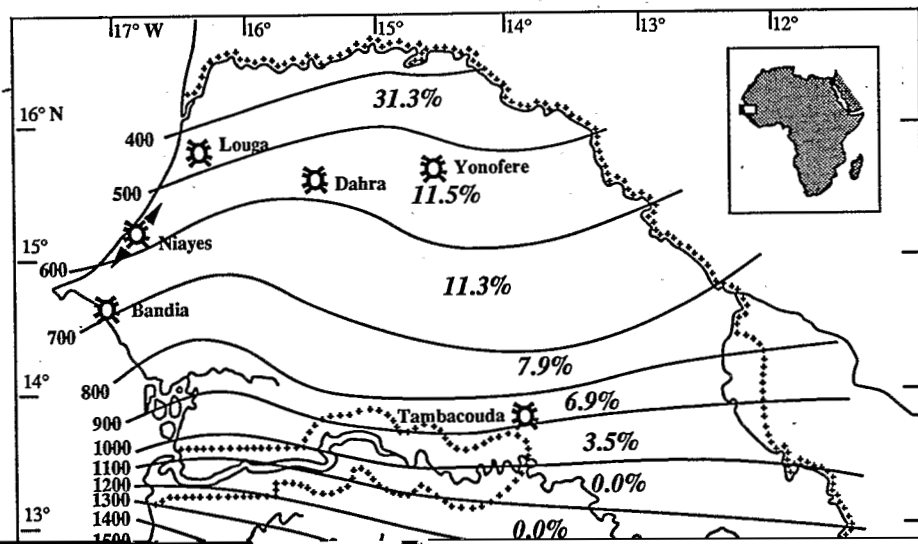


TABLE 3
Relative abundances of adult ixodid ticks on domestic sheep, cattle, and goats in selected sites and bioclimatic zones in Senegal

Tick species*	Relative abundance in zones and villages†						
	Sahelian		Sahelo-Sudanian			Sudano-Guinean	
	Dahra	Yonofere	Louga	Niayes	Bandia	Tambacounda	Kolda
<i>H. truncatum</i>	+++	+++		+++	+++	++	+
<i>H. impeltatum</i>	+++	+	+		+		
<i>H. m. rufipes</i>	+	+		++	++	+++	+
<i>H. impressum</i>				+	+		
<i>H. dromedarii</i>	•						
<i>R. guilhoni</i>	+++	+++	+++	+	+++		+
<i>R. e. evertsi</i>	•	+		+	+	+++	
<i>R. sulcatus</i>				+	+		+
<i>R. senegalensis</i>				+		•	
<i>R. lunulatus</i>						•	
<i>R. muhsamae</i>					+	•	
<i>A. variegatum</i>				+++	++	+	+++
<i>B. decoloratus</i>				+++	+	•	
<i>B. geigy</i>							+++

* Genera are *Hyalomma*, *Amblyomma*, *Boophilus*, and *Rhipicephalus*.
† Very abundant, +++; moderately abundant, ++; rare, +; extremely rare, •, and absent (no mark). Based on our observations in Dahra, Yonofere, and Bandia and reanalyzed previously published data from studies by Gueye and collaborators for Louga,²³ Niayes,²⁷ Tambacounda,²⁴ and Kolda.²⁵

sheep in southern Senegal, low-level transmission was suggested by the presence of human antibodies, and by the previous results of Chukhikhin and others⁴ showing 8.6% of cattle (n = 93) from that area with antibodies. Indeed, cattle may represent a more sensitive indicator of low level CCHF virus circulation, as they can be 10-fold more heavily infested by *Hyalomma* ticks than are sheep,³² and, on average, they are longer-lived.

The tick vectors that maintain CCHF virus transmission in Senegal are unknown, although our results indicate that ≥ 1 *Hyalomma* species are important.³² Results from other studies have suggested a correlation between *Hyalomma* tick abundance and virus transmission,¹ even though numerous species from 6 other genera have been shown to be infected.³ Our study demonstrated a positive correlation between the spatial patterns of *H. truncatum*, *H. impetatum*, and *H. m. rufipes* abundance, and that of IgG prevalence in humans and sheep. A similar relationship with *R. guilhoni* was also apparent. Although *A. variegatum* and other *Rhipicephalus* species were present where evidence of CCHF virus transmission was found, these ticks were most abundant in the central and southern sites where virus circulation appears less active. *Hyalomma* ticks predominated in areas where transmission to humans and sheep was most intense, and CCHF virus has been isolated in Senegal from those 3 species that were most abundant.³ Thus, certain *Hyalomma* ticks are circumstantially implicated in the enzootic cycle. In addition, 4 species of 3 other genera have been shown to be infected in Senegal,³ notably *R. guilhoni* and *A. variegatum*. Epidemic transmission may include some of these ticks. Controlled studies of horizontal and vertical transmission using native vertebrate hosts are needed to examine the actual role of each potential vector.

Human cases of CCHF are seen rarely in West Africa, enigmatic in that seroprevalences are at

cases of CCHF occur without diagnosis. Deaths in remote villages may go unreported or may be reported without etiology. Thus, underestimation of severe disease and fatalities could be considerable. Because CCHF virus appears to be weakly- or non-pathogenic to domestic ungulates, and is rarely diagnosed in humans, only large-scale epidemiological studies will expose the true intensity of virus circulation. More systematic, prospective serological studies and increased attention to the etiologic agent in hemorrhagic fever cases may reveal widespread transmission of CCHF virus and its true frequency as a cause of human morbidity and mortality.

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BOOKS REVIEWED

M.D.: One Doctor's Adventures Among the Famous and Infamous from the Jungles of Panama to a Park Avenue Practice, by B. H. KEAN with TRACY DAHLBY. XII + 402 pages. Ballantine Books, New York. 1990. \$19.95.

I hate to tell people that I am a parasitologist because I always get the same responses. Most often the person I'm talking to changes the subject, since he probably has no idea of the functions of a parasitologist. The second most common response is, "It must be really fascinating to study ESP." Sometimes, the person has had a stool examination at one time, and thinks that parasitologists spend all their time looking at feces. These people usually ask me what made me decide to choose such an "interesting" career. I used to have trouble answering this question. Well, not anymore. Now I simply tell them to read Ben Kean's book.

I count myself among the 5,000 or so Cornell Medical School students who have heard Kean lecture on parasitology. Some teachers of parasitology give dense, detailed lectures about life cycles, epidemiology, symptomatology, and control. Not Ben Kean. He tells stories. Out of a 50 minute lecture period, he may tell anecdotes for 40 minutes and then show a life cycle and a few pathology sections just before the bell rings. But, he always makes his listeners so interested in the diseases that they then go home and learn the details from the textbook. As a student, I was enthralled by the picture he painted of a strange and primitive, yet critically important, field. It was during one of his lectures that I decided to become a parasitologist.

This book contains Kean's best parasite stories. He recounts in detail, for example, his experiences with an elderly uranium baron and his entourage (including a voluptuous blond mistress named "Baby") as the

boss is purged of a tapeworm. And how he was caught by a female physician while watching a movie starlet gyrate nude in an attempt to make her ascaris dizzy. There are also fascinating medical detective stories, such as how he discovered the cause of an epidemic of trichinosis among telephone company employees and the cause of an epidemic of toxoplasmosis among medical students.

Kean's non-tropical adventures are equally amusing and interesting. There are wonderful sections about post-war Germany and about the Shah of Iran (his patient), which give a doctor's perspective on important historical events. He tells about his meeting with FDR to discuss whether sharks really ate downed airmen and his debilitating attack of turista just prior to his meeting with the Pope. And his stories about his famous patients, such as Salvador Dali, Oscar Hammerstein, Martina Navratilova, and Frank Loesser, are funny and touching (this chapter could have been entitled "Feces of the Rich and Famous").

Kean was born in Indiana and grew up in Greenwich Village. After finishing his medical training, he spent the war years as a pathologist at the Gorgas Memorial Laboratory in Panama and several years after the war in the U.S. military as a health officer in Germany. He returned to New York and began a double life as a physician on Park Avenue and a professor in academia. In both worlds, Kean has had a knack for adventure, and he has collected some truly fascinating and funny anecdotes in this book.

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