Field Investigations of an Outbreak of Ebola Hemorrhagic Fever, Kikwit, Democratic Republic of the Congo, 1995: Arthropod Studies

Paul Reiter, Michael Turell, Russell Coleman, Barry Miller, Gary Maupin, Jorge Liz, Ana Kuehne, James Barth, Joan Geisbert, David Dohm, Jason Glick, James Pecor, Richard Robbins, Peter Jahrling, Clarence Peters, and Thomas Ksiazek

Dengue Branch, Centers for Disease Control and Prevention (CDC), San Juan, Puerto Rico; US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland; Arbovirus Branch, CDC, Fort Collins, Colorado; Viral and Rickettsial Zoonoses Branch and Special Pathogens Branch, CDC, Atlanta, Georgia; Department of Entomology, Walter Reed Army Institute of Research, and Armed Forces Pest Management Board, Walter Reed Army Medical Center, Washington DC

During the final weeks of a 6-month epidemic of Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo, an extensive collection of arthropods was made in an attempt to learn more of the natural history of the disease. A reconstruction of the activities of the likely primary case, a 42-year-old man who lived in the city, indicated that he probably acquired his infection in a partly forested area 15 km from his home. Collections were made throughout this area, along the route he followed from the city, and at various sites in the city itself. No Ebola virus was isolated, but a description of the collections and the ecotopes involved is given for comparison with future studies of other outbreaks.

In the first 6 months of 1995, a major outbreak of Ebola (EBO) hemorrhagic fever (EHF) occurred in Kikwit, Democratic Republic of the Congo (DRC). Of 317 cases reported, 244 (77%) were fatal [1].

By coincidence, a popular account of previous outbreaks of hemorrhagic disease [2] and a movie of a fictional outbreak in the United States (*Outbreak*, Warner Brothers, released March 15, 1995) had recently achieved worldwide success. This synchrony of popular fiction with actual events energized the news media to capitalize on the Kikwit story, focusing on the frightening pathology, contagious nature, high mortality, and enigmatic origins of the disease, all in the scenario of a remote city in the African rain forest. The attention generated by this unprecedented level of publicity lent a special urgency to scientific investigation. An international team, coordinated by the World Health Organization, arrived in early May. One month later, when sufficient epidemiologic information was available, a second group of specialists was dispatched to search for the origins of the virus and the factors that had led to its emergence. By this time, the epidemic was in its final stages. (The last identified case died on July 16.)

Despite investigations of three previous outbreaks, virtually nothing was known of the natural history of EBO. The geographic, temporal, and ecologic setting of transmission was unclear. The only suggestion of a reservoir was that bats had been present in a cotton factory that was the workplace of the first identifiable cases during three outbreaks in southern Sudan [3–5], and they had also been present in a Kenyan cave where two infections of Marburg hemorrhagic fever (MHF), a closely related filovirus, were assumed to have been acquired [6]. There was no evidence for or against transmission by arthropods, although a case in Rhodesia (now Zimbabwe) coincided with a lesion "compatible with a horsefly or spider bite" [7], and a single study had suggested that Marburg virus could replicate in *Aedes (Stegomyia) aegypti* mosquitoes after intrathoracic inoculation [8]. However, since many of the diseases that the public associate with the tropics are vector borne, there was strong pressure to include arthropods in the work of the international teams.

Herein, we report on that work, and discuss its merits in light of the failure to detect virus in the large quantity of material that was collected. The search for a vertebrate host is presented separately [9].

Background to the Investigation

The outbreak became known to the outside world in April 1995 but had probably begun in early January of that year. All cases appeared to have originated from 1 person [1], a 42-year-old man who had presumably made contact with the natural reservoir in the last week of 1994. For this reason, much of the search for that reservoir centered on the daily routine and environment of this putative primary case (identified as C1).

Kikwit and its surroundings. Kikwit (5°2′ S, 18°48′ E; population ~200,000) lies ~400 km west by southwest of the national capital, Kinshasa, and is the largest city in the Bandundu region. It is a terminal port on the river Kwilu, a tributary of the Kasai, which flows into the river Congo. Most of the inhabitants are Bantu. The dominant ethnic groups are Kwango

Reprints or correspondence: Dr. Paul Reiter, Chief, Entomology Section, CDC Dengue Branch, 2 Calle Casia, San Juan, Puerto Rico 00921-3200 (ipr1@cdc.gov).

The Journal of Infectious Diseases 1999;179(Suppl 1):S148–54

0022-1899/99/79S1–0024$02.00

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1999		**00-00-1999 to 00-00-1999**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Field Investigations of an Outbreak of Ebola Hemorrhagic Fever, Kikwit, Democratic Republic of the Congo, 1995: Arthropod Studies	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Walter Reed Army Institute of Research,Department of Entomology,Washington,DC,20307**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**7**	

and Kasai. Several African languages are spoken in the area, but French is the lingua franca.

European settlement began in 1901, and the region became a flourishing center of trade and agricultural production. Extensive rubber plantations later gave way to cultivation of oil palm, groundnuts, cassava, and maize. Today, however, Kikwit is an impoverished, declining city in a country that has become among the poorest in the world. The road to the capital, once a journey of about 4 h, is now almost impassable during rainy weather. Roads and footpaths in the city are also severely eroded. Public utilities are virtually nonfunctional, and most homes are without domestic water or sewage systems. At night, there is virtually no street lighting. Education and health facilities are severely limited, and there is no local radio or newspaper.

Most homes are built of mud and wattle, with thatched or corrugated iron roofs. Indeed, much of the city has more the appearance of a conglomeration of traditional villages than a modern urban area; there are many small subsistence plots where cassava, maize, and other staples are grown, and most families keep sheep, goats, chickens, and other domestic animals. Many species of wild birds are common, as are rats and other small mammals.

Local agricultural produce is still shipped to Kinshasa, and there is paid work on the oil palm plantations, but most of the inhabitants survive by subsistence farming and petty trade. Indeed, a striking feature of the city is the number of people who commute to the surrounding countryside: At daybreak, thousands can be seen leaving the city, mainly on foot, and they return at nightfall laden with agricultural produce, firewood, charcoal, and other items. Among these returning crowds are cattle and other domestic livestock, many from far afield, that are driven to the city markets for slaughter. Other meats, such as monkey, pangolin, guinea pig, and rat, plus a variety of dried insects and other creatures are also sold for food.

Geobotanically, the region is part of the Guineo-Congolese and Zambesian transitional region, a low plateau with dense gallery forest along the numerous river courses and derived savanna on the uplands. The growth of the human population over the past 30 years has practically eliminated the primal forest around the city, as an ever-increasing radius of land has been cleared for timber harvest, fuel wood, and the cultivation of food staples.

The climate is moist equatorial. Mean daily temperatures are almost constant throughout the year (monthly mean, 24.2°C; range of monthly means, 23.6–24.5°C), but there is a well-defined rainy season from October to April [10]. Thus, C1 became infected during the rainiest period of the year, whereas our investigations were done at the height of the dry season, when rainfall was rare, but a heavy mist and dew often formed at sundown.

Daily routine of the primary case. The following description is given in detail to emphasize the variety of ecotopes that C1 encountered during his normal day, any of which could presumably have harbored the putative EBO reservoir.

C1 lived in the city with his extended family of 20–30 people. The family compound was relatively large and well maintained. Surviving members told us that his daily routine involved a 12-h round trip of >30 km in the country. His two principal occupations were cultivation (maize and cassava) and charcoal production. He also trapped rodents and other small animals for food.

C1 left home at 6:30 A.M., shortly after sunrise, and cycled via a bridge across the Kwilu River to a paved road that runs eastward along the northern flanks of the river valley. After 9 km, he turned onto a dirt logging track. Nearby, on the banks of a tributary river, was a small family compound, where he broke the first stage of his journey to drink and bathe. He then followed the track for 4 km, passing through cultivated plots (mainly cassava and maize), fallow land with trees (mostly secondary growth), and small patches of primary forest. A single family compound about midway along this section was the only other permanent human habitation in the area.

He habitually left his bicycle at the rotted hollow stump of a large tree. From there, he walked for ~150 m through maize and cassava plots into a woodland area known as Mbwambala, where he spent the rest of the day. Two kilometers of foot track connected his activities in this area (figure 1). The track led south, first through more patches of cultivation on level ground and then down a slope for ~150 m to a point where a side track led to the site of his charcoal mound.

The mound was rectangular, roughly 4 m × 2 m, and set in a pit dug ~1.5 m into the soft earth. During the firing phase, it probably stood ~1.75 m above the ground. The extent of the surrounding clearing indicated that the site had been worked for several years. At the time of his death, C1 had completed a production cycle and had removed most of the charcoal. Nothing of note was observed in the vicinity except for a small but deep (60 cm) hole dug among the roots of an adjacent tree, perhaps for excavation of a root or other edible item.

The land around the charcoal mound was mainly secondary woodland. Much of the understory was dominated by a leafy, 1- to 2-m-high brush plant, *Chromolaena (Eupatorium) odoratum*, which was dense and difficult to penetrate. A scattering of smooth-boled trees, some up to 45 m tall, survived from the original forest, but many of these were dead or in a deteriorated condition. The only patch of seemingly undisturbed primary forest was on an almost vertical face ~150 m west of the charcoal mound.

Below the side track, the main track led down a steep slope to more level ground, where C1 had cultivated a small patch of maize. The crop had been harvested, perhaps shortly before he became infected.

C1 had a second cultivation patch ~1.5 km from the first. He reached this through cultivated and wooded land, clambering up and down several steep, muddy slopes. Felled trees served to bridge a small river that was almost stagnant at the time of our investigation but probably flowed more swiftly during the rainy season. On the west side of this river, the

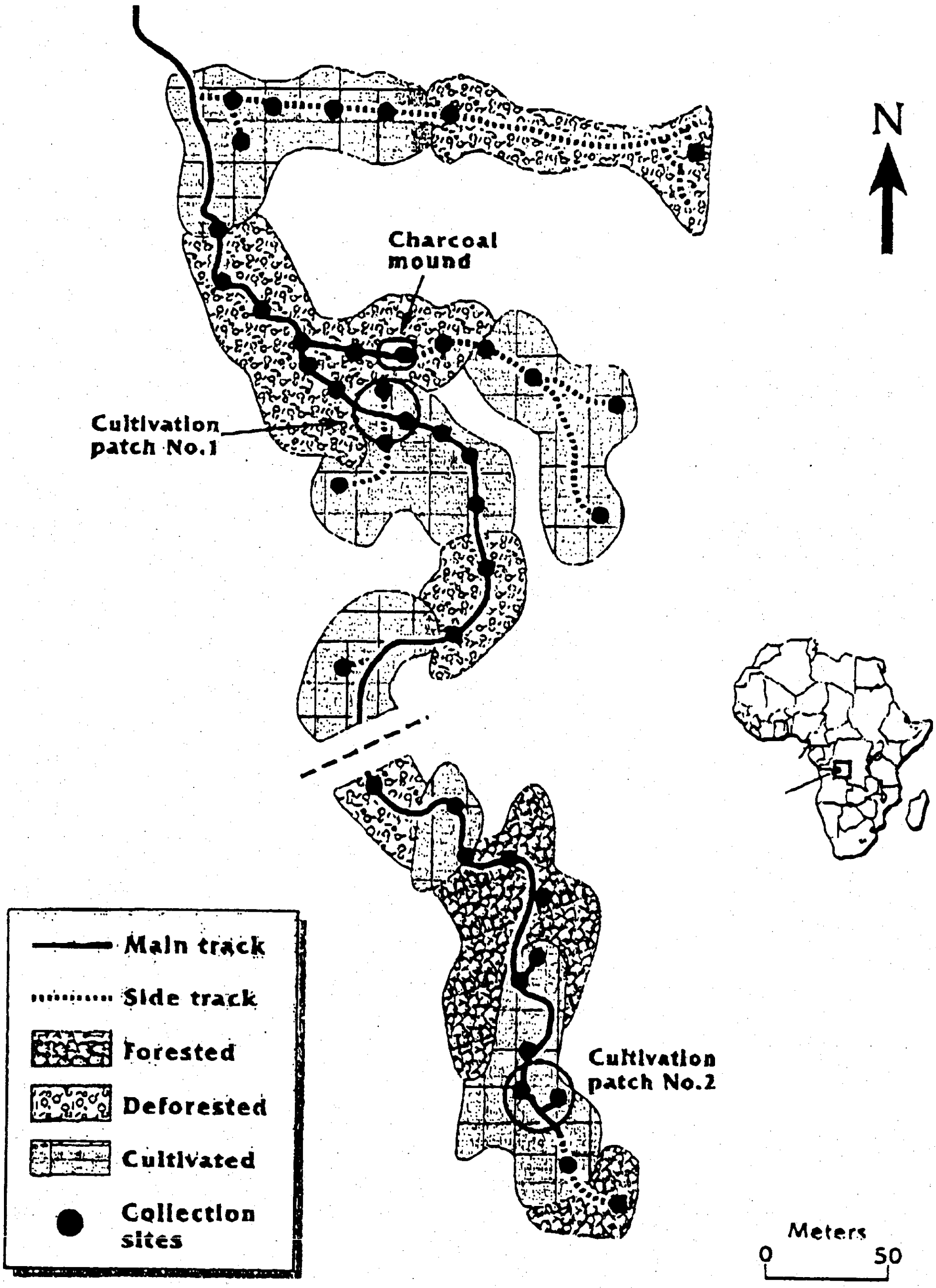

Figure 1. Collection sites in Mbwambala woodland. Principal area of activity for primary Ebola hemorrhagic fever case in weeks preceding his illness.

track led down a precipitous slope, which retained much of its primary forest. On the east side, it passed through fallow land bordered with dense, tall, grassy vegetation. After fording a small stream, it ended at a steep south-facing slope, C1's second cultivated site. At the foot of this slope, bordered by the stream, was a level patch of marshy grass and plants. In a drier part of this patch he had erected a small shelter, where he rested on the ground in the hotter part of the day. C1 had cultivated maize on the lower levels of the slope and cassava on the land above. Since his death, the patch had been reclaimed by secondary vegetation, dominated by *C. odoratum*. At the top of the slope was a patch of dense woodland, much of which was primary vegetation.

During the week that he became infected, C1, assisted by his wife and at least 2 daughters, had been harvesting maize in his second cultivated patch. The work involved unsheathing the cobs and stripping the grain. Neither this nor any other of his activities were unique: The same work was being done at the same time by many other families in the surrounding woodland. Thus, during our investigations, C1's track was al-

most deserted, apparently for fear of EHF, but plenty of people were present in the surrounding area. Two men were felling trees and operating a charcoal mound in the woodland above his second cultivated site, and a steep slope on the opposite side of the stream was planted with maize and cassava. Snares for trapping small rodents and other food items were quite common, and survivors of C1's family confirmed that he had operated such devices himself. Indeed, local rumor maintained that he had acquired EHF by stealing a rat from the trap of a neighbor.

Arthropod Collections

Arthropods were collected from 10 June to 14 July 1995. The focus was on hematophagous species, but samples of non-biting arthropods were also retained. Without any hint as to which orders were most likely to yield virus, the effort was directed at collecting as many specimens from as many different groups as possible. There was no firm evidence that C1 had been infected in the woodland area, so collections also were made in the city and at sites along his route from the city to Mbwambala. City collections were centered around homes where ≥4 residents had contracted EHF and at Kikwit General Hospital. Last, ectoparasites were collected from domesticated animals, especially cattle driven in from the surrounding countryside for slaughter.

Light traps. Battery-powered light traps were set at 40 sites along C1's track and along 3 adjacent transects chosen to represent all the apparent biotopes in the woodland area. Each device was supplemented with CO_2, when available, from a bag of dry ice (imported by plane from South Africa or Switzerland). On other nights, about half of the traps were supplemented with a live guinea pig in a perforated plastic container.

Most traps were suspended ~1.5 m above the ground. Near to the charcoal mound, two were hoisted to the level of the lower canopy, 6–8 m above the ground. These were operated without rain covers, as this appeared to augment the catch of phlebotomine sandflies. Traps were also run in an area of primary forest close to the Kwilu River (12 traps), along the river itself (5 traps), and between the isolation ward and the morgue at the hospital (2 traps).

Gravid traps. CDC gravid traps were used at five sites in the woodland area. They were first used with a leaf infusion and later with dry ice or a live guinea pig.

Goat-baited trap. A small goat was tethered under a mosquito bed net in the pit of the charcoal mound. A gap of ~12 cm between the lower edge of the net and the earth allowed entry of insects, which were collected in the morning by battery-powered aspirator [11].

Sticky traps. Sticky tape and paper coated with a sticky material was placed overnight at the entrance to a number of holes and animal burrows along C1's route, around the goat-baited trap, around the animal and CO_2-baited gravid traps, and on the floor of the morgue. The aim was to collect host-seeking ticks or other arthropods attracted to these sites, but the method was not effective, perhaps because of the heavy nocturnal dew.

Malaise trap. A trap was operated for a few days next to C1's bower, but no hematophagous arthropods were captured, and the collections, mainly small Diptera and Lepidoptera and Orthoptera, were badly damaged by the dew.

Backpack aspirators. Mosquitoes resting indoors were collected by 3 teams, 2 men per team, who visited about 400 houses in Kikwit, focusing on the vicinity of homes where ≥4 cases of EHF had been reported. Collections were also made at Kikwit General Hospital, in the EHF convalescent ward, and in the morgue.

Manual collections. A wide range of standard methods was used to collect non–host-associated ticks, including CO_2 traps, flagging, dragging, and swabbing of burrows and tree holes. A single specimen (a nymphal *Haemaphysalis* species) was the sole product of these collections. Several recently felled trees were scoured, and two were deliberately felled for collections. All had hollow cavities with evidence of bat habitation, but no ticks were found.

Six collectors, operating in pairs, removed ectoparasites (i.e., ticks, fleas, and lice) from cattle and other animals. Most of the cattle had been driven on the hoof into the city from a 50-km radius of surrounding countryside, some from as far as 75 km away. Collections were also made from domestic animals and birds living in and around Kikwit. A few ticks were found on wild animals, such as tree pangolins, cane rats, and palm civets.

Bedbug collections were made from bedding in 76 homes, 11 of which had harbored 20 recent fatal cases of EHF.

Collections of arthropods landing on humans were proscribed by safety regulations, but 11 tsetse were captured along the tributary to the Kwilu River, close to the road.

Processing of specimens. Specimens were brought to Kikwit, immobilized by short exposure to low temperature, sorted by major taxonomic group, and transferred to liquid nitrogen. After shipment on dry ice to the United States, they were identified to species on a chill table, pooled (<25 per pool), and stored at −70°C. Pools were triturated in 2 mL of diluent (10% heat-inactivated fetal bovine serum in medium 199 with Earle's salts, antibiotics, and $NaHCO_3$), divided into multiple aliquots, and returned to storage. Aliquots from 10 such pools were combined into "super pools" and tested for the presence of virus under biosafety level 4 conditions by intracranial inoculation into newborn ICR mice (Charles River Breeding Laboratories, Wilmington, MA) and strain 13 guinea pigs and by cell culture in Vero cells.

Results

Arthropods. A total of 34,985 arthropod specimens were collected (table 1), of which 27,843 were selected for virus isolation, identified to family or species (table 2), and processed

Table 1. Arthropod collections, Kikwit and rural surroundings, June to July 1995.

Arthropod	No.
Mosquitoes	18,878
Bedbugs	9139
Ticks	6166
Sandflies	341
Fleas	198
Nonbiting flies	149
Lice	103
Tsetse flies	11
Total	34,985

in 90 super pools. Of the 15,118 mosquitoes that were tested (54% of the total), 4,651 (31%) were *Culex quinquefasciatus* and *Anopheles (Celia)* species (probably of the *gambiae* complex), all of which were captured indoors in urban Kikwit. The bulk of the remainder were captured in rural sites, mainly by light trap.

The human bedbug *(Cimex hemipterus)* was the next largest group, with an average of 109 captured per home. Nearly all of the 6166 ticks were collected from dogs (51%) or domestic cattle (42%), as reflected in the species composition of the catch. Most of the lice and fleas were from domestic dogs, cats, and ducks.

Viruses. No EBO virus was isolated from any of the super pools. A single isolate of a bunyavirus (Bwamba group) was made from a superpool of *Anopheles (Celia)* species captured by backpack aspirator at indoor sites. The virus was subsequently reisolated from the original pool, which came from inside a home in the city. No other isolates were made.

Discussion

A hallmark of the filoviruses is their apparent rarity or at least the rarity of their transfer to humans. Indeed, in Kikwit, as in all previous outbreaks, the impressive number of people who did not acquire the virus, despite being involved in the same activities in the same environment as the unlucky individual who did, is as striking as the number of people who subsequently became infected after contact with the primary case. In this context, the chances of encountering an arthropod vector, if one exists, are probably dauntingly small. However, zoonoses are not test-tube relationships between 1 or 2 species but complex systems that operate in a complex environment. In Kikwit, we were able to focus closely on the activities of the putative primary case and the environment where he presumably acquired his infection. Thus, although our investigations did not yield virus, they did provide information that may be useful in investigations of future outbreak areas.

Much of our effort centered on C1's forest activities because previous human infections with EBO and Marburg viruses may all have involved contact with a forest reservoir. In the Congo Basin of northern DRC, the Yambuku [12] and Tandala [13] outbreaks of EHF were in the zone of transition from tropical rain forest to savanna.

In southern Sudan, a 1976 outbreak of EHF in Nzara (population ~20,000) began in workers at the local cotton factory, but the primary case lived 10 km from that factory in a remote homestead set in dense woodlands [3], also in the transition

Table 2. Number of arthropod specimens pooled and tested for Ebola virus from total collected in Kikwit, Democratic Republic of the Congo, June to July 1995.

Arthropod	No. pooled and tested
Mosquitoes	
Anopheles (Cellia) species	3157
Anopheles longipalpis	1
Coquillettidia metallica	173
Coquillettidia (Coquillettidia) species	106
Culex cinereus	779
Culex (Culex) species	7368
Culiseta fraseri	9
Uranotaenia bilineata	1
Aedes aegypti	83
Aedes (Aedimorphus) species	40
Coquillettidia microannulata	378
Coquillettidia (Coquillettidia) annettii	422
Culex quinquefasciatus	1494
Culex (Culiciomyia) species	368
Mansonia africana	739
Total mosquitoes	15,118
Phlebotomines	
Sergentomyia (Neophlebotomus) species	113
Sergentomyia schwetzi	9
Sergentomyia species	2
Total	124
Bed bugs	
Cimex hemipterus	6538
Fleas	
Ctenocephalides felis	43
Echidnophaga gallinacea	101
Total	144
Lice	
Linognathidae	9
Menoporidae	69
Philopteridae	10
Trichodectidae	15
Total lice	103
Ticks	
Rhipicephalus appendiculatus	25
Rhipicephalus longus	25
Rhipicephalus longus/appendiculatus	3
Rhipicephalus sanguineus	2570
Rhipicephalus species	27
Haemaphysalis paraleachi	736
Amblyomma variegatum	1775
Boophilus decoloratus	655
Total	5816
Total arthropods	27,843

NOTE. Use of "species" indicates that exact species was not determined.

zone of the northern boundary of the Congo forest [4]. A second wave of cases that originated in the same factory appeared unrelated to the original chain of transmission, but here again, each family lived in a remote homestead [3]. In 1979, yet another outbreak was traced to a primary case at the same factory, but a review of the absentee and illness records failed to incriminate the site as an active source of the infection [5].

Likewise, 2 human cases of MHF in Kenya, which were separated by an interval of 7 years, appeared to be associated with entering a cave on Mount Elgon [6, 14]; however, access to the cave was by a foot trail through primary rain forest encircled by land that had been partially logged and cultivated, an area strongly reminiscent of the site of C1's activities near Kikwit. It is entirely possible that the infections were acquired in this forest, and there is serologic evidence of filovirus infection among people living in a partly forested area at the base of the mountain, including a well-documented fatal case with no travel history to the cave [15].

A feature common to all the infection areas is the presence of disturbed forest and the "commuting activities" of infected persons between that forest and urban centers. It may be that accessibility reveals the presence of EBO virus by enabling patients to seek medical attention, but it is also conceivable that tree-felling brings the reservoir to ground level or that the ecologic changes associated with logging promote species that are associated with the virus, augmenting the probability of their contact with humans. Even the nomadic forest pygmies of Southeast Cameroon, 17% of whom were seropositive for EBO virus infection, were influenced by an "extensive road system built by the logging companies, so that the forest is becoming more visible and accessible, and bandas (camps) that used to be 30 km deep into the forest now may be only 3 km from a road" (Webb PA; personal communication, from report to CDC, 1980). This description is strongly reminiscent of the Kikwit area, and it would be interesting to compare the ecology of all the outbreak areas that have been studied. Unfortunately, virtually no information is available, perhaps because failure to isolate virus persuaded previous investigators that publication was not worthwhile.

In arbovirus studies, the proportion of infected specimens captured in the field is usually measurable in fractions of a percent. Therefore, maximum effort is made to obtain large numbers of the specific vectors involved by using methods best suited to collect them. In Kikwit, we did not have the luxury of knowing which order of arthropod to collect, so we opted to collect the maximum number of species and specimens from as many sites as possible. However, some ecotopes, such as the upper canopy of large forest trees, were beyond our reach. The final listing (table 1) may appear impressive in numbers, but ~60% of the total catch were urban species. These were unlikely to have made contact with the unknown reservoir, although they clearly had the highest possibility of contact with viremic patients. *C. quinquefasciatus* was the dominant species (table 2), followed by mosquitoes of the *Anopheles gambiae* complex. EBO virus has been reported to replicate after inoculation in adult *A. aegypti* [8], a species that was also present; however, recent studies by Turell et al. [16] have failed to repeat this finding, and replication was not observed in *C. quinquefasciatus* or *Anopheles stephensi*.

Collections outside the city were less likely to yield virus. Many mosquitoes collected by light trap are unfed nullipars, so they have not had contact with any potential host species. Dry ice increases the proportion that has gone through at least one gonotrophic cycle, and the use of live guinea pigs may have had the same effect. However, without information on the preferred hosts of the captured insects, we have no way of knowing whether they are likely to be primatophilic. We did not make human-bait collections, so we have no information on primatophilic woodland *Aedes* species, which are active during the day and not attracted to light traps. Moreover, the arthropod fauna in the middle of the rainy season, when C1 became infected, may have been very different from that during the driest time of the year, when we made our collections. Last, the dense overgrowth of secondary vegetation on his abandoned land clearly constituted a change in the ecology of the area.

An alternative approach to our study would have been to ignore the events in the field and concentrate on transmission studies in the laboratory. Indeed, after our return, several species of mosquitoes, cockroaches, ticks, spiders, and other arthropods were inoculated with EBO virus and showed no evidence of replication [16]. However, this work was done with an Asian strain of the virus (subtype Reston), which is markedly different from the African viruses, both in replication rate and pathology [17]. Moreover, such studies do not rule out the possibility that a species not included in the tests may be receptive to infection.

A third approach could have been to restrict field studies to the vertebrate population, assuming that this includes the reservoir. Once the reservoir was identified, attention could have shifted to likely modes of infection, including studies of hematophagous arthropods associated with the relevant species. However, despite a major effort, the vertebrate collections in Kikwit also did not yield any EBO virus and were subject to the same problems of sampling logistics [9].

Last, seasonality is important in disease transmission, even in the humid tropics. Our studies should have begun soon after C1 was infected, but this was impossible because >4 months had passed before we even learned of the outbreak. We would have preferred to delay our efforts until the rainy season, but the urgency of making a visible response to the widely publicized human tragedy was an important factor in the decision-making process. Indeed, heavy media coverage, as illustrated by the presence of numerous international television crews in Kikwit, has become a significant element in such field investigations and needs to be addressed if it is not to distort scientific research.

Acknowledgments

Heartfelt thanks go to our team of cheerful, hard-working collectors: Alain Ndumandele, Alphonse Musamu, Appolinaire Bilier,

Adolf Bin Bwalungu, Laurent Musalu, Etienne Mbula, Jean Kisuka, Jean Lundu, Samuel Ndeke, and William Ntama. Figure 1 was drawn by Willy-R. Sangibala N'Kumat and Godefroid Mubanga Nzo-Ayum, both of the Bureau de Cartographie de l'I.S.P., Kikwit, with the assistance of Alphonse Kimbiti Kalume and Ikama Daki. Marlon Wolcott prepared the map for publication. We also gratefully acknowledge the logistic support of Ethleen Lloyd (CDC), Mark Pelletier (USAID, Kinshasa), and Lynette Simon (USAID, Kinshasa). Last, our mission would have been far less enjoyable without the cheerful dinnertime discussions with our landlord and host, George Quintas.

References

1. Khan AS, Tshioko FK, Heymann DL, et al., for the Commission de Lutte contre les Epidémies à Kikwit. The reemergence of Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995. J Infect Dis 1999; 179(suppl 1):S76–86.
2. Preston R. The hot zone. New York: Random House, 1994.
3. World Health Organization. Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team. Bull World Health Org 1978;56:247–70.
4. Arata AA, Johnson B. Approaches towards studies on potential reservoirs of viral haemorrhagic fever in southern Sudan (1977). In: Pattyn SR, ed. Ebola virus haemorrhagic fever. Amsterdam: Elsevier/North–Holland Biomedical Press, 1978:129–35.
5. Baron RC, McCormick JB, Zubeir OA. Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. Bull World Health Organization 1983;61:997–1003.
6. Smith DH, Johnson BK, Isaacson M, Swanepoel R, Johnson KM, Killey M. Marburg-virus disease in Kenya. Lancet 1982;1:816–20.
7. Conrad JL, Isaacson M, Burnett Smith E, et al. Epidemiologic investigation of Marburg virus disease, southern Africa, 1975. Am J Trop Med Hyg 1978;27:1210–5.
8. Kunz C, Hofmann H, Aspöck H. Die vermehrung des "Marburg-Virus" in *Aedes aegypti*. Zentralbl Bakteriol I Orig 1968;208:347–9.
9. Leirs H, Mills JN, Krebs JW, et al. Search for the Ebola virus reservoir in Kikwit, Democratic Republic of the Congo: reflections on a vertebrate collection. J Infect Dis 1999;179(suppl 1):S155–63.
10. National Oceanic and Atmospheric Administration. National Climatic Data Service. Global Historical Climatology Network. http://www.ncdc.noaa.gov/ol/climate/research/ghcn/ghcn.html. Version 1. August 1992.
11. Clark GG, Seda H, Gubler DJ. Use of the "CDC backpack aspirator" for surveillance of *Aedes aegypti* in San Juan, Puerto Rico. J Am Mosq Ctrl Assoc 1994;10:119–24.
12. Brès P. Report of the Informal Consultation on the Marburg virus-like disease outbreaks in the Sudan and Zaire in 1976, held at the London School of Hygiene and Tropical Medicine, 4 and 5 January 1977. World Health Organization Report 1977;VIR/77.1.
13. Heymann DL, Weisfeld JS, Webb PA, Johnson KM, Cairns T, Berquist H. Ebola hemorrhagic fever: Tandala, Zaire, 1977–1978. J Infect Dis 1980;142:372–6.
14. Johnson ED, Johnson BK, Silverstein D, et al. Characterization of a new Marburg virus isolated from a 1987 fatal case in Kenya. Arch Virol 1996;11:101–14.
15. Teepe RGC, Johnson BK, Ocheng D, et al. A probable case of Ebola virus haemorrhagic fever in Kenya. East Afr Med J 1983;60:718–22.
16. Turell MJ, Bressler DS, Rossi CA. Lack of virus replication in arthropods after intrathoracic inoculation of Ebola Reston virus. Am J Trop Med Hyg 1996;55:89–90.
17. Fisher-Hoch SP, Brammer TL, Trappier SG, et al. Pathogenic potential of filoviruses: role of geographic origin of primate host and virus strain. J Infect Dis 1992;166:753–63.