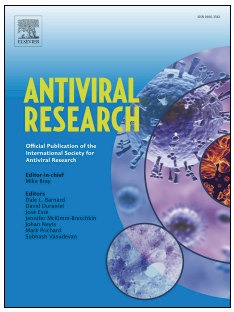

GloPID-R report on chikungunya, o'nyong-nyong and Mayaro virus, part 2: Epidemiological distribution of o'nyong-nyong virus

L. Pezzi, A.D. LaBeaud, C.B. Reusken, J.F. Drexler, N. Vasilakis, M. Diallo, F. Simon, T. Jaenisch, P. Gallian, A. Sall, A.B. Failloux, S.C. Weaver, X. de Lamballerie, on behalf of GloPID-R chikungunya, o'nyong-nyong and Mayaro virus Working Group

PII: S0166-3542(19)30512-1

DOI: https://doi.org/10.1016/j.antiviral.2019.104611

Reference: AVR 104611

To appear in: *Antiviral Research*

Received Date: 8 September 2019

Accepted Date: 17 September 2019

Please cite this article as: Pezzi, L., LaBeaud, A.D., Reusken, C.B., Drexler, J.F., Vasilakis, N., Diallo, M., Simon, F., Jaenisch, T., Gallian, P., Sall, A., Failloux, A.B., Weaver, S.C., de Lamballerie, X., on behalf of GloPID-R chikungunya, o'nyong-nyong and Mayaro virus Working Group, Boyer, S., Brasil, P., Busch, M., Diamond, M.S., Drebot, M.A., Kohl, A., Lecuit, M., Lourenço-de-Oliveira, R., Neyts, J., Lfp, N., Ribeiro, G.S., Rios, M., Rodriguez-Morales, A.J., Rosa-Freitas, M.G., Simmons, G., Siqueira, A.M., Vega Rua, A., GloPID-R report on chikungunya, o'nyong-nyong and Mayaro virus, part 2: Epidemiological distribution of o'nyong-nyong virus, *Antiviral Research* (2019), doi: https://doi.org/10.1016/j.antiviral.2019.104611.

GloPID-R report on chikungunya, o'nyong-nyong and Mayaro virus, part 2: Epidemiological distribution of o'nyong-nyong virus

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1. Introduction

O'nyong-nyong virus (ONNV) is a mosquito-borne alphavirus (family *Togaviridae*), primarily transmitted through the bite of *Anopheles funestus* and *A. gambiae* mosquitoes. After its first isolation from Gulu, Uganda in 1959, ONNV was detected several times in humans and mosquitoes [1]; so far, its known geographical distribution is confined to the African continent. It is highly probable that ONNV has been misdiagnosed and/or underreported, because of the circulation in Africa of pathogens causing similar illnesses (*i.e.*, *Plasmodium falciparum,* chikungunya virus (CHIKV), dengue virus (DENV), and other arboviruses) and of the difficult interpretation of serological results (due to cross-reactivity between antibodies against ONNV and those against CHIKV) [2].

2. GloPID-R chikungunya (CHIKV), o'nyong-nyong (ONNV) and Mayaro virus (MAYV) Working Group

The GloPID-R (Global Research Collaboration for Infectious Disease Preparedness, https://www.glopid-r.org/) chikungunya (CHIKV), o'nyong-nyong (ONNV) and Mayaro virus (MAYV) Working Group has been established to investigate the natural history, epidemiology and clinical aspects of these viruses; the objective was to identify knowledge gaps and to propose recommendations for direct future investigations and rectification measures. After the first report dedicated to diagnostic aspects of CHIKV, ONNV and MAYV [2], here we present an assessment of the available information regarding the epidemiological distribution of ONNV. It will be followed by a report on CHIKV and MAYV distribution.

3. Sources of data

Data from the first viral isolation until December 2018 were collected, including both acute cases in humans (confirmed by PCR+ and/or presence of IgM) as well as results of serological studies showing past infections in humans (presence of IgG). Data were obtained from health organizations and from the peer-reviewed literature concerning African countries. We consulted different public health alert systems websites, including:

- World Health Organization (WHO) - 'Disease outbreak news (DON)' section
- Program for Monitoring Emerging Diseases (ProMED);

- European Centre for Disease Prevention and Control (ECDC) through 'Communicable disease threats report (CDTR)';
- Institute of health of each African country, through periodic bulletins on viral infections (when available).

In addition, a comprehensive review of literature using PubMED was conducted with the search terms 'O'nyong-nyong' and the name of each African country. Finally, viral sequences available in GenBank were checked, in order to assess the countries where ONNV-positive human samples have been collected.

In the article and in Tables 1-2 of Supplementary data we reported details about ONNV detection: number of cases, year, localization and technique(s) used to confirm the infection. We classified the sources of our data in 5 categories:

- Declared outbreaks
- Single case reports (mainly about returning travelers)
- Surveillance studies, aiming to identify the etiology of acute illnesses confirmed by PCR and/or serology
- Serosurveys (to evaluate previous exposition to the virus, confirmed by serological tools)
- GenBank sequences, present on the database without additional information on the isolated strains

In this report, experts provided a picture of ONNV geographic distribution through Africa over the last 60 years; in addition, they identified gaps of knowledge and made recommendations in order to suggest research priorities in the field.

3. ONNV distribution

An account of the epidemiological findings about ONNV human cases is shown below. Further details are presented in Table 1 (West Africa) and Table 2 (Central and East Africa) of Supplementary data.

I. West Africa

A. Côte d'Ivoire

1) Outbreak

First reported epidemic caused by Igbo-Ora virus (later determined to be a strain of ONNV). Cases were confirmed in Central Côte d'Ivoire in 1984-1985 by serological tests or by virus isolation. Entomological investigation allowed to detect the virus in *A. funestus* and *A. gambiae* [3].

2) Outbreak

ONNV outbreak was declared in Nicla Border Camp in western Côte d'Ivoire, among Liberian refugees [4]. Cases were confirmed positive by serological tests or PCR.

B. Ghana

1) Serosurvey

A seroprevalence study was performed in travelers returning from tropical Africa in 1975-1977 [5]. Sera from patients with evidence of past infections were tested by haemagglutination inhibition (HI), complement fixation (CF), and indirect immunofluorescence (IF) tests.

C. Nigeria

1) Case report

Two Igbo-Ora virus strains were isolated from two febrile children in Oyo State, south-western Nigeria, in 1966 [6].

2) Case report

An Igbo-Ora virus strain was isolated from an adult patient living in Oyo State, southwestern Nigeria, in 1969 [6].

3) Surveillance study

The seroprevalence study was performed in travellers returning from tropical Africa [5] in 1975-1977. Sera from patients with evidence of past infections were tested by haemagglutination inhibition (HI), complement fixation (CF), and indirect immunofluorescence (IF) tests. One ONNV infection dated back to June 1974, another one to December 1975. A third patient was infected in Nigeria or in Sierra Leone, with no information about the year.

4) Serosurvey

Seroprevalence study performed among voluntary donors in Oyo State, southwestern Nigeria [7]. Sera were tested by HI. Cross-reactivity with antibodies against CHIKV was observed.

D. Senegal

1) Outbreak

A ONNV outbreak involving both eastern and western Africa (Uganda, Kenya, Mozambique, Senegal, Malawi, Tanzania, Cameroon, Central African Republic, Democratic Republic of the Congo) in 1959-1962 accounted for more than 2 million cases in eastern Africa alone [1, 5]. *A. funestus* was identified as the main vector [3, 4]. The first ONNV strain was isolated from Gulu (Uganda) in 1959.

II. Central and East Africa

A. Cameroon

1) Outbreak

A ONNV outbreak involving both eastern and western Africa (Uganda, Kenya, Mozambique, Senegal, Malawi, Tanzania, Cameroon, Central African Republic, Democratic Republic of the Congo) in 1959-1962 accounted for more than 2 million cases in eastern Africa alone [1, 8]. *A. funestus* was identified as the main vector [9, 10]. The first ONNV strain was isolated from Gulu (Uganda) in 1959.

2) Serosurvey

A seroprevalence study performed in 2000-2003 involved adults in nine rural villages in Cameroon. Sera were tested for ONNV by PRNT (plaque-reduction neutralization test). Cross-reactivity with antibodies against CHIKV was observed [11].

3) Surveillance study

A study was conducted in 2004-2005 in the Fako Division (southwest Cameroon). Sera were collected from febrile patients and tested by haemagglutination inhibition (HI) and complement fixation (CF) tests, with 34,2% and 33,3% of positive results, respectively. Cross-reactivity with antibodies against CHIKV was observed [12].

B. Central African Republic

1) Outbreak

A ONNV outbreak involving both eastern and western Africa (Uganda, Kenya, Mozambique, Senegal, Malawi, Tanzania, Cameroon, Central African Republic, Democratic Republic of the Congo) in 1959-1962 accounted for more than 2 million cases in eastern Africa alone [1, 8]. *A. funestus* was identified as the main vector [9, 10]. The first ONNV strain was isolated from Gulu (Uganda) in 1959.

2) GenBank sequence (no additional information)

An Igbo-Ora strain of ONNV was isolated from human blood sample in 1985. GenBank accession number available in Table 2- Supplementary data.

C. Chad

1) Case report

Imported case - France ex Chad (southern part of the country) [13]. Sera was IgM+ and IgG+ anti-ONNV by ELISA; virus was isolated from peripheral blood mononuclear cells.

D. Democratic Republic of the Congo

1) Outbreak

A ONNV outbreak involving both eastern and western Africa (Uganda, Kenya, Mozambique, Senegal, Malawi, Tanzania, Cameroon, Central African Republic, Democratic Republic of the Congo) in 1959-1962 accounted for more than 2 million cases in eastern Africa alone [1, 8]. *A. funestus* was identified as the main vector [9, 10]. The first ONNV strain was isolated from Gulu (Uganda) in 1959.

E. Kenya

1) Outbreak

A ONNV outbreak involving both eastern and western Africa (Uganda, Kenya, Mozambique, Senegal, Malawi, Tanzania, Cameroon, Central African Republic, Democratic Republic of the Congo) in 1959-1962 accounted for more than 2 million cases in eastern Africa alone [1, 8]. *A. funestus* was identified as the main vector [9, 10]. The first ONNV strain was isolated from Gulu (Uganda) in 1959.

2) Serosurvey

Seroprevalence study was conducted in two sites in Western Kenya in 1973: Kalo Plain (67,3% of positivity rate for ONNV) and Bunyala (72,3%) [14]. Tests on sera were performed with HI.

3) Outbreak

A ONNV outbreak was first detected in south-central Uganda and then spread to Kenya and Tanzania in 1996-1997 [15]. A case-finding serosurvey conducted in January and February 1997 in affected sites in Uganda estimated infection rates at 45-68%. Cross-reactivity with antibodies against CHIKV was observed.

4) Outbreak

ONNV cases were confirmed in Mombasa and Malindi districts (eastern Kenya) in 2004 (ProMED 20041216.3325).

5) Serosurvey

Seroprevalence study was performed on sera collected in Kwale County, eastern Kenya, in 2009 [16]. Sera were tested with IgG ELISA and PRNT (plaque reduction neutralization test). Cross-reactivity with antibodies against CHIKV was observed, with 168 additional sera with high titres for both viruses with PRNT.

6) Case report

Imported case - Germany ex Kenya in 2013 [17]. After a travel in eastern Africa, paired sera were tested by immunofluorescence (IF) and virus neutralization (VNT) tests.

F. Malawi

1) Outbreak

A ONNV outbreak involving both eastern and western Africa (Uganda, Kenya, Mozambique, Senegal, Malawi, Tanzania, Cameroon, Central African Republic, Democratic Republic of the Congo) in 1959-1962 accounted for more than 2 million cases in eastern Africa alone [1, 8]. *A. funestus* was identified as the main vector [9, 10]. The first ONNV strain was isolated from Gulu (Uganda) in 1959.

G. Mozambique

1) Outbreak

A ONNV outbreak involving both eastern and western Africa (Uganda, Kenya, Mozambique, Senegal, Malawi, Tanzania, Cameroon, Central African Republic, Democratic Republic of the Congo) in 1959-1962 accounted for more than 2 million cases in eastern Africa alone [1, 8]. *A. funestus* was identified as the main vector [9, 10]. The first ONNV strain was isolated from Gulu (Uganda) in 1959.

H. South Sudan

1) Outbreak

During an outbreak of haemorrhagic fever syndrome in 2015-2016, 5 sera collected from patients and tested with PCR, PRNT and ELISA were ONNV+ [18].

I. Tanzania

1) Outbreak

A ONNV outbreak involving both eastern and western Africa (Uganda, Kenya, Mozambique, Senegal, Malawi, Tanzania, Cameroon, Central African Republic, Democratic Republic of the Congo) in 1959-1962 accounted for more than 2 million cases in eastern Africa alone [1, 5]. *A. funestus* was identified as the main vector [3, 4]. The first ONNV strain was isolated from Gulu (Uganda) in 1959.

2) Outbreak

A ONNV outbreak was first detected in south-central Uganda and then spread to Kenya and Tanzania in 1996-1997 [15]. A case-finding serosurvey conducted in January and February 1997 in affected sites in Uganda estimated infection rates at 45-68%. Cross-reactivity with antibodies against CHIKV was observed.

J. Uganda

1) Outbreak

A ONNV outbreak involving both eastern and western Africa (Uganda, Kenya, Mozambique, Senegal, Malawi, Tanzania, Cameroon, Central African Republic, Democratic Republic of the Congo) in 1959-1962 accounted for more than 2 million cases in eastern Africa alone [1, 5]. A. funestus was identified as the main vector [3, 4]. The first ONNV strain was isolated from Gulu (Uganda) in 1959.

2) Outbreak

A ONNV outbreak was first detected in south-central Uganda and then spread to Kenya and Tanzania in 1996-1997 [15]. A case-finding serosurvey conducted in January and February 1997 in affected sites in Uganda estimated infection rates at 45-68%. Cross-reactivity with antibodies against CHIKV was observed.

4. Discussion

In this paper, we report cases of ONNV infections in humans; however, the assessment of ONNV circulation in Africa remains a difficult task for several reasons.

First, the absence of surveillance in most African countries led to fragmentary data including mainly isolated case reports of local people or returning travelers, and sporadic outbreaks. Returning travelers can serve as sentinels, providing information on recent circulation of an infectious pathogen, especially in countries where laboratory diagnosis, especially seroneutralization, is difficult to implement. However, the diagnosis of imported ONNV infections should not only constitute a detection for its own sake, but also provide evidence of viral circulation and transmission in source countries that often do not have the diagnostic capacity to rule in ONNV. Similarly, outbreaks have been reported but not followed by systematic seroprevalence, cross-sectional or follow-up studies; their implementation is necessary in order to evaluate the real scale of ONNV circulation and to identify possible *sequelae* post-infection. This is valid especially for countries that have already experienced large-scale outbreaks (*i.e.* Kenya, Uganda, Tanzania), and/or where ONNV circulation is suspected to be endemic [16].

Second, the scarce seroprevalence data currently available are not robust, due to the cross-reactivity with antibodies raised against closely related alphaviruses. In particular, in several serosurveys described in the article it was hard to discriminate infections caused by ONNV and those caused by CHIKV, since antibodies against the two viruses strongly cross-react if tested by immunofluorescence test (IF), enzyme-linked immunosorbent assay (ELISA), complement fixation test (CF) or haemagglutination inhibition Test (HI) [5, 7, 12]. Even neutralization tests, normally the gold standard for specificity, are cross-reactive, sometimes in an asymmetric manner [2]. For example, LaBeaud *et al*. observed that 38% of ONNV- and CHIKV-positive samples had high titers for both alphaviruses using PRNT, and were then classified as “equivocal” [16]. The presence of antibodies against ONNV and CHIKV may sometimes be the result of infection by both viruses (which would imply that infection by one pathogen does not provide protection against the other), or may be due to massive cross-reactivity because of the antigenic similarity between the two viruses. However, strong cross-protection has been reported in studies of CHIKV vaccines, suggesting that most cross-reactivity results from single infections [19]. Since ONNV and CHIKV are phylogenetically close, some degree of cross-protection is highly probable; dual reactivity against both ONNV and CHIKV is likely to reflect the limited discriminatory capacity of

available neutralization assays. Another explanation could be the circulation of a novel third virus that induces antibodies that cross-neutralize both CHIKV and ONNV.

Third, the scarcity of available ONNV genetic data limits molecular epidemiology studies and makes difficult to map viral spread throughout Africa. According to the 9th report of the International Committee on Taxonomy of Viruses (ICTV) [20], ONNV forms with CHIKV a monophyletic group within the Semliki Forest complex. Phylogenetic analysis with partial genome sequences available from GenBank shows that ONNV isolates constitute a distinct evolutionary branch apart from all CHIKV strains, with two major clades among ONNV strains (Figure 2) [21]. The relationship among viral strains does not depend on sample year and/or location, suggesting frequent movement of ONNV through the African continent. Igbo Ora virus, first isolated in 1966 from Nigerian patients and sometimes considered as a separate virus, is closely related to an ONNV strain isolated during 1996-97 outbreak in Uganda, supporting the idea that it should be considered a member of species ONNV [20, 22].

The analysis of the distribution of ONNV cases in time and space suggests a certain periodicity of outbreaks and geographical limitations in viral spread. ONNV has been capable of causing two known large-scale epidemics, one in 1959-1962 and the following one in 1996-1997. As concerns geographical distribution of ONNV cases, figure 1 shows a restriction to sub-Saharan countries in both East and West Africa. Since ONNV is transmitted by malaria vector mosquitoes (*Anopheles* species), it is not surprising that our ONNV map and those reporting *Anopheles* and malaria cases distribution are overlapping [23, 24]. The relationship between ONNV and malaria transmission remains to be investigated. An unusual drop in malaria transmission was noted at the same time as intense circulation of ONNV in southern Uganda during the 1959-1962 epidemic, in a period of the year with favorable meteorological conditions for high mosquito densities [25]. This observation raises interesting questions regarding the growth and transmission of malaria parasites (*Plasmodium falciparum*) in the *anopheles* mosquitoes infected by ONNV.

Investigating the relationship between ONNV and co-circulating pathogens (primarily CHIKV), as well as factors that limit ONNV distribution at regular intervals to some sub-Saharan countries other than the diffusion of competent vectors (*i.e.* the presence of natural reservoirs) would most probably improve our understanding of ONNV epidemiology.

According to available data on ONNV distribution, experts identified several gaps of knowledge and provided adapted recommendations (Paragraphs 5 and 6). The aim was to identify and suggest research priorities in the field.

5. Gaps of knowledge

A. Disease surveillance and epidemiology

Despite ONNV detection in humans, vertebrates and mosquitoes throughout African continent, the surveillance strategy remains unsatisfactory. In some African countries, evidence for ONNV circulation relies on small identified outbreaks (South Sudan, Côte d'Ivoire) or single case reports (Chad, Ghana); in others, few seroprevalence studies have been performed (Nigeria, Kenya) but the robustness of data is disputable. Altogether, the scarcity of currently available information does not allow to provide a clear picture of the actual scale of virus spread.

B. Entomological and environmental surveillance

Despite the epidemiologic studies described above, fundamental information on ONNV maintenance, presumably through an enzootic cycle, remains wanting. This information includes both enzootic reservoir or amplification hosts, enzootic vectors, and mechanisms of human-mosquito-human epidemic emergence.

C. Laboratory tests for seroprevalence studies

The close phylogenetic relationship between ONNV and CHIKV explains why anti-ONNV and anti-CHIKV antibodies can barely be distinguished. This is very difficult –if not impossible- using standard ELISA or immunofluorescence test [2], but even seroneutralization tests often generate equivocal results. Accordingly, the choice of the best laboratory test to use in seroprevalence studies is crucial, but it is doubtful that adapted and convenient serological tools and interpretation algorithms are currently available to expedite accurate (and large scale) seroepidemiological studies.

D. Diagnosis of acute cases

Very few incident cases are reported, most probably because diagnostic tests that should be used at the acute phase of the disease are rarely available. IgM serological assays have not been evaluated, most probably poorly discriminate with CHIKV, and commercial kits do not exist. Very few molecular assays for ONNV detection exist, and their performances (in terms of specificity and sensitivity) have never been evaluated through comparative studies. In practice they are rarely available and used [2]. Information about kinetics of viral load are scarce and concern just sera samples; other body fluids have never been tested. No International Standards (IS) are currently available and External Quality Assessment (EQAs) have never been organized to evaluate laboratories capacity to diagnose ONNV infection.

E. Cross-protection

It is unknown whether individuals infected by ONNV or CHIKV can secondarily be infected by the other virus. It is presumed, due to the close antigenic relationship, that the infection by one virus protects against subsequent infection by the other, but since the seroneutralizing relationship is not symmetric (antibodies to CHIKV provide better protection against ONNV than the opposite), this remains to be confirmed. In addition, serological patterns associated with sequential exposure to the two viruses are not characterized.

F. Natural history

The natural history of the disease deserves to be better investigated, including acute manifestations and long-term sequelae. Moreover, whereas a variety of long-term (mostly rheumatologic) complications of the disease have been observed for the related chikungunya virus since the Indian Ocean outbreak (constituting an actual "post-CHIK" syndrome), longitudinal studies of ONNV infections have not been performed to determine whether a "post-ONN" syndrome exists.

6. Expert recommendations

A. Disease surveillance and epidemiology

Specific efforts are required for improving our knowledge about ONNV circulation and epidemiology at a large scale (including clinical epidemiology). ONNV should be more frequently included in molecular diagnostic panels for febrile illnesses of unknown origin in Africa, and seroprevalence studies should be performed. This obviously requires improved diagnostic assays and access.

B. Entomological and environmental surveillance

Entomology studies (including investigation of possible vector switches) and characterization of the natural enzootic cycle in reservoirs and vectors should be promoted.

C. Laboratory tests for seroprevalence studies

Accurate assays and algorithms allowing to detect IgG antibodies to ONNV and to distinguish them from IgG to CHIKV are needed. At this stage, performing seroneutralization confirmation is mandatory after performing a screening assay (*e.g.* ELISA, HI or IF assays) and allows asserting ONNV circulation when titers against ONNV are clearly higher than those against CHIKV. However, a proportion of samples remains without clear identification, in particular in case of co-circulation of both viruses [16]. Clear interpretation guidelines or new specific assays are required and reference serological standards are needed (possibly produced from non-human primates, [2]).

D. Diagnosis of acute cases

For diagnosis at the acute stage of the disease, viral co-circulation requires the development of molecular and serological multiplex tools to differentiate ONNV from CHIKV in Africa. Identification and analysis of acute cases is mandatory to better know the natural history of the disease and identify severe and complicated forms. Viremia kinetics and viral loads in different body fluids should be better documented, as well as the kinetics of immune response. International Standards (IS) should be made available, both molecular and serological. Moreover, External Quality Assessments (EQAs) should be organized in order to assess laboratory capacity of detecting ONNV with both molecular and serological tools.

E. Cross-protection

Cross-protection studies between ONNV and related alphaviruses, in particular CHIKV, should be implemented. This could be done using samples from both naturally exposed humans and experimentally infected non-human primates.

F. Natural history

Studies should be performed in order to better characterize the natural history of ONN disease and to identify long term sequelae post infection.

Acknowledgement

Publication fees were covered by the Global Research Collaboration for Infectious Disease Preparedness (GloPID-R).

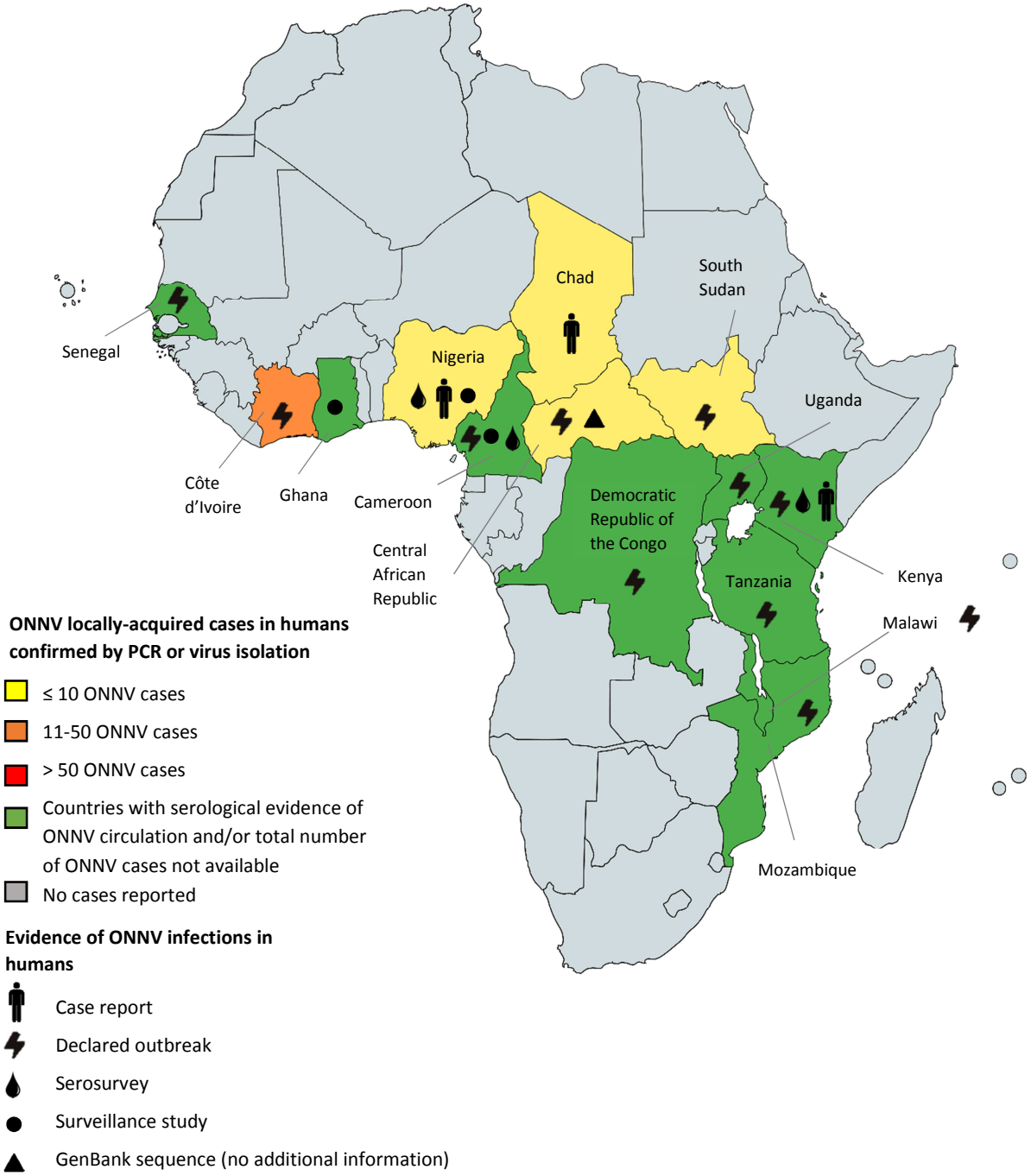

Figure 1. Acute ONNV cases in humans confirmed by PCR or virus isolation. Countries (in green) where ONNV infections have been confirmed by HI, VNT, PRNT, IF or ELISA are not classified in the map since massive cross-reactivity with related alphaviruses affects serological assays.

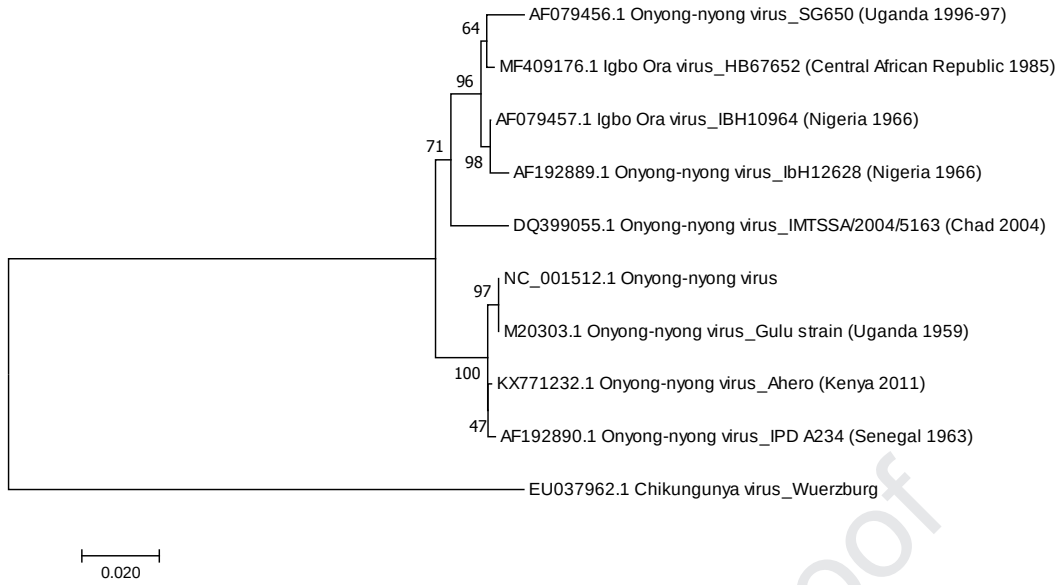

Figure 2. Phylogenetic analysis of 9 ONNV strains (including Igbo Ora, considered an ONNV strain) present in GenBank. Neighbor-joining phylogeny tree was generated using p-distance model with 1000 bootstrap replication. 1,044 sites were included from partial E2-E1 sequences. Tip labels indicate GenBank accession number, strain name, country and year of isolation. For NC_001512, sampling country and year are not available.

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Highlights: (3-5, 125 characters max including spaces)

- The real scale of O'nyong-nyong virus circulation in Africa is poorly understood.
- Co-circulation of related viruses causing similar clinical pictures, like chikungunya virus, makes specific diagnosis hard.
- Massive cross-reactivity affects serological tools necessary to perform seroprevalence studies.
- Surveillance studies should be performed to assess the real burden of the disease in Africa.
- The natural history of the disease deserves further investigation, especially long-term complications.