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Managing infectious disease in developing countries: studies following epidemics of yellow fever, o'nyong-nyong fever, and leptospirosis

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Chapter 5

O'nyong-nyong Fever in South-Central Uganda, 1996-1997: Description of the Epidemic and Results of a Household-based Seroprevalence Survey

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Methods

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Abstract

O'nyong-nyong (ONN) fever, caused by infection with a mosquito-borne central African alphavirus, is an acute, nonfatal illness characterized by polyarthralgia. During 1996-1997, south-central Uganda experienced the second ONN fever epidemic ever recognized. During January and early February 1997, active case finding and a household cluster serosurvey were conducted in two affected and two comparison areas. A confirmed case was defined as an acute illness with fever and polyarthralgia occurring within the previous 9 months, plus serologic confirmation or isolation of ONN virus from blood. In affected ($n = 129$) and comparison ($n = 115$) areas, the estimated infection rates were 45% and 3%, respectively, and the estimated attack rates were 29% and 0%, respectively, for an apparent-to-inapparent infection ratio of nearly 2 in affected areas. In villages sampled near Lake Kijanebalola, Rakai District, the estimated infection and attack rates were 68% and 41%, respectively, and 55% of sampled households had experienced ≥ 1 ONN fever case. In conclusion, this epidemic was focused near lakes and swamps, where it was associated with high infection and attack rates.

Key Words: O'nyong-nyong virus; alphavirus; arbovirus; epidemiology; Uganda; seroprevalence

Introduction

O'nyong-nyong (ONN) virus is a mosquito-borne alphavirus that, during 1959-1962, caused a major central African epidemic that began in northern Uganda and involved an estimated 2 million patients in Kenya, Tanzania, and Uganda alone.¹ This self-limiting and previously unknown disease was characterized by debilitating polyarthralgia, often accompanied by skin rash and lymphadenitis.² During 1959-1962, 46 strains of ONN virus were isolated from acutely ill patients in 15 locations in Uganda, Kenya, and Tanzania.¹ Although no fatal cases of ONN fever were documented, the epidemic was associated with substantial morbidity. Labor-intensive production and agricultural industries were significantly affected in some areas, with 25% or more of the labor force - often 10% at one time - missing at least 5 days of work.³ Two of the region's major malaria vectors, *Anopheles gambiae* and *An. funestus*, were implicated as the primary epidemic vectors of this virus.^{4 5} Although the epidemic waned in Uganda during 1961, ONN fever cases were documented in an area extending from Mozambique to Senegal during 1960-1962.⁶ In 1978, a strain of ONN virus was isolated from *An. funestus* collected in western Kenya.⁷ Although no cases of ONN fever were documented after 1962, results of seroprevalence studies suggested that sporadic human infections with ONN virus continued to occur within the region.^{8 9 10 11} The enzootic vectors and natural reservoir hosts of ONN virus remain unknown.

In mid-1996, following an apparent absence of approximately 35 years, the first recognized re-emergence of ONN fever occurred in south-central Uganda.¹² The earliest cases were noted in the mostly rural Rakai District. Within a few weeks, the epidemic had extended to portions of the adjacent Masaka and Mbarara districts. Vertebrate studies conducted during the epidemic failed to incriminate rodents or mongooses as amplifying hosts of the virus.¹³ Although a strain of ONN virus was isolated from *Mansonia uniformis* mosquitoes, *An. funestus* was implicated as the primary epidemic vector.¹⁴ Results of molecular virologic studies suggested that a single genotype of ONN virus circulated during this epidemic, and indicated that this genotype shared a high degree of genetic and protein sequence homology with an ONN virus strain isolated in northern Uganda in 1959 during the previous epidemic.¹⁵

This paper provides a general description of the 1996-1997 epidemic and results of a serologic and virologic survey of humans conducted during January and early February 1997 in the affected region. A more detailed description of clinical features and an analysis of the utility of a purely clinical case definition for surveillance purposes will be published separately (*Chapter 6*).

Methods

Description of the epidemic area. The Rakai District, with a 1997 population of ~444,500, is irregularly shaped with a surface area of ~4,100 square km, and lies just below the

equator. Lake Victoria and Tanzania form its eastern and southern borders, respectively (figure 1). Its topography consists mostly of heavily vegetated rolling hills. Numerous small-to medium-sized lakes occur in the district's interior, often fringed by papyrus swamps. The largest interior lake is the centrally located Lake Kijanebalola. The district's equatorial climate is moderated by the elevation of the land, typically 1,200-1,500 m above sea level. Rainy seasons occur in March-April and October-November. Few improved roads exist in the district. In the countryside, inhabitants typically live in houses constructed of mud and sticks or of crude bricks, with roofs made of thatch or corrugated metal, usually with open eaves. Most inhabitants engage in subsistence farming (e.g., bananas, coffee, cassava, mango, jack fruit, pineapple, sorghum, tobacco, sweet potatoes) or fishing or both. Small, government-run hospitals operate in a few of the largest towns, while outpatient clinics and dispensaries are maintained in many of the smaller towns and villages. Practitioners of folk medicine are common throughout the area.

Active case finding

For purposes of this study, a presumptive clinical case of ONN fever was defined as an acute febrile illness with polyarthralgia occurring within the previous 9 months (May 1996 to February 1997).

Two teams of researchers surveyed the Rakai District and adjacent portions of the Masaka and Mbarara districts region during January 23-25, 1997. In selected towns or villages, village leaders, health-care workers, folk medicine practitioners, druggists, and other inhabitants were questioned about the occurrence in their area of a disease matching the description of ONN fever. The term "o'nyong-nyong" derives from a northern Ugandan dialect¹⁶ and is generally meaningless in southern Uganda, where more common local terms for ONN fever included "kyikonyogo" (roughly translated as "beaten on the joints with a stick") and "kyikutiiya". Informational leads were pursued and persons meeting the clinical case definition were located, interviewed, and sometimes bled. In this manner, major North-South and East-West transects of the Rakai District and adjacent portions of the adjacent Masaka and Mbarara districts were crudely sampled.

Selection of study sites

Four sites were selected for more intensive study: two sites at which cases (either current, recent, or both) meeting the clinical case definition of ONN fever were relatively abundant ("affected sites"), and two comparison sites at which few, if any, such cases were reported. One affected site (Site #1) included two villages located on the shores of Lake Kijanebalola, central Rakai District: on the southern lakeshore, Kasiika village, Byakabanda Subcounty, Kooki County, and approximately 10 km away on the northern lakeshore, Bbaale village (also selected for entomologic¹⁴ and vertebrate¹³ studies), Kagamba Subcounty, Kooki

County. The northernmost affected site chosen (Site #2) consisted of three villages located away from major lakes or swamps, but near riverine and marshy habitat: Bwingana village, Malongo Subcounty, Bukoto County, Masaka District, and Kigando and Nakaseta villages, located approximately 20 km away in Kaliiro Subcounty, Kabula County, northern Rakai District. The two comparison sites were Kiyooza village – located approximately 10 km southeast of the southern shore of Lake Kijanebalola in Byakabanda Subcounty, Kooki County, central Rakai District (Site #3) – and Kitto village in Kalisizo Subcounty and Mabaale village in Kyotera Subcounty, Kyotera County, northeastern Rakai District (Site #4), located 20-30 km northeast of Lake Kijanebalola (figure 1).

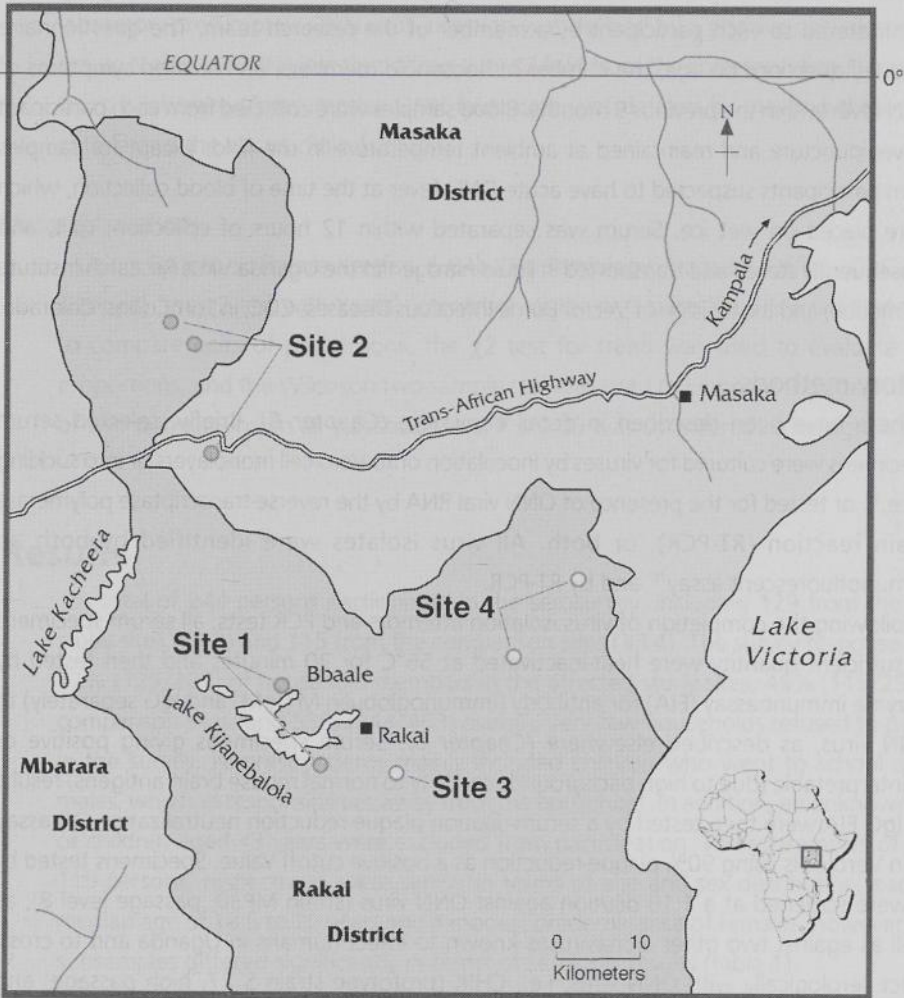


Figure 1. Map of south-central Uganda showing study sites

○ ONN Fever affected study sites: site 1, 2

○ Comparison study sites: site 3,4

Sampling methods

At each of the four study sites, a cluster survey of households was conducted in villages. Based on a modification of World Health Organization-Expanded Programme on Immunization vaccine coverage survey method, sample villages were divided into four sectors and a sector was randomly selected by flipping a coin twice.¹⁷ At the center of the selected sector, a cardinal direction was randomly selected by spinning a bottle or writing pen. Every second household was selected. If a household refused to participate, the next household in the cardinal direction was selected. Households were visited once. Blood samples were requested from all household members present, excluding those aged <3 years. A standardized questionnaire was verbally translated into the local language and administered to each participant by a member of the research team. The questionnaire included questions on age, the number of household members, and recalled symptoms of ONN fever within the previous 9 months. Blood samples were collected from each participant by venipuncture and maintained at ambient temperature in the field, except for samples from participants suspected to have acute ONN fever at the time of blood collection, which were placed on wet ice. Serum was separated within 12 hours of collection, split, and subsequently stored and transported in liquid nitrogen to the Uganda Virus Research Institute in Entebbe, and the Division of Vector-Borne Infectious Diseases, CDC, in Fort Collins, Colorado.

Laboratory methods

These have been described in detail elsewhere (*Chapter 6*). Briefly, selected serum specimens were cultured for viruses by inoculation onto Vero cell monolayers or into suckling mice,¹⁸ or tested for the presence of ONN viral RNA by the reverse-transcriptase polymerase chain reaction (RT-PCR), or both. All virus isolates were identified by both an immunofluorescent assay¹⁹ and by RT-PCR.

Following the completion of virus isolation attempts and PCR tests, all serum specimens of sufficient quantity were heat-inactivated at 56°C for 30 minutes and then tested by enzyme immunoassay (EIA) for antibody (immunoglobulin M [IgM] and IgG separately) to ONN virus, as described elsewhere (*Chapter 6*). Serum specimens giving positive or uninterpretable (due to high background reactivity to normal mouse brain antigens) results by IgG EIA were then tested by a serum-dilution plaque-reduction neutralization (N) assay²⁰ in Vero cells using 90% plaque-reduction as a positive cutoff value. Specimens tested by N were screened at a 1:10 dilution against ONN virus (strain MP30, passage level 8), as well as against two other alphaviruses known to infect humans in Uganda and to cross-react serologically with ONN virus, i.e., CHIK (prototype strain S 27, high passage) and Sindbis (SIN, prototype strain Ar 339, passage level 14) viruses.²¹ Using serial 2-fold dilutions, endpoint N titers were then determined for specimens that gave positive screening N results against one or more of these viruses.

Case definitions

A laboratory-confirmed ONN virus infection was defined by one or more of the following: virus isolation, a positive IgM EIA, or a positive convalescent-phase serum N titer (≥ 10) to ONN virus that was ≥ 4 -fold higher than the corresponding N titer to CHIK virus. Similarly, a confirmed case of ONN fever was defined by laboratory confirmation and meeting the above clinical criteria for presumptive ONN fever (i.e., a history of an acute febrile illness with polyarthralgia within the previous 9 months). Individuals who met these laboratory criteria but not these clinical criteria were considered to have had inapparent ONN virus infections.

ONN virus infections in humans commonly result in the development of similar titers of N antibodies against both ONN virus and the closely related CHIK virus.²² Therefore, a laboratory-presumptive ONN virus infection was defined by a positive convalescent-phase serum N titer to ONN virus that differed by ≤ 2 -fold from the corresponding N titer to CHIK virus; a laboratory-presumptive ONN fever case was defined by meeting these serologic criteria and the above clinical criteria.

Statistical methods

Using EpiInfo software version 6.04b (Epidemiology Program Office, CDC, Atlanta, Georgia), the χ^2 test with Yates's correction or the two-tailed Fisher's exact test was used to compare pairs of proportions, the χ^2 test for trend was used to evaluate series of proportions, and the Wilcoxon two-sample test was used to compare the age distributions of two populations. In all statistical tests, $P \leq 0.05$ was considered to be significant.

Results

A total of 244 persons participated in the serosurvey, including 129 from the affected study sites (#1-2) and 115 from the comparison sites (#3-4). The survey response rate was 62% (129/207) of household members in the affected study sites, 45% (115/256) in the comparison sites, and 53% (244/463) overall. Very few households refused to participate in the survey. Nonrespondents mainly included children who went to school and adult males, who had responsibilities away from the household. In addition, an unknown number of children aged < 3 years were excluded from participation. The subsamples of 129 and 115 persons, respectively, were similar in terms of age and sex distribution, each with a median age of 18.5 to 20 years and a modest predominance of females; however, the two subsamples differed significantly in terms of serologic results (table 1).

Among the 129 persons sampled at study sites #1 and #2, current, recent, or previous ONN virus infections were laboratory-confirmed in 36 (28%), and no infections with CHIK or SIN viruses were confirmed. Of these 36 laboratory-confirmed ONN virus infections, 2

Table 1. Demographic characteristics of, and frequency of o'nyong-nyong (ONN), chikungunya (CHIK), and Sindbis (SIN) virus infections in, persons (n = 244) sampled from ONN fever-affected and comparison study sites, south-central Uganda, 1996-1997

Characteristic	Affected sites (#1-2)	Comparison sites (#3-4)	P *
Sample size	129	115	
Median age in years (range)	20 (3-91)	18.5 (3-80)	0.8
No. females (%)	71 (55)	67 (58)	0.7
No. laboratory-confirmed ONN virus infections (%)	36 (28)	2 (2)	<0.001
No. virus isolations/no. Attempts	2/14	0/7	
No. IgM EIA-positive	12 [†]	1	
No. neutralization test-positive	30	1	
No. meeting clinical criteria for recent ONN fever [‡]	25	0	
No. laboratory-presumptive ONN virus infections [§] (%)	22 (17)	2 (2)	<0.001
No. meeting clinical criteria for recent ONN fever [‡]	12	0	
No. laboratory-confirmed CHIK virus infections (%)	0	1 (1) [¶]	0.5
No. laboratory-confirmed SIN virus infections	0	0	

* By the two-tailed Fisher's exact test except for age distribution, for which the Wilcoxon two-sample test was used.

[†] Two were positive by both IgM EIA and neutralization.

[‡] Fever and joint pain within the previous 9 months

[§] Positive neutralization titers against ONN and CHIK viruses differed by <4-fold.

[¶] Based on single convalescent-phase neutralization titer of 320. No compatible illness reported within the previous 9 months.

were confirmed by virus isolation alone (of 14 attempts), 10 by IgM EIA alone, 22 by N alone (median titer, 80; range, 20-320), and 2 by a combination of IgM EIA and N (titer, 40 and 160). Of these 36 patients, 25 met the clinical criteria for recent (within the previous 9 months) ONN fever cases, for an estimated attack rate of 19% (25/129) and apparent-to-inapparent infection ratio of 2.3 (25/[36-25]) (table 1). Of the 22 persons with previous ONN virus infections confirmed by N alone, 17 were aged ≤ 36 years and 13 met the clinical criteria for recent ONN fever.

At study sites #1 and #2, an additional 22 (17%) of 129 persons met the serologic criteria for laboratory-presumptive ONN virus infections, and 12 of these 22 met the clinical criteria for recent ONN fever (table 1). These 22 persons and the 36 persons with laboratory-confirmed ONN virus infections did not differ significantly in terms of age or sex distribution (data not shown) or the proportion who met the clinical criteria for recent ONN fever (12/22 vs. 25/36, $p=0.4$ by the χ^2 test). Therefore, the 36 laboratory-confirmed and the 22 laboratory-presumptive ONN virus infections were combined for further analysis, as were the 25 laboratory-confirmed and the 12 laboratory-presumptive ONN fever cases. These combined totals gave an estimated infection rate of 45% (58/129), an estimated attack rate of 29% (37/129), and an estimated apparent-to-inapparent infection ratio of 1.8 (37/[58-37]). The infection rate among females was higher than that among males, but the

difference was not significant (35/71 or 49% vs. 23/58 or 40%, respectively, $p=0.4$ by χ^2). Infection rates were inversely related to age, but this trend did not reach statistical significance ($p=0.2$, χ^2 test for trend; figure 2). The attack rate among females was higher than that among males, but the difference was not significant (24/71 or 34% vs. 13/58 or 22%, respectively, $p=0.2$ by χ^2). Attack rates were not significantly related to age ($p=0.1$, χ^2 test for trend; figure 2).

Among the 58 persons with laboratory-confirmed or -presumptive ONN virus infections at study sites #1 and #2, the median age was 15 years (range, 3-91) and 35 (60%) were females. Among the 37 laboratory-confirmed or -presumptive ONN fever cases, the median

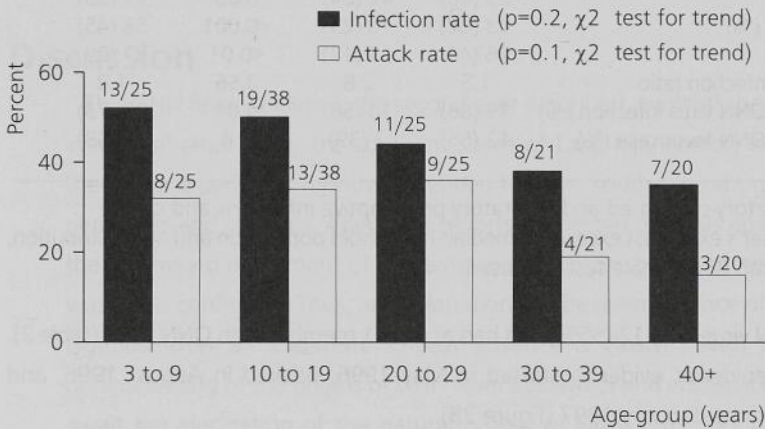


Figure 2. Infection and attack rates by age-group, o'nyong-nyong fever-affected areas, south-central Uganda, 1996-1997

date of illness onset was in September 1996 (range, May 1996 through January 1997) (figure 3A); the median age was 15 years (range, 3-52); 24 (65%) cases were in females; and 30 (86%) and 13 (37%) involved a rash or lymphadenitis, respectively (information on rash and lymphadenitis was available in 35 of 37 cases). In terms of the distribution of age, sex, and N titers (among those who were N positive), these 37 persons did not differ significantly from the 21 persons with laboratory-confirmed or -presumptive ONN virus infections who did not meet the study clinical criteria for recent ONN fever (data not shown).

The survey response rates at Site #1 and Site #2 were not significantly different (57% vs. 69%, respectively, $p = 0.09$, Fisher's exact test). Females constituted a higher percentage of respondents at Site #2 than at Site #1 (64% vs. 46%, respectively, $p = 0.05$, Fisher's exact test) (table 2). The estimated ONN virus infection rates and ONN fever attack rates were higher at site #1 than at site #2. At site #1, an infection rate of 68% (43/63), an attack rate of 41% (26/63), and an apparent:inapparent infection ratio of 1.5 (26/17) were estimated. Of 22 households sampled at Site #1, 19 (86%) had had at least 1 member

Table 2. Demographic characteristics, estimated o'nyong-nyong (ONN) virus infection rates, estimated ONN fever attack rates, and estimated apparent:inapparent ONN virus infection ratios in persons (n = 129) sampled from ONN fever-affected study sites, south-central Uganda, 1996-1997*

Characteristic	Study Site			
	#1	#2	P [†]	Total
No. households sampled	22	18		40
Total household population	111	96		207
Median household population (range)	5 (2-8)	5 (3-13)	0.9	5 (2-13)
No. persons sampled (%)	63 (57)	66 (69)	0.09	129 (62)
Median age in years (range)	16 (3-91)	20 (4-64)	0.2	20 (3-91)
No. females (%)	29 (46)	42 (64)	0.05	71 (55)
No. ONN virus infections (%)	43 (68)	15 (23)	<0.001	58 (45)
No. ONN fever cases (%)	26 (41)	11 (17)	<0.01	37 (29)
Apparent-to-inapparent infection ratio	1.5	2.8	0.56	1.8
No. households with ≥ 1 ONN virus infection (%)	19 (86)	10 (56)	0.04	29 (73)
No. households with ≥ 1 ONN fever case (%)	12 (55)	7 (39)	0.4	19 (48)

* Including both laboratory-confirmed and laboratory-presumptive infections and cases.

† By the two-tailed Fisher's exact test except for median household population and age distribution, for which the Wilcoxon two-sample test was used.

infected with ONN virus, and 12 (55%) had had at least 1 member with ONN fever (table 2). At this site, the epidemic evidently started in May 1996, peaked in August 1996, and continued at least into January 1997 (figure 3B).

At study site #2, an infection rate of 23% (15/66), an attack rate of 17% (11/66), and an apparent:inapparent infection ratio of 2.8 (11/4) were estimated. Of 18 households sampled at Site #2, 10 (56%) had had at least 1 member infected with ONN virus, and 7 (39%) had had at least 1 member with ONN fever (table 2). At this site, the epidemic evidently started in September 1996 and continued at least into early 1997 (figure 3B).

Of the 115 individuals sampled from 35 households at comparison study sites #3 and

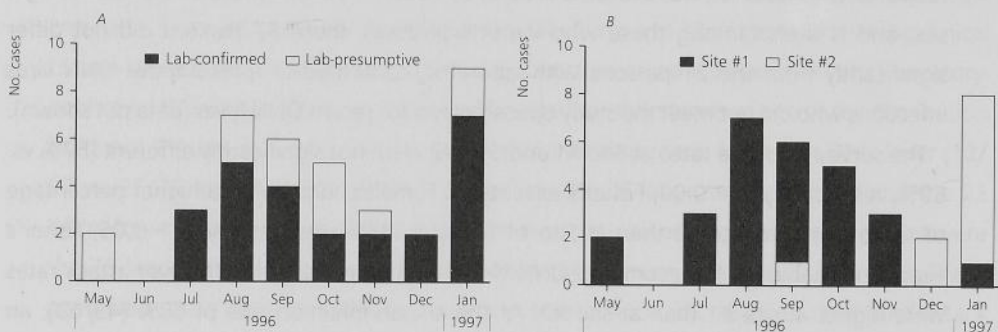


Figure 3. Dates of onset in 37 laboratory-confirmed or -presumptive cases of o'nyong-nyong (ONN) fever, south-central Uganda, 1996-1997, by A) case status and B) ONN fever-affected study site

#4, 1 was anti-ONN virus IgM-positive, 1 had evidence of a previous ONN virus infection by N, 1 had evidence of a previous CHIK virus infection by N, 2 had positive N titers to both ONN and CHIK viruses that differed from each other by $2\leq$ -fold, none were confirmed to have had recent cases of ONN fever, none had confirmed SIN virus infections, and none of 7 persons cultured for virus were positive (table 1). Of the 4 (3%) persons with confirmed or presumptive previous ONN virus infections, the IgM-positive person was an asymptomatic 16-year-old sampled at study site #3, while all the others were aged >36 years and sampled at study site #4.

Discussion

This ONN fever epidemic, the second ever described, evidently began during mid-1996 in villages situated along the southern shore of Lake Kijanebalola in the Rakai District and then, over a period of months, extended to other south-central Ugandan villages situated near lakes and swamps. Anecdotal reports suggested that this epidemic was triggered by the northward movement of a viremic person or persons from nearby Tanzania, but this was never confirmed. Thus, an explanation for the re-emergence of epidemic ONN fever in Uganda after an apparent 35-year absence is elusive. Even the most rudimentary understanding of the origins of ONN fever epidemics and endemic cases (if they occur) will await the elucidation of the natural history of ONN virus, including its enzootic cycles, reservoirs, and inter-epidemic vectors.

The previous ONN fever epidemic in Uganda evolved into a major regional epidemic that swept across much of Central Africa over more than a 2-year period,³ presumably caused largely by the movement of viremic humans. It is unknown why the epidemic of 1996-1997 apparently did not expand in a comparable fashion, especially given the fact that a major East-West trans-Central African highway traverses the northern portion of the Rakai District. Interestingly, unconfirmed reports of the extension of this epidemic were received during mid-1997 from parts of the Masaka District and from far west-central Uganda. Subsequently, 5 ONN fever cases with clinical onset in April-May 1998 were serologically confirmed from the far southern portion of the Rakai District [unpublished data].

In the current study, large differences in infection and attack rates between ONN fever-affected study sites and comparison sites were documented. In affected areas, the estimated overall ONN virus infection rate and ONN fever attack rate were 45% and 29%, respectively. In comparison areas, only 1 recent ONN virus infection was documented (based on the presence of IgM antibody) and no recent clinical ONN fever cases were identified. In one instance, the distance between an affected site (#1) and a comparison (#3) site was only a few kilometers. These marked geographic differences in human infection rates presumably

reflect the relative proximity to larval habitat of *An. funestus*, the most likely primary vector of ONN virus in this epidemic.¹⁴ Larvae of this mosquito species are found most commonly in larger bodies of clear, permanent water, such as lake shores, river margins, and swamps.²³ Because of this extreme geographic discontinuity and the relatively small population sampled, extrapolation of the results of this study to the general population of south-central Uganda is unfeasible.

Whether ONN fever was endemic in Central Africa during the inter-epidemic period of 1962-1996 remains speculative. It is conceivable that sporadic cases and smaller epidemics occurred in the region during that interval but were unrecognized or unreported. In 1978, a strain of ONN virus was isolated from *An. funestus* collected in western Kenya.⁷ Furthermore, results of seroprevalence surveys suggest that sporadic human infections with ONN virus occurred in east-central Africa during the inter-epidemic period, although no cases of ONN fever, per se, were reported.⁸⁻¹¹ In west-central Africa, however, cases of Igbo-Ora fever were recognized during that time period, and Igbo-Ora fever may yet be shown to be synonymous with ONN fever. Igbo-Ora virus is closely related to both ONN and CHIK viruses but distinguishable from them on cross-complement fixation tests.²⁴ This virus was first isolated in 1966 from Nigerian patients²⁵ and subsequently — as late as 1985 — from patients, *An. funestus*, and *An. gambiae* sampled in Ivory Coast.²⁶ The published clinical descriptions of Igbo-Ora fever are strikingly similar to those of ONN fever, except that, in Igbo-Ora fever, lymphadenitis was not commonly observed.²⁴⁻²⁶ Recent studies found 98% nucleic acid and 99% amino acid sequence homologies between a 1966 human isolate of Igbo-Ora virus from Nigeria and a 1996 human isolate of ONN virus from Uganda, which suggests that Igbo-Ora virus is a strain of ONN virus.¹⁵ Additional field observations will be needed to clarify whether or not these two viruses are clinically, epidemiologically, or ecologically distinct.

If ONN virus was endemic in south-central Uganda during the inter-epidemic period, an increasing prevalence of N antibodies to ONN virus associated with increasing age would be expected among persons born after the previous ONN fever epidemic had ended and who had no recent illnesses suggestive of ONN fever. However, among the 62 persons sampled in the current study from ONN fever-affected study sites (#1 and #2) and who were tested for N antibody to ONN virus, who had no recent illnesses suggestive of ONN fever, and who were born after 1962, seroprevalence actually decreased with increasing age, although the trend was not statistically significant (data not shown). These data are difficult to interpret, however, because individuals who were infected during the inter-epidemic period are impossible to distinguish from those subclinically infected during the recent epidemic. Furthermore, the serosurvey response rate was 62% (129/207), which may have biased survey results (see below).

In the current study, an apparent-to-inapparent infection ratio of 1.8 (37/21) was estimated. During the previous ONN fever epidemic in Uganda, an apparent-to-inapparent infection ratio of 4 (25/6) was estimated, also based on a retrospective study design.²² Such a relatively minor — and statistically insignificant — difference could be attributable to differences in the clinical case definitions used. For example, Williams and colleagues²² considered as clinical cases all study subjects who said they had suffered from “ONN fever” (with or without joint pain). This definition is somewhat more liberal than the clinical case definition used in the current study, which required the presence of joint pain as well as fever. Obviously, for many infectious diseases including ONN fever, apparent-to-inapparent infection ratios are difficult to estimate accurately, especially from retrospective studies, and depend largely on how clinical cases are defined. They also depend on the accuracy of clinical information, such as self-reports of fever, which can be unreliable.²⁷ Nevertheless, among those alphaviruses that typically cause self-limited diseases characterized by fever and joint pain, apparent-to-inapparent infection ratios of 1 or more are not unusual. For example, estimates of apparent-to-inapparent infection ratios were 4 for Mayaro virus²⁸ and from 0.02 to 3 for Ross River virus.²⁹

The current study has several limitations. First, the study was undertaken when the epidemic was waning in most affected villages in the midst of a dry season, and thus a largely retrospective design was necessary. Consequently, among a population experiencing frequent self-limited febrile illnesses, study participants were asked to recall details of an illness that occurred as many as 9 months previously. Secondly, ONN fever has never been well-studied in a prospective manner, and thus the optimal clinical case definition has yet to be determined. And although the case definition used in the current study (fever with joint pain) was probably highly sensitive, it may have been relatively nonspecific (*Chapter 6*). For these reasons, some misclassification of clinical cases in each direction undoubtedly occurred, and this could have affected estimates of clinical attack rates and apparent-to-inapparent infection ratios. Finally, the overall serosurvey response rate of 53% (244 of 463 household members) is low and, anecdotally, we observed that more adult males than females refused participation in the study. Young children are under-represented in the sample as well. Whether or not this biased the survey results is unknown, but they should be interpreted cautiously nonetheless.

With the possible exception of the routine use of mosquito bed nets (which was denied by virtually all 244 persons sampled in the present study; data not shown), no practical measures currently exist for the widespread prevention and control of ONN fever or other diseases transmitted by *An. funestus* and *An. gambiae*. Housing and living conditions common to the region make widespread vector exclusion measures impractical in the short term. Poverty and remoteness of many villages work against the widespread availability

of insect repellents. No vaccine against ONN virus exists. Anecdotal reports suggest that patients who developed apparent ONN fever in the Rakai District in 1996-1997 were often initially misdiagnosed with, and treated for, malaria. Thus, the cornerstone of an appropriate public health response to an epidemic of ONN fever generally should be the ascertainment of the geographic distribution and movement of the epidemic, and the dissemination of accurate information concerning the clinical and diagnostic features of the illness, the lack of specific treatment options, and the proper application of personal preventive measures. Communication between public health officials in neighboring countries in the region is essential.

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