O'nyong-nyong fever: a neglected mosquito-borne viral disease

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ABSTRACT

O'nyong nyong virus (ONNV), a mosquito-borne Alphavirus, is primarily transmitted through the bite of *Anopheles funestus* and *Anopheles gambiae* mosquitoes, which are also malaria parasite vectors in Africa. The virus, first isolated in Uganda in 1959, is endemic in sub-Saharan Africa and has caused several major outbreaks both in West and East Africa. ONNV fever, characterized by severe arthralgia, is similar to chikungunya fever, with the exception of cervical lymphadenitis, which is peculiar to the former. Prevention measures do not differ from those adopted against malaria parasite transmission. Effective vaccines and drugs are not available, but animal models suggest that vaccine candidates against CHIKV may also confer protection against ONNV.

KEYWORDS

O'nyong-nyong;
chikungunya; alphavirus;
epidemiology

O'nyong nyong virus (ONNV) is a mosquito-borne virus endemic to sub-Saharan Africa. It was first isolated during an epidemic that started in Uganda in 1959 and spread regionally, passing through Kenya and Lake Victoria's shores to southeastern Africa by 1962 [1]. The Acholi people of northwestern Uganda called the disease 'o'nyong-nyong,' meaning 'very painful weakening of joints,' which underscores the importance of the severe arthralgia characteristic of infection [1].

The causative agent

ONNV belongs to the genus *Alphavirus* of the family *Togaviridae*, and is closely related to other members of the Semliki Forest antigenic complex that cause arthritis, including chikungunya (CHIKV), Mayaro (MAYV), and Ross River viruses (RRV) [2,3]. In particular, CHIKV and ONNV form a monophyletic group within the Semliki Forest complex, where ONNV isolates form a robust, distinct clade apart from all CHIKV strains, which probably diverged at least thousands of years ago [2]. Phylogenetic analysis shows that ONNV strains isolated from Gulu, Uganda, in 1959 and from Senegal in 1963, are closely related, while strains from Nigeria 1966, Uganda 1996, and Chad 2004 cluster in a distinct clade [4]. Thus, the relationships among ONNV strains appear to be independent of location and/or year of viral collection, suggesting frequent movement of viruses within Africa [4]. Analyses of partial genome sequences available from the Genbank library (genomic sequences excluding the untranslated regions and two highly variable regions in the nsP3 and capsid protein genes) confirm two major

clades of ONNV (Figure 1). A strain called Igbo-Ora virus, named for the village where it was first isolated, has been described in West Africa [3]. It was first isolated in 1966 from Nigerian patients and then from *Anopheles* spp. mosquitoes in the Ivory Coast [5,6]. Our analysis indicates that it is closely related to the Uganda 1996 outbreak ONNV strain, supporting the previous conclusion that it is a variant of ONNV [7]. The sample year and location of the EVA887 strain is not reported (GenBank Accession # NC001512) and the Kenya 2001 ONNV strain has a nearly identical sequence to that of the Uganda Gulu strain from 1959 [only 23 nucleotide differences, inconsistent with typical rates of alphavirus divergence (10^{-3} – 10^{-4} subst/nt/year, e.g. 4×10^{-4} for CHIKV) over 42 years] [8], suggesting that the Kenya strain could have resulted from laboratory contamination (Figure 1).

Transmission

Although ONNV is a close relative of CHIKV, which is transmitted by *Aedes* spp. mosquitoes in both enzootic and epidemic cycles, it is unique among alphaviruses and one of very few arthropod-borne viruses transmitted by night-feeding anopheline mosquitoes. These include *Anopheles funestus* and *Anopheles gambiae*, which are also malaria vectors [1,9,10]. Unlike *Aedes* spp. mosquitoes, larvae of anopheline mosquitoes are typically found in larger bodies of clear, permanent water, such as lake shores, river margins, and swamps. While CHIKV is not very infectious for *An. gambiae*, strain variability is observed for ONNV in its ability to infect *An. gambiae* and *Aedes aegypti*; however, this variability could be

Figure 1. Maximum likelihood tree of Semliki Forest Virus Complex based on complete open reading frame sequences excluding the highly variable regions in the nsP3 and capsid protein genes. The tree is mid-point rooted. Bootstrap values were estimated based on 1000 replicates of a Neighbor-Joining tree using the best-fit GTR+I+G substitution model; those higher than 70 are labeled on the major branches. Major chikungunya virus lineages are also shown: ECSA (East/Central/South African), West African and Asian.

explained, at least in part, by the fact that the viruses were serially passaged in mice and that there were differences in passage history among ONNV strains [11]. Studies comparing chimeric, infectious cDNA clone-derived viruses containing genes from both ONNV and CHIKV demonstrated that all of the ONNV structural proteins are necessary for infection of *An. gambiae* mosquitoes [12]. However, recent findings with additional chimeras indicate that nonstructural protein 3 (nsP3) of ONNV is sufficient to confer CHIKV infection competence for anopheline mosquitoes, and a fitness advantage of ONNV in anopheline mosquitoes could be provided by the presence of an opal termination codon near the 3' end of the nsP3 gene, near nsP4 [13,14]. Experimental studies have also identified atypical sites of initial ONNV infection and dissemination patterns in *An. gambiae* not frequently observed in *Aedes* spp. infections [15]. Finally, vector competence appears to be conditioned by innate immune response in *An. gambiae* [16].

ONNV has caused large epidemics in East Africa involving millions of persons, followed by its apparent disappearance for long time periods. During the interepidemic periods, ONNV is probably maintained through an enzootic cycle, whose actors (i.e., enzootic vectors and natural reservoir hosts) remain unknown.

Clinical features

The differential diagnosis with chikungunya fever may be difficult on clinical grounds alone. The initial symptoms and signs of ONNV infection are represented by fever, joint pains (without effusions), primarily in the large joints, headache, generalized maculopapular skin rash (often itchy), posterior cervical lymphadenopathy, and conjunctivitis (red eyes); bleeding gums or nosebleeds are rarely reported [17,18].

The incubation period prior to acute illness due to alphaviruses is usually short, but was estimated to be around 8 days for ONNV fever [19]. An investigation conducted during the 1961 Uganda outbreak found a

combination of joint pains, rash, and lymphadenopathy in about 40% of cases, whereas the same combination (with or without fever) was reported by only 23% of laboratory-confirmed cases during a 1996–1997 outbreak [17,19]. This variability in clinical presentation may reflect the use of different case-definitions. With regard to the clinical case-definition, the combination of fever, polyarthralgia and lymphadenopathy is associated with the best combination of specificity (83%) and sensitivity (61%). In typical cases, the illness lasts for few days, and patients are immobilized for a median of 4 days. Persistent joint pains occur in a minority of patients [20]. Differences in clinical presentation among ONNV strains have not been reported, with the exception of lymphadenitis, which was reported to be less commonly observed in Igbo–Ora (see below) outbreaks [6,20]. However, since Igbo–Ora is considered a strain of ONNV, differences in the clinical presentation should be interpreted with caution.

The apparent-to-inapparent infection ratio has been estimated at 2:1, but higher estimates have been obtained in some studies depending on the design. Although the illness is typically self-limiting and fatal cases have not been reported, outbreaks may cause considerable morbidity and loss of work days [20].

Laboratory diagnosis may be difficult due to serologic cross-reactions with CHIKV and possibly other alphaviruses co-circulating in Africa. In particular, reciprocal plaque-reduction neutralization tests (PRNT) are needed to distinguish humoral immunity (typically IgG) from ONNV v. CHIKV [2].

Viral isolation from blood is frequently possible during the first week of illness, particularly in the first three days after onset. Viremia can also be confirmed by reverse transcription-polymerase chain reaction (RT-PCR) detection of viral RNA [17]. Detectable levels of serum IgM antibodies to ONNV typically appear during the second week of illness and persist for about 2 months, although persistence has been detected in a few cases for 6 months or more [17].

Epidemiology and geographical distribution of ONNV in Africa

After its first identification, ONNV was isolated several times from humans and mosquitoes in East Africa [21,22]. Then, following the 1959–1962 epidemic, which involved both eastern (Uganda, Kenya, Tanzania, Malawi, and Mozambique) and western Africa (Democratic Republic of Congo, Central African Republic, Cameroon, and Senegal), accounting for more than 2 million cases in eastern Africa alone [23–26], ONNV activity seemed to disappear [27].

Although no cases were documented in these regions after 1962, ONNV was isolated from a pool of *An. funestus* mosquitoes collected in western Kenya in 1978 [10]. Moreover, seroprevalence studies suggested continued

transmission and human exposure until ONNV fever reemerged in south-central Uganda in mid-1996 [18,20]. During this outbreak, the earliest cases were identified in the rural Rakai District, and then extended to the adjacent districts of Masaka and Mbarara [20]. However, even within the affected areas, there was large variation in the number of cases reported in different sites, with infection rates ranging from 3% to 45% and estimated attack rates of ONNV fever ranging from 3 to 29% [20]. Another study conducted in Coastal Kenya showed higher IgG prevalence (56%) for ONNV compared with CHIKV (6%). IgG prevalence was lower among children compared with adults, but younger adults were more likely to be seropositive than older adults [28], suggesting an increased risk of transmission of children in recent decades and/or waning immunity in adults. Overall, this apparently high level of exposure was unexpected, since the last known outbreak in that region occurred in 1961. Further studies revealed a low IgG prevalence (2.6%) for alphaviruses in selected lake/river basins of Kenya, but anti-ONNV antibodies were more frequently detected than anti-CHIKV antibodies, which is surprising since a major CHIKV infection outbreak began in Kenya in 2004 [29]; among alphaviruses, the overall seroprevalence was lowest for CHIKV and Sindbis viruses (0.26%), 0.8% for ONNV, and 1.3% for Semliki Forest virus (SFV) [30]. Finally, a study conducted in the Kano Plain of Kenya estimated an average annual incidence of ONNV infection of about 10% [31].

With regard to West Africa and the Sahel, ONNV infection was confirmed in a French soldier returning

from Chad [4]. ONNV circulation was identified among febrile patients in southwestern Cameroon but not among healthy volunteers in northwestern Cameroon, where only anti-CHIKV antibodies were found [32,33]. Further studies conducted in rural Cameroon found similar seroprevalence by plaque-reduction neutralization tests (PRNT) against ONNV and CHIKV in the same area; although cross-reactions could not be ruled out, high antibody titers suggested co-circulation of both viruses [34]. Serologic evidence of human and animal ONNV infection was reported also in Nigeria, where the virus was also isolated from human cases, in Ghana and Sierra Leone [35–37], and an outbreaks were reported in the Ivory Coast and among Liberian refugees [6,38]. Finally, antibodies to ONNV were found in wildlife in the Central African countries like the Democratic Republic of Congo and Gabon [39]. A map of African countries with known ONNV circulation is presented in Figure 2.

Although few cases of ONNV fever have been identified in travelers returning from Africa to Europe [4,37,40]; this diagnosis should be considered in patients presenting with a dengue-like illness.

Treatment and prevention

There is no specific treatment for ONNV fever, and the illness is usually a self-limited. Mortality associated with ONNV fever has not been reported.

The immunological response to ONNV has been investigated in animal models such as type I interferon

Figure 2. Map of Africa showing countries where ONNV activity in humans, mosquitoes, or wildlife has been identified.

receptor knockout (A129) mice, highlighting the importance of innate responses in initially controlling infection, while the adaptive immune system was found to be relatively unimportant in protection against acute ONNV infection [41].

A vaccine against ONNV is not available, although experience in vaccine development for other alphaviruses suggests feasibility. Due to their antigenic similarity, a single dose of a highly attenuated CHIKV vaccine candidate (CHIKV/IRES) was found to elicit a strong cross-neutralizing antibody response and to confer protection against ONNV challenge in the A129 mouse model [42]. Thus, CHIKV candidate vaccines, including several that are in clinical trials [43], have the potential to protect humans not only against CHIKV but also against ONNV infection.

Prevention of transmission is challenging because during epidemics ONNV is apparently transmitted from human-to-human via *Anopheles spp.* mosquitoes. However, interventions implemented against malaria, from bed nets to personal protection against mosquito bites, may reduce the risk of ONNV transmission both at the individual and the population levels.

In conclusion, ONNV morbidity in Africa is undoubtedly underestimated, due to limited surveillance and under-diagnosis, and both the enzootic source and the epidemic potential of ONNV remains undefined. Although past outbreaks are not known to have spread beyond Africa, increases in air travel will heighten this risk during future epidemics if anopheline species in other regions of the world are capable of transmission. Preparedness plans are needed in order to address response to ONNV fever outbreaks, by improving surveillance to understand endemic exposure and epidemic emergence, by improving diagnosis of ONNV infection, and by developing improved prevention and treatment products.

Disclosure statement

No potential conflict of interest was reported by the authors.

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