O'Nyong-Nyong Fever in South-Central Uganda, 1996–1997: Clinical Features and Validation of a Clinical Case Definition for Surveillance Purposes

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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. Among 391 persons interviewed and sampled, 40 cases of confirmed and 21 of presumptive, well-characterized acute, recent, or previous ONN fever were identified through active case-finding efforts or during a household serosurvey and by the application of clinical and laboratory criteria. Among confirmed cases, the knees and ankles were the joints most commonly affected. The median duration of arthralgia was 6 days (range, 2–21 days) and of immobilization was 4 days (range, 1–14 days). In the majority, generalized skin rash was reported, and nearly half had lymphadenopathy, mainly of the cervical region. Viremia was documented in 16 cases, primarily during the first 3 days of illness, and in some of these, body temperature was normal. During this epidemic, the combination of fever, arthralgia, and lymphadenopathy had a specificity of 83% and a sensitivity of 61% in the identification of cases of ONN fever and thus could be useful for surveillance purposes.

O'nyong-nyong (ONN) virus is a mosquito-borne alphavirus that belongs to the Semliki Forest serological complex [1]. As such, it is closely related both genetically and clinically to chikungunya (CHIK), Mayaro, and Ross River viruses, which cause CHIK fever [2], Mayaro virus disease [3], and epidemic polyarthritis [4], respectively.

During 1959–1962, ONN virus caused a major central African epidemic of ONN fever that began in northern Uganda and involved an estimated 2 million patients in Kenya, Tanzania, and Uganda alone [5]. Typical clinical features included low-grade fever, symmetrical polyarthralgia, lymphadenopathy (particularly of the posterior cervical region), and a generalized papular or maculopapular exanthem [6]. Although some patients experienced prolonged joint pain during the recovery phase, no fatal cases or permanent sequelae were observed. Two of the region's major malaria vectors, *Anopheles funestus* and *Anopheles gambiae,* were implicated as the principal epidemic vectors of ONN virus [7, 8]. Although the epidemic waned in Uganda during 1961, ONN fever cases were documented in an area extending from Mozambique to Senegal during 1960–1962 [9].

After the epidemic of 1959–1962, results of seroprevalence studies suggested that sporadic human infections with ONN virus continued to occur within the region, though no cases of ONN fever were documented after 1962 [10–13]. Although a strain of ONN virus was isolated from *An. funestus* collected in western Kenya [14], the enzootic vectors and natural reservoir hosts of ONN virus remain unknown.

Beginning in mid-1996, after an apparent absence of ~35 years, the first recognized reemergence of ONN fever occurred in south-central Uganda [15]. The epidemic was focused near lakes and swamps, where it was associated with high infection and attack rates and an apparent-to-inapparent infection ratio of ~2 : 1 [16]. *An. funestus* was implicated as the primary epi-

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demic vector [17]. Vertebrate studies conducted during the epidemic failed to incriminate rodents or mongooses as amplifying hosts of the virus [18]. Results of molecular virological studies suggested that a single genotype of ONN virus circulated during this epidemic and indicated that this genotype shared a high degree of genetic homology with a strain isolated in northern Uganda during 1959 [19].

Here we describe the clinical features of the 1996–1997 epidemic, based on case ascertainment efforts during September and October 1996 and during January and early February 1997. This study was largely retrospective but included some acute ONN fever cases as well. Additionally, we evaluated a variety of purely clinical case definitions of ONN fever for surveillance purposes.

Methods

Case ascertainment. Beginning in June 1996, clinicians and local health officials in the mostly rural Rakai District of south-central Uganda noted an increase in cases of an acute febrile illness with polyarthralgia and, often, skin rash [15]. The typical case was characterized by onset of low-grade fever and malaise, followed ~2 days later by symmetrical polyarthralgia and often by lymphadenopathy, especially of the posterior cervical region, followed ~2 days later by a generalized maculopapular rash that was commonly pruritic. Patients usually had full recovery within 1–2 weeks of clinical onset, though some patients experienced more prolonged arthralgia.

Subsequently, in September and October 1996, Ugandan Ministry of Health officials conducted active case finding and collected blood samples and limited clinical data from selected residents of the Rakai District, including some who were acutely ill with symptoms and signs suggestive of ONN fever. Tests conducted at the Uganda Virus Research Institute and the Centers for Disease Control and Prevention (CDC) confirmed the presence of ONN virus in samples from some acutely ill patients.

During late January and early February 1997, Ugandan scientists were joined by a multinational team of researchers to conduct more detailed epidemiological, entomological, and vertebrate studies of the affected area [16]. The initial step was a preliminary, rapid, and crude survey of the region by automobile. Village leaders, health-care workers, folk medicine practitioners, druggists, and other inhabitants were questioned about the occurrence in their area of suspected ONN fever cases. Informational leads were pursued, and suspected case patients were located and interviewed and sometimes had blood samples obtained.

Subsequently, a cluster survey of households was conducted in selected villages [16], based on a modification of the World Health Organization–Expanded Programme on Immunization vaccine coverage survey method [20]. Blood samples were requested from all household members, excluding those aged <3 years. A standardized questionnaire was verbally translated into the local language and administered to each study participant by a member of the research team. This questionnaire included questions on age, the number of household members, symptoms of ONN fever recalled within the previous 9 months, and the approximate date of onset of symptoms.

Case definitions. Clinical criteria for an ONN fever case were an acute febrile illness with polyarthralgia and occurrence within the previous 9 months (May 1996 to February 1997). Among patients meeting these criteria, confirmed cases were defined as those associated with any of the following laboratory evidence of current or previous infection with ONN virus: virus isolation, the presence of serum IgM antibody to ONN virus, a positive convalescent-phase serum neutralizing antibody titer to ONN virus that was ⩾40 and was 4-fold or more higher than the corresponding neutralizing antibody titer to CHIK virus, or a positive convalescent-phase serum neutralizing antibody titer to ONN virus of 20 with a corresponding neutralizing antibody titer to CHIK virus of <10. Because ONN virus infections in humans commonly result in the development of similar titers of neutralizing antibodies to both ONN and CHIK viruses [21], presumptive ONN fever cases were defined as those associated with the presence of a positive convalescent-phase serum neutralizing antibody titer to ONN virus (⩾10) that differed by 2-fold or less from the corresponding neutralizing antibody titer to CHIK virus.

Laboratory methods. Single blood samples were collected from each study 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. For all specimens, serum was separated within 12 hr 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.

Serum specimens collected ⩽21 days after clinical onset were cultured for viruses by inoculation onto Vero cell monolayers or into suckling mice [22], or were tested for the presence of ONN viral genetic material by the reverse transcriptase–polymerase chain reaction (RT-PCR), or both. All virus isolates were identified by both an immunofluorescent assay [23] and RT-PCR.

For RT-PCR, 140 μL of starting material was extracted from serum, tissue culture fluid, or 10% suckling mouse brain suspension by use of the QIAamp viral RNA kit (QIAGEN, Valencia, CA) following the manufacturer's protocol. RNA was resuspended in a final volume of 100 μL of RNase-free water. ONN virus–specific primers (5′-CTGACTGCGGGGAGGGACACT-3′ [8698–8718] and 5′-ATCGTAATAACATCTTTGGTAGGTC-3′ [9765–9791]) were designed with the PrimerSelect module of the Lasergene software package (DNASTAR, Madison, WI) by use of the published genomic sequence of ONN virus strain MP30 ("Gulu") [24]. Five microliters of purified RNA was combined with 200 pmol of each ONN virus–specific primer, and the RT-PCR reaction was performed by use of the TITAN One Tube RT-PCR kit (Boehringer Mannheim Biochemicals, Indianapolis) following the manufacturer's protocol. Samples that generated a double-stranded DNA band of the correct size (1093 bp) were further analyzed by DNA sequencing. The 1093-bp DNA fragment was purified by electrophoresis on a 1% agarose gel followed by gel excision and extraction with the QIAquick gel extraction kit (QIAGEN); 500 ng of this material was then sequenced by use of the Taq DyeDeoxy Terminator Cycle sequencing kit (Perkin-Elmer/Applied Biosystems, Foster City, CA) and the same primers used for amplification. The

sequence data were aligned and edited with the SeqMan module of the Lasergene package.

After virus isolation attempts and PCR tests, all serum specimens were heat-inactivated at 56°C for 30 min and then tested by EIA for antibody (IgM and IgG separately) to ONN virus. Antigens were derived from suckling mouse passage 14 of ONN virus strain MP30 (kindly provided by Drs. Laura Chandler, Robert Tesh, and Robert Shope, University of Texas at Galveston). IgM antibody–capture EIA was done by using a modification of a previously published procedure [25]. After the wells of a 96-well Immunlon 2 microtiter plate (Dynex Technologies, Chantilly, VA) were coated with a predetermined quantity (75 μL/well) of goat anti-human IgM antibody in bicarbonate-carbonate buffer (pH 9.6) overnight at 4°C, wells were blocked with 200 μL of 5% skim milk and 0.5% Tween 20 in PBS for 30 min at room temperature. Serum samples (diluted 1 : 400 in PBS/0.05% Tween 20 and incubated at 37°C for 1 hr) were then tested in triplicate with ONN virus antigen (incubated overnight at 4°C) derived from infected, sucrose-acetone-extracted suckling mouse brain and with normal uninfected mouse brain antigen control prepared by the same method. In each well, 50 μL of an alphavirus group–reactive murine monoclonal antibody (MAb 2A2C-3) conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratories, West Grove, PA), diluted 1 : 4000 in blocking buffer, was added and allowed to incubate at 37°C for 1 hr. Bound enzyme-conjugate was detected with 75 μL of the substrate 3,3′,5,5′-tetramethylbenzidine (GIBCO Bethesda Research Laboratories, Gaithersburg, MD) per well. After this solution was incubated at room temperature for precisely 10 min, the reaction was stopped with 1 *N* H_2SO_4 (50 μL/well), and the absorbance at 450 nm (A_{450}) was measured [26]. Reactions were judged as positive if the signal-to-noise ratio (P/N) of the test sample was ⩾2.0 (A_{450} of the sample [P] divided by the A_{450} of a normal [N] human antibody control). Viral antigen–specific antibody reactivities were also compared with reactivities with normal mouse brain antigen. The test result was considered uninterpretable if the A_{450} of the test sample with viral antigen was less than or equal to twice the A_{450} of the test serum with normal mouse brain antigen.

IgG EIA was done by using a modification of a previously published procedure [27]. For this assay, purified ONN viral antigen was not directly coated into microtiter plates, but rather antigen was captured from infected, sucrose-acetone-extracted suckling mouse brain by use of a predetermined quantity of the alphavirus group–reactive capture MAb, 1A4B-6 [28]. This MAb was coated onto the wells overnight at 4°C in bicarbonate coating buffer, pH 9.6. Each well was then blocked with 200 μL of 3% normal goat serum in PBS with 0.1% Tween 20 for 30 min at room temperature. ONN viral antigen was then added and allowed to incubate overnight at 4°C. Negative-antigen control wells were also prepared by use of normal mouse brain antigen. Test serum samples and normal and positive human serum controls were added in triplicate at a 1 : 400 dilution and incubated at 37°C for 1 hr. Bound antiviral antibody was detected by adding alkaline phosphatase–conjugated goat anti-mouse IgG Fc-specific antibody (Jackson ImmunoResearch Laboratories) diluted 1 : 1000 in blocking buffer and by incubating at 37°C for 1 hr, followed by the addition of substrate (3 mg/mL *p*-nitrophenyl phosphate [Sigma 104; Sigma, St. Louis] in 1 *M* Tris-HCl, pH 8.0) and incubation for 30 min at room temperature. The reaction was then stopped with 35 μL of 3 *M* NaOH per well and the A_{405} was measured. As with the IgM EIA, P/N ratios were calculated, and serum samples with P/N values of ⩾2.0 were considered positive. The test result was considered uninterpretable if the A_{405} of the test sample with viral antigen was less than or equal to twice the A_{405} of the test serum with normal mouse brain antigen.

Serum specimens giving positive or uninterpretable (because of high background reactivity to normal mouse brain antigens) results by IgG EIA were tested by a serum-dilution plaque-reduction neutralization assay [29] in Vero cells, with use of 90% plaque reduction as a positive cutoff value. Specimens tested by neutralization were screened at a 1 : 10 dilution against ONN virus (strain MP30, passage level 8) as well as against 2 other alphaviruses known to infect humans in Uganda and to cross-react serologically with ONN virus: CHIK (prototype strain S 27, high passage) and Sindbis (prototype strain Ar 339, passage level 14) viruses [24]. By means of serial 2-fold dilutions, end-point neutralization tests were then done on specimens that gave positive screening neutralization results against one or more of these viruses.

Evaluation of clinical case definitions for surveillance purposes. By designating a positive ONN virus culture result or the presence of serum IgM antibody to ONN virus as the reference standard for infection, we evaluated various combinations of self-reported clinical features (fever, joint pain, rash, and lymphadenopathy) in terms of sensitivity and specificity. To minimize problems with recall, only cases in which the clinical onset was <6 weeks prior to interview and specimen collection were included.

Statistical methods. By use of EpiInfo software version 6.04b (Epidemiology Program Office, CDC, Atlanta, Georgia), the 2-tailed Fisher's exact test was used to compare pairs of proportions, and the Wilcoxon 2-sample test was used to compare the distributions of ordered categorical variables in 2 populations. In all statistical tests, $P \leqslant .05$ was considered to be significant.

Results

Serum samples and at least partial clinical data were collected from a total of 391 persons. Of these, 85 were sampled in September and October 1996, and 306 were sampled during January and February 1997. Of the latter, 62 were sampled during case ascertainment efforts and 244 [16] were sampled during the serosurvey. Of these 391 persons, 121 (31%) had laboratory evidence of a current, recent, or past infection with ONN virus (figure 1), 3 (0.8%) had evidence of a past infection with CHIK virus (based on comparative neutralizing antibody titers), and none had convincing laboratory evidence of a previous Sindbis virus infection. Of these 121 persons, 74 (61%) had confirmatory laboratory evidence of a current, recent, or past infection with ONN virus: 31 (42%) by IgM antibody testing, 27 (36%) by neutralization, and 16 (22%) by virus isolation (among 96 persons cultured). ONN virus was the only virus isolated during the study. The remaining 47 (39%) of these 121 persons had presumptive laboratory evidence of a past ONN virus infection (figure 1). Of these 121 persons, 68 (56%) reported a current or recent (within the previous 9 months) illness characterized by fever and joint pain and thus met the study clinical criteria

for ONN fever. Of these 68 persons, 47 (69%) were considered as having laboratory-confirmed cases and 21 (31%) were considered as having laboratory-presumptive cases (figure 1).

Of these 47 confirmed cases, 23 (49%) were confirmed by a positive serum IgM antibody test, 14 (30%) by neutralization and 10 (21%) by virus isolation (among 24 persons cultured). Complete questionnaire data were available for 40 (85%) of these 47 cases. Of these 40 cases, 20 (50%) were confirmed by a positive serum IgM antibody test, 14 (35%) by neutralization and 6 (15%) by virus isolation (among 20 persons cultured). Demographic and certain other characteristics of these 40 patients are shown in table 1. Symptoms reported by these patients are shown in table 2. By definition, all reported fever and joint pain. Knees and ankles were the joints most commonly affected. The median duration of joint pain was 6 days (range, 2–21 days) and of immobilization was 4 days (range, 1–14 days); these were unrelated to age or sex. The majority of patients reported headache and skin rash. Of those reporting skin rash, most described it as generalized and itchy. Nearly half of the patients reported lymphadenopathy and red eyes. Of those reporting lymphadenopathy, almost all reported involvement of the neck (figure 2). Bleeding of the gums was reported by one patient. No fatalities were reported.

Among the 20 IgM antibody–positive confirmed cases for which complete data were available, the median interval from onset of symptoms to sample collection was 19 days (range, 7–204 days). The prevalence of IgM seropositivity appeared to decrease ~60 days after the onset of illness (figure 3) (16 positive results among 23 patients sampled within 60 days of clinical onset vs. 4 positive results among 17 patients sampled >60 days after onset, $P < .01$). The patient with persistent IgM antibody to ONN virus ~204 days after clinical onset was a 28-year-old man who described a week-long illness consisting of fever, joint pain, generalized rash, and cervical lymphadenopathy. His serum sample showed neutralizing antibody titers of 10 and <10 against ONN virus and CHIK virus, respectively.

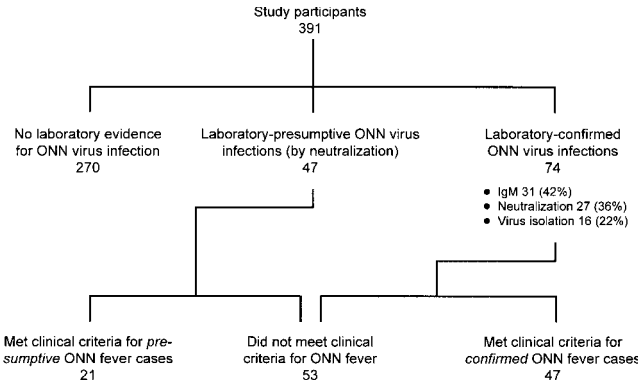

Figure 1. Summary of results of applying laboratory and clinical criteria for o'nyong-nyong (ONN) fever to 391 study participants in south-central Uganda, 1996–1997.

Among the 6 confirmed cases in which ONN virus was isolated and for which complete clinical data were available, the reported duration of illness at the time of sample collection was 1–3 days in all cases. Median body temperature measured at the time of sample collection in these cases was 37.4°C (range, 36.7°C–39.3°C), and temperatures were normal (<37.1°C) in 2 of these cases. Of the 10 other ONN virus–positive patients identified in the study, 1 reported fever but no joint pain, and 1 reported joint pain but no fever (and, thus, their cases did not meet the study clinical criteria); 4 reported both fever and joint pain (and thus their cases met the study clinical criteria) but a lack of complete clinical details precluded their inclusion in the group of 40 cases selected for detailed analysis; and clinical data were unavailable for 4 patients sampled in September 1996.

Table 1. Summary of demographic and other characteristics of total study population, and of 40 well-characterized laboratory-confirmed and 21 laboratory-presumptive o'nyong-nyong fever cases, south-central Uganda, 1996–1997.

Characteristic	Total study population	Clinically well-characterized laboratory-confirmed cases	Laboratory-presumptive cases	P[a]
n	391[b]	40	21	—
Age, years				
Median	20[b]	20	15	.9[c]
Range	3–91	5–80	3–45	—
No. female (%)	187[b] (56)	25 (62.5)	14 (67)	.8[d]
Date of symptom onset				
Median	—	Late Dec. 1996	Mid-Sept. 1996	—
Range	—	July 1996–Jan. 1997	May 1996–Jan. 1997	—
Interval from symptom onset to interview and blood sample collection, days				
Median	—	33	141	.004[c]
Range	—	1–204	12–261	—

[a] Comparing laboratory-confirmed and laboratory-presumptive cases.
[b] Age and sex data were unavailable for 67 and 60 persons, respectively, sampled during September–October 1996.
[c] Wilcoxon two-sample test.
[d] Two-tailed Fisher's exact test.

Table 2. Symptoms reported for 40 well-characterized laboratory-confirmed and 21 laboratory-presumptive o'nyong-nyong fever cases, south-central Uganda, 1996–1997.

Symptom	Proportion of patients reporting symptom (%)	
	Well-characterized laboratory-confirmed cases	Laboratory-presumptive cases
Fever	40/40[a] (100)	21/21[a] (100)
Joint pain	40/40[a] (100)	21/21[a] (100)
Knees	36/40 (90)	19/21 (90)
Ankles	33/40 (83)	15/21 (71)
Elbows	30/40 (75)	16/21 (76)
Wrists	30/40 (75)	14/21 (67)
Fingers	25/40 (63)	11/21 (52)
Immobilization	31/40 (78)	19/21 (90)
Headache	33/40 (83)	12/21 (57)
Rash	28/40 (70)	19/21 (90)
Generalized	22/28 (79)	13/16[b] (81)
Itchy	24/28 (86)	16/19 (84)
Lymphadenopathy	18/40 (45)	10/21 (48)
Cervical	15/16[b] (94)	5/9[b] (56)
Inguinal	9/16[b] (56)	7/9[b] (78)
Axillary	7/16[b] (44)	3/9[b] (33)
Red eyes	18/40 (45)	13/21 (62)
Bleeding gums	1/40 (3)	0/21
Nosebleed	0/40	1/21 (5)

[a] By definition (see text).
[b] Responses were missing in a few cases.

The 21 laboratory-presumptive ONN fever cases were demographically similar to the 40 well-characterized laboratory-confirmed cases (table 1). The median interval from reported onset of symptoms to interview and blood collection was significantly longer among laboratory-presumptive cases than among laboratory-confirmed cases. By definition, fever and joint pain were reported in all 21 laboratory-presumptive cases (table 2). Knees, elbows, and ankles were the joints most commonly affected. The median duration of joint pain was 7 days (range, 1–90 days) and of immobilization (reported in 19 of 21 cases) was 4 days (range, 1–28 days). The majority of patients reported headache and skin rash. Of those reporting skin rash, most described it as generalized and itchy. Nearly half of the patients reported lymphadenopathy and more than half reported red eyes. Of those reporting lymphadenopathy, half reported involvement of the neck. Nosebleed was reported by one patient. Statistically, symptoms in laboratory-confirmed and laboratory-presumptive cases differed only in terms of the proportions reporting cervical lymphadenopathy among those reporting lymphadenopathy (15/16 vs. 5/9; $P = .04$). No fatalities were reported.

Serum specimens from 76 (19%) of the 391 study participants were tested for ONN virus by both culture and RT-PCR. The concordance of culture (the reference standard) and PCR results was 96% (10 specimens were both culture- and PCR-positive; 63 specimens were both culture- and PCR-negative; 3 specimens were culture-positive and PCR-negative; and no specimens were culture-negative and PCR-positive). Compared with culture, PCR in this study demonstrated the following characteristics: sensitivity, 77% (10/13); specificity, 100% (63/63); predictive value of a positive test, 100% (10/10); and predictive value of a negative test, 95% (63/66).

The results of evaluating the sensitivity and specificity of various combinations of clinical features for surveillance purposes are shown in table 3.

Discussion

This is the second series of ONN fever cases ever described. Despite differences in the way the 2 case series were constructed and evaluated, the clinical features that characterized the current series are virtually indistinguishable from those that characterized the previous one [6]. The high degree of genetic homology observed between ONN virus strains circulating during the 2 epidemics [19] may help to explain the apparent consistency of clinical features between them.

The northern Ugandan series of nearly 21,000 ONN fever cases reported by Shore [6] was constructed retrospectively from outpatient and inpatient medical records and community sur-

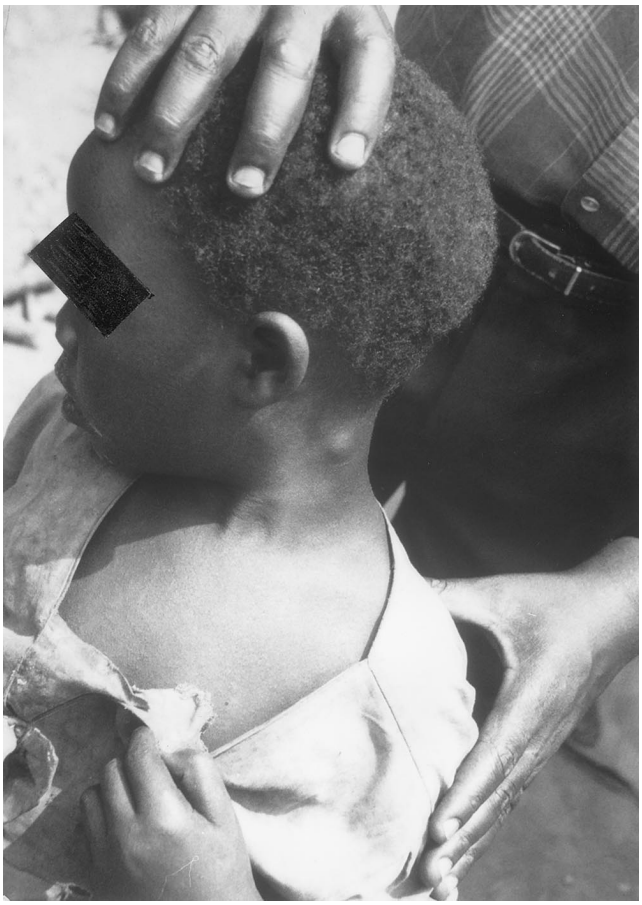

Figure 2. Patient with acute, culture-confirmed o'nyong-nyong fever and posterior cervical lymphadenopathy.

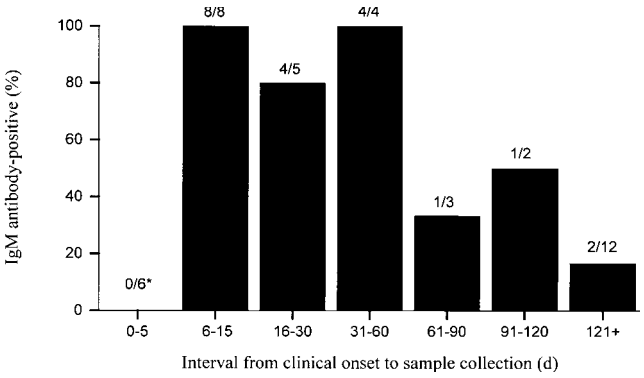

Figure 3. Prevalence (no. positive/no. tested) of IgM antibody to o'nyong-nyong (ONN) virus among 40 well-characterized laboratory-confirmed ONN fever cases, south-central Uganda, 1996–1997.

veys within the epidemic area. A case was defined in strictly clinical terms as an illness with joint pain with or without fever; there were no laboratory criteria. Thus, misclassification of cases in both directions was probably common. Interestingly, in 28% of nearly 600 clinically diagnosed cases in which a single measurement of body temperature was documented at the time of presentation, the temperature was normal (i.e., <37.1°C) [6].

The current case series also was constructed largely retrospectively, though some acute cases were observed. A case was defined by the presence of both joint pain and fever, as well as by specific laboratory criteria, including virus isolation or the presence of specific antibody in serum. Thus, we are reasonably confident that the ONN fever cases included in our series, especially the 47 laboratory-confirmed cases, are true cases. However, because fever (either self-reported or researcher observed) was included as a clinical case criterion, clinically less severe cases may be underrepresented in our series. Interestingly, though only a handful of acute cases were observed during the current study, among the 6 viremic cases in which both joint pain and fever were reported by patients, body temperature was found to be normal (as defined above by Shore [6]) in 2 cases. Therefore, in some cases of ONN "fever," elevated body temperature either is intermittent or does not occur at all, even during the acute, viremic phase of the illness. Afebrile viremia is also common in cases of epidemic polyarthritis [4].

Other observations by Shore [6] included that the combination of joint pains, rash, and lymphadenopathy occurred in ~40% of all cases, that the posterior cervical lymph nodes were commonly affected, that ONN fever more commonly affected females, that "some patients complained of epistaxis," and that, rarely, ONN fever can recur in individual patients, with episodes separated by a month or more. By comparison, in the current study, an illness characterized by the combination of joint pains, rash, and lymphadenopathy (with or without fever) was reported by only 17 (23%) of 74 persons with confirmatory laboratory evidence of current, recent, or past ONN virus infection. And, while 39 (64%) of 61 well-characterized laboratory-confirmed or laboratory-presumptive ONN fever cases identified in the current study were in females (table 1), this proportion is not significantly different from the proportion of females in the total sample. Anecdotally, we observed that more males than females refused participation in the study. Thus, results of this study provide no evidence that females are at greater risk than males for ONN fever. Although 2 (3%) of 61 well-characterized laboratory-confirmed or laboratory-presumptive ONN fever case-patients reported nosebleed or bleeding gums (table 2), a causal relationship between ONN fever and these mild, uncommonly reported hemorrhagic symptoms has not been established. In fact, the frequency of these symptoms among ONN fever cases may be no greater than among the general population. Finally, the present study documented no recurrent ONN fever cases, though its largely retrospective study design makes it impossible to rule out their occurrence. Despite the statement by Shore [6] to the contrary, which was based on solely clinical observations without virological confirmation, no convincing evidence exists to suggest that recurrent clinical infections occur with ONN virus or other members of the Semliki Forest antigenic complex.

As in the case series of Shore [6], lymphadenopathy, especially of the cervical region, was commonly reported as a clinical manifestation of ONN fever in the current case series. Shore [6] was of the opinion that by comparison, lymphadenopathy rarely if ever occurred in CHIK fever cases and that this fact could aid in distinguishing between these 2 diseases, which are otherwise very similar clinically [2]. More recent clinical descriptions of CHIK fever, however, have documented that lymphadenopathy, especially of the inguinal region, commonly occurs and that the posterior cervical nodes are affected in at least a few cases [30–32].

In the current study, we had no difficulty in isolating ONN virus from acutely ill patients. That this virus can readily be isolated from ONN fever patients during the acute phase is not a new observation. During 1959–1960, 46 ONN virus strains were isolated from acutely ill patients sampled in Uganda, Kenya, and Tanzania [5]. Furthermore, all of these strains were isolated from samples collected during days 1–6 of illness, and all but 2 strains were isolated from samples collected during days 1–3 of illness. Thus, our results were nearly identical to those of Williams et al. [5] in that in all 6 virus-positive cases for which dates of onset and complete clinical data were available, samples for culture had been collected during days 1–3 of illness. Therefore, as in most cases of CHIK fever [30], Mayaro virus disease [3, 33], and epidemic polyarthritis [34], in ONN fever cases a brief window of viremia commonly exists during the first few days of illness.

The current study is the first to evaluate the dynamics of the IgM antibody response to ONN virus infection. Although the data were derived from tests of single serum samples collected largely retrospectively, they suggest that detectable levels of se-

Table 3. Results of evaluation of sensitivity and specificity of various combinations of clinical features by designating positive result for culture of o'nyong-nyong (ONN) virus or presence of serum IgM antibody to ONN virus as reference standard for infection.

Clinical features	No. of evaluable study participants	Sensitivity (%)	Specificity (%)
Fever + joint pain	37	100	31
Fever + joint pain + (rash or lymphadenopathy)	33	76	58
Fever + joint pain + rash	34	68	58
Fever + joint pain + lymphadenopathy	35	61	83
Fever + joint pain + rash + lymphadenopathy	33	48	83

rum IgM antibody to ONN virus typically appear during the second week of illness and persist for ~2 months, though in a few cases, detectable IgM antibody may persist for 6 months or more. These dynamics are similar to those observed in epidemic polyarthritis [4, 35].

In the current study, we were unable to estimate the incubation period of ONN fever because mosquito exposure in these rural populations is ubiquitous and virtually continuous. The origin of the estimate by Shore [6] of ⩾8 days is unclear. Typical incubation periods of diseases caused by other members of the Semliki Forest complex include 3 days (range, 2–12 days) for CHIK fever [2, 6] and 9 days (range, 3–21 days) for epidemic polyarthritis [36].

Although ONN fever appears to be uniformly nonfatal, it causes substantial morbidity. The results of the current study suggest that in typical ONN fever cases, patients are immobilized for a median of 4 days, ranging as high as 2 weeks. During the epidemic of 1959–1962, labor-intensive production and agricultural industries were significantly affected in some areas, with ⩾25% of the labor force—often 10% at one time—missing at least 5 days of work [37]. Thus, although the social and economic costs of a large ONN fever epidemic have not been formally studied, they are undoubtedly significant.

Ideally, the public health response to a suspected epidemic of ONN fever or other viral illness would include sophisticated and timely laboratory support, both to confirm the etiologic agent and to help track the epidemic geographically and temporally. Unfortunately, in developing countries, strong laboratory support for epidemic investigations is often either unavailable or not available in a timely fashion. For this reason, we evaluated various purely clinical case definitions of ONN fever for public health surveillance purposes. The results suggest that the combination of fever, polyarthralgia, and lymphadenopathy is associated with the best combination of specificity (83%) and sensitivity (61%) (table 3). In the future, such a clinical case definition could assist public health officials throughout central Africa both by triggering appropriate laboratory-based investigations of suspected ONN fever epidemics and in tracking those ONN fever epidemics that are already laboratory confirmed. Obviously, because of its limited sensitivity and specificity, such a definition may be useful as a surveillance tool during epidemics but has no place in the evaluation of sporadic cases or in the clinical diagnosis or management of individual patients.

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 had occurred as many as 9 months earlier. Second, because blood samples were obtained from study participants only once, seroconversion to ONN virus was not available as a confirmatory test for recent infection. For this reason, we considered the presence of serum IgM antibody to ONN virus in single specimens as confirmatory evidence of recent infection. Finally, because virological evidence for cocirculation of CHIK virus during this ONN fever epidemic was absent, we did not test study participants for IgM antibody to CHIK virus. When tested by EIA, some patients recently infected with CHIK virus have been shown to have cross-reactive IgM antibody to ONN virus, albeit at much lower titers [38]. This raises the possibility that some ONN virus IgM antibody–positive participants in the current study had recently been infected with CHIK virus instead of ONN virus, although this seems unlikely. Nevertheless, for the above reasons, some misclassification of clinical cases in each direction undoubtedly occurred.

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