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Orthobunyavirus Antibodies Among Humans in Selected Parts of the Rift Valley and Northeastern Kenya

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Abstract

Ngari, Bunyamwera, Ilesha, and Germiston viruses are among the mosquito-borne human pathogens in the *Orthobunyavirus* genus, family Bunyaviridae, associated with febrile illness. Although the four orthobunyaviruses have been isolated from mosquito and/or tick vectors sampled from different geographic regions in Kenya, little is known of human exposure in such areas. We conducted a serologic investigation to determine whether orthobunyaviruses commonly infect humans in Kenya. Orthobunyavirus-specific antibodies were detected by plaque reduction neutralization tests in 89 (25.8%) of 345 persons tested. Multivariable analysis revealed age and residence in northeastern Kenya as risk factors. Implementation of acute febrile illness surveillance in northeastern Kenya will help to detect such infections.

Keywords

Orthobunyavirus; Bunyaviridae; Arboviruses; Kenya

Introduction

Bunyamwera virus (BUNV) and Ngari virus (NRIV) have recently been isolated from mosquitoes and ticks in northeastern Kenya (Blaney Jr et al. 2001, Lwande et al. 2013, Ochieng et al. 2013). Likewise, Ilesha virus (ILEV) and Germiston virus (GERV) have previously been isolated from mosquitoes in western Kenya (Johnson et al. 1977). These viruses belong to the *Orthobunyavirus* genus of the family Bunyaviridae and possess a

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Author Disclosure Statement

No conflicting financial interests exist.

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Centers for Disease Control and Prevention or the institutions with which the authors are affiliated tripartite, single-stranded, negative-sense RNA genome. Some members of the genus including these four viruses are known to cause disease in humans with varying pathological consequences (Karabatsos 1985).

Although orthobunyaviruses have been isolated from arthropod vectors during surveillance exercises in Kenya, no clear evidence exists for incidence of human infection in this region because diagnostic laboratories seldom test for these infections. Moreover, viruses of this genus comprise a neglected but potentially deadly group of viruses given their potential to undergo genetic reassortment with altered pathogenic manifestations, as evidenced by the recent outbreak of Schmallenberg virus in Europe that resulted in malformations in ruminants (Wisloff et al. 2014) and the emergence of NRIV in hemorrhagic fever outbreaks in East Africa (Gerrard et al. 2004, Briese et al. 2006). We investigated whether orthobunyaviruses commonly infect humans in three different regions of Kenya, as an indication of past clinical or subclinical infection.

Materials and Methods

The present study obtained specimens from an Integrated Response System for Emerging Infectious Diseases in East Africa, also known as the Arbovirus Incidence and Diversity (AVID) project. This project brought together a consortium of implementing institutions consisting of health, veterinary, wildlife, and vector experts to take an integrated approach to arbovirus surveillance and research. The main aim of the project is to improve the prediction and prevention of Rift Valley fever virus and other emerging arboviruses and to develop a model for response that could be expanded to other emerging diseases in the East African region. The project is interested in the discovery of both known and unknown viruses causing emerging infectious diseases. Serum samples were obtained from 345 febrile patients at three health facilities in Kenya between January, 2009, and April, 2012. Specimens were drawn from patients attending Sangailu Dispensary ($n = 94$) and Kotile Health Centre ($n = 118$), the main facilities that serve most of the inhabitants of Ijara subcounty of Garissa County in northeastern Kenya. Specimens were also drawn from the Mai Mahiu Health Centre ($n = 133$) in Naivasha, a subcounty within Nakuru County of the Rift Valley Province of Kenya, a region from which no isolations of these viruses have been made. Ethical approval was obtained from the Kenya Medical Research Institute, Kenya.

All sera were screened at a dilution of 1:20 by a plaque reduction neutralization test (PRNT) using BUNV (strain GSA/S4/11232), NRIV (strain TND/S1/19801), ILEV, and GERV (both obtained from the Centers for Disease Control and Prevention [CDC], Fort Collins, CO) as described (Blitvich et al. 2012). Reactive sera were titrated to determine the highest dilution neutralizing 90% or greater of the virus. For etiological diagnosis, the PRNT_{90} antibody titer was required to be four-fold or more higher than that of the other virus tested.

Results

Neutralizing antibodies to any of the four orthobunyaviruses were detected in 89 (25.8%) of 345 study participants. Thirty-three participants (9.6%) had neutralizing antibodies to more than one virus, with PRNT titers ranging from 20 to 640 and the majority of cross-neutralization between ILEV and BUNV (Table 1). These specimens were titrated and analyzed by comparative PRNT to identify the *Orthobunyavirus* responsible for seropositivity. Eight specimens were resolved, whereas 25 remained undetermined (Table 1). The observed cross-neutralization may represent persons doubly infected years ago, and, as such, trace amounts of antibodies remaining were insufficient to yield a four-fold or more difference in titer between the implicated viruses. Alternatively, there could be other yet to be identified orthobunyaviruses circulating within the same region. Although we did not screen for Shokwe virus, previously isolated in Kenya, antibodies against the virus do not neutralize any of the viruses tested in the current study (Karabatsos 1985). Additionally, it is possible that some of the patients seropositive for NRIV may have been infected with Batai virus, although this virus has not been isolated in Kenya.

Logistic regression analysis showed that risk for infection increased significantly with age ($p \leq 0.0001$). The proportion of participants with neutralizing antibodies against orthobunyaviruses was significantly higher in Sangailu (52.1%) compared to Kotile (32.2%) ($p < 0.0001$), probably due to its close proximity to the Boni Forest National Reserve, whereas Naivasha had the lowest proportion (1.5%). There was no gender difference in the proportion of participants with neutralizing antibodies against orthobunyaviruses.

Discussion

We found that 25.8% of 345 participants in our study had evidence of *Orthobunyavirus* exposure. The proportion of neutralizing antibodies was higher in participants 15 year or older, indicating that exposure to these viruses may have occurred during the Rift Valley fever outbreak in 1997–1998, where NRIV was isolated from hemorrhagic cases (Gerrard et al. 2004). Neutralizing antibodies in participants younger than 10 years may indicate ongoing interepidemic transmission and need to be investigated further. The proportion of participants with neutralizing antibodies increased with age, suggesting endemicity of these viruses in northeastern Kenya. Differences in the proportion of participants with neutralizing antibodies between subcounties can be explained by differences in climatic conditions that may influence economic activities of residents. Pastoralism as a source of livelihood and the nomadic nature of inhabitants of northeastern Kenya may promote the spread of these viruses due to their close interaction with domestic animals and possible interaction of their animals with wild animals (which may be reservoirs of these viruses) during grazing (Lwande et al. 2012). Additionally, mosquito vectors associated with previous isolations of these viruses, including *Aedes* (*Ae.*) *mcintoshi*, *Ae. tricholabis*, *Ae. ochraceus*, *Culex pipiens*, *Anopheles (An.) gambiae*, *An. phareoensis*, *Mansoni* (*Mn*) *africanus*, and *Mn. uniformis* (Gonzalez and Georges 1988, Logan et al. 1991, Traorelamizana et al. 2001, Crabtree et al. 2009), are abundant in northeastern Kenya (Lutomiah et al. 2013) and may likely be transmitters of these viruses. Additionally, we have previously determined that *An. gambiae* is a competent vector for Bunyamwera and Ngari viruses (Odhiambo et al. 2014).

All patients in the parent study sought care for unspecified fevers, but we could not determine whether any of these febrile illnesses resulted from infection by these viruses because there was no follow-up to obtain convalescent sample for determination of rise in antibody titer. Moreover, no immunoglobulin M (IgM)-capture enzyme-linked immunosorbent assay (ELISA) for orthobunyavirus diagnosis exists. Additionally, we were unsuccessful in isolation of these viruses from reactive sera by inoculation of reactive sera on confluent Vero cells culture and by reverse transcription (RT)-PCR using *Orthobunyavirus* genus primers, suggesting that these are likely past infections. However, viremia in humans is known to be transient and of low magnitude, hence RT-PCR may be ineffective for detection of *Orthobunyavirus* RNA in serum specimens (Blitvich et al. 2012). Given our inability to include Shokwe and Batai viruses in our analysis due to biosecurity concerns, definitive, specific diagnosis is limited by a lack of comprehensive diversity in the panel of viruses that were used for PRNT.

Conclusion

The present study demonstrates serological evidence of *Orthobunyavirus* activity in the Sangailu and Kotile regions of Garissa County in northeastern Kenya, thus requiring inclusion of orthobunyaviruses among the unrecognized causes of illness in humans within the region.

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References

Blaney JE Jr, Johnson DH, Firestone CY, Hanson CT, et al. Chemical mutagenesis of dengue virus type 4 yields mutant viruses which are temperature sensitive in Vero cells or human liver cells and attenuated in mice. J Virol. 2001; 75:9731–9740. [PubMed: 11559806]

Blitvich BJ, Saiyasombat R, Talavera-Aguilar LG, Garcia-Rejon JE, et al. Orthobunyavirus antibodies in humans, Yucatan Peninsula, Mexico. Emerg Infect Dis. 2012; 18:1629–1632. [PubMed: 23017592]

Briese T, Bird B, Kapoor V, Nichol ST, et al. Batai and Ngari viruses: M segment reassortment and association with severe febrile disease outbreaks in East Africa. J Virol. 2006; 80:5627–5630. [PubMed: 16699043]

Crabtree M, Sang R, Lutomiah J, Richardson J, et al. Arbovirus surveillance of mosquitoes collected at sites of active Rift Valley Fever virus transmission: Kenya, 2006–2007. J Med Entomol. 2009; 46:961–964. [PubMed: 19658258]

Gerrard SR, Li L, Barrett AD, Nichol ST. Ngari virus is a Bunyamwera virus reassortant that can be associated with large outbreaks of hemorrhagic fever in Africa. J Virol. 2004; 78:8922–8926. [PubMed: 15280501]

Gonzalez, JP.; Georges, AJ. Bunyaviral fevers: Bunyamwera, Ilesha, Germiston, Bwamba and Tataguine. In: Monath, T., editor. The Arboviruses: Epidemiology and Ecology. Boca Raton, FL: CRC Press; 1988.

Johnson BK, Shockley P, Chanas AC, Squires EJ, et al. Arbovirus isolations from mosquitoes: Kano Plain, Kenya. Trans R Soc Trop Med Hyg. 1977; 71:518–521. [PubMed: 24288]

Karabatsos N. Supplement to international catalogue of arboviruses including certain other viruses of vertebrates. Am J Trop Medicine Hyg. 1978; 27(2 Pt 2 Suppl):372.

Logan TM, Linthicum KJ, Davies FG, Binepal YS, et al. Isolation of Rift Valley fever virus from mosquitoes (Diptera: Culicidae) collected during an outbreak in domestic animals in Kenya. J Med Entomol. 1991; 28:293–295. [PubMed: 1676073]

Lutomiah J, Bast J, Clark J, Richardson J, et al. Abundance, diversity, and distribution of mosquito vectors in selected ecological regions of Kenya: Public health implications. J Vector Ecol. 2013; 38:134–142. [PubMed: 23701618]

Lwande OW, Irura Z, Tigoi C, Chepkorir E, et al. Sero-prevalence of Crimean Congo hemorrhagic fever virus in Ijara District, Kenya. Vector Borne Zoonotic Dis. 2012; 12:727–732. [PubMed: 22925021]

Lwande OW, Lutomiah J, Obanda V, Gakuya F, et al. Isolation of tick and mosquito-borne arboviruses from ticks sampled from livestock and wild animal hosts in Ijara District, Kenya. Vector Borne Zoonotic Dis. 2013; 13:637–642. [PubMed: 23805790]

Ochieng C, Lutomiah J, Makio A, Koka H, et al. Mosquito-borne arbovirus surveillance at selected sites in diverse ecological zones of Kenya; 2007–2012. Virol J. 2013; 10:140. [PubMed: 23663381]

Odhiambo C, Venter M, Chepkorir E, Mbaika S, et al. Vector competence of selected mosquito species in kenya for Ngari and Bunyamwera viruses. J Med Entomol. 2014; 51:1248–1253. [PubMed: 26309314]

Traore-lamizana M, Fontenille D, Diallo M, Ba Y, et al. Arbovirus surveillance from 1990 to 1995 in the Barkedji area (Ferlo) of Senegal, a possible natural focus of Rift Valley Fever virus. J Med Entomol. 2001; 38:480–492. [PubMed: 11476327]

Wisloff H, Nordvik BS, Sviland S, Tonnessen R. First documented clinical case of Schmallenberg virus in Norway: Fetal malformations in a calf. Vet Rec. 2014; 174:120. [PubMed: 24399664]

Table 1

End Point Titers of Serum Samples Collected from Persons in Northeastern Kenya and Analyzed by Using Comparative PRNT

	Demographic characteristics				*PRNT$_{90}$ titers				
Patient ID	**Collection year**	**Residence**	**Age (years)**	**Sex**	**BUNV**	**NRIV**	**ILEV**	**GERV**	**Inferred infection**
HSA010062	2009	Sangailu	50	M	160	160	160	80	UND
HSA010005	2009	Sangailu	50	F	160	–	20	–	BUNV
HSA010010	2009	Sangailu	60	M	–	40	40	–	UND
HSA010074	2010	Sangailu	30	M	20	160	–	–	NRIV
HSA010094	2010	Sangailu	20	F	160	80	–	–	UND
HSA010109	2010	Sangailu	60	F	40	20	–	–	UND
HSA010303	2010	Sangailu	60	M	40	–	40	–	UND
HSA010312	2010	Sangailu	20	F	40	80	320	160	UND
HSA010314	2010	Sangailu	20	F	80	–	40	–	UND
HSA010389	2010	Sangailu	47	M	80	40		–	UND
HSA010395	2010	Sangailu	60	M	80	–	20	–	BUNV
HSA010752	2011	Sangailu	16	M	20	160	–	–	NRIV
HSA010779	2011	Sangailu	30	F	20	40	–	–	UND
HSA010780	2011	Sangailu	50	F	80	40	40	–	UND
HSA010856	2011	Sangailu	80	F	40	–	20	–	UND
HSA010888	2011	Sangailu	29	M	80	–	40	–	UND
HSA010918	2011	Sangailu	30	M	20	160	40	–	NRIV
HSA010947	2011	Sangailu	90	F	20	80	40	–	UND
HSA010988	2012	Sangailu	38	M	–	160	40	–	NRIV
HSA050018	2011	Kotile	17	M	80	–	20	–	BUNV
HSA050075	2011	Kotile	37	M	40	40	–	20	UND
HSA050083	2011	Kotile	35	M	80	–	80	40	UND
HSA050102	2011	Kotile	36	M	80	160	40	80	UND
HSA050124	2011	Kotile	30	M	–	640	160	–	NRIV
HSA050137	2011	Kotile	24	F	160	20	160	40	UND
HSA050146	2011	Kotile	19	M	20	80	–	–	NRIV
HSA050220	2011	Kotile	76	M	–	80	80	40	UND

	Demographic characteristics				*PRNT$_{90}$ titers				
Patient ID	**Collection year**	**Residence**	**Age (years)**	**Sex**	**BUNV**	**NRIV**	**ILEV**	**GERV**	**Inferred infection**
HSA050222	2011	Kotile	73	M	–	80	80	160	UND
HSA050225	2011	Kotile	30	M	80	320	160	160	UND
HSA050286	2012	Kotile	37	F	80	160	160	80	UND
HSA050308	2012	Kotile	8	F	40	20	20	–	UND
HSA050388	2012	Kotile	55	F	20	–	40	–	UND
HSA050399	2012	Kotile	50	F	20	40	–	–	UND

PRNT, plaque reduction neutralization test; M, male; UND, undetermined orthobunyavirus; F, female, BUNV, Bunyamwera virus; NRIV, Ngari virus; ILEV, Ilesha virus; GERV, Germiston virus; −, titer < 20;