Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Detection of a novel putative phlebovirus and first isolation of Dugbe virus from ticks in Accra, Ghana

Daisuke Kobayashi[a,b,1], Mitsuko Ohashi[a], Joseph H.N. Osei[c], Esinam Agbosu[d], Millicent Opoku[c], Alfred Agbekudzi[c], Joannitta Joannides[c], Ryosuke Fujita[b,e], Toshinori Sasaki[b], J.H. Kofi Bonney[d], Samuel Dadzie[c], Haruhiko Isawa[b,*], Kyoko Sawabe[b], Nobuo Ohta[a]

[a] Department of Environmental Parasitology, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-0034, Japan
[b] Department of Medical Entomology, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan
[c] Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana, P.O. Box LG 581, Legon, Accra, Ghana
[d] Department of Virology, Noguchi Memorial Institute for Medical Research, University of Ghana, P.O. Box LG 581, Legon, Accra, Ghana
[e] Japan Agency for Medical Research and Development, 1-7-1 Ote-machi, Chiyoda-ku, Tokyo 100-0004, Japan

ARTICLE INFO

Keywords:
Africa
Ghana
Dugbe virus
Nairovirus
Phlebovirus
Surveillance
Tick

ABSTRACT

Ticks are ectoparasites that transmit various types of human and animal pathogens. In particular, emerging and re-emerging diseases caused by tick-borne viruses are public health concerns around the world. However, in many countries of the sub-Saharan African region, epidemiological information on tick-borne viral infections is limited, and their prevalence and distribution remain largely unknown. In this study, we conducted surveillance on ticks to detect medically important tick-borne bunyaviruses in three study sites in and near to Accra, the capital city of Ghana, in 2015. Domestic dogs and cattle were surveyed and were found to be infested with various tick species belonging to the genera *Rhipicephalus*, *Amblyomma* and *Haemaphysalis*. Importantly, we detected a novel putative phlebovirus in *Rhipicephalus* ticks, and successfully isolated a new strain of Dugbe virus from *Am. variegatum* ticks. To our knowledge, this is the first report of tick-associated viruses in Ghana other than Crimean-Congo hemorrhagic fever virus.

1. Introduction

Ticks are known as important vectors of human and animal pathogens including protozoa, bacteria and viruses. Recently, emerging infectious diseases caused by novel tick-borne viruses such as severe fever with thrombocytopenia syndrome virus, Heartland virus, and Bourbon virus have been discovered in some parts of the world (Yu et al., 2011; Savage et al., 2013; Kosoy et al., 2015). On the other hand, reported cases of infection with Crimean-Congo hemorrhagic fever virus (CCHFV) and tick-borne encephalitis virus, both of which had been found more than half a century ago, are increasing in epidemic proportions, and expansion of their geographical distributions have become global public health concerns as re-emerging viral diseases (Estrada-Peña and de la Fuente, 2014). In the sub-Saharan African region, tick-borne bacterial infections including tick-borne relapsing fever and African tick bite fever are common and well documented (Hotez and Kamath, 2009). However, information on tick-borne viral diseases remains limited, with their prevalence and distribution remaining largely unknown.

In the Republic of Ghana, located in West Africa, tick-borne viral diseases in human have not yet been reported, although many potential viral vectors are distributed nationwide (e.g. *Hyalomma marginatum rufipes* as a vector of CCHFV, *Rhipicephalus decoloratus* as a vector of Bhanja virus, and *Amblyomma variegatum* as a vector of Dugbe virus) (Ntiamoa-Baidu et al., 2004) and CCHFV was recently detected in ixodid ticks (Akuffo et al., 2016). In fact, human or other animal infections with several tick-borne viruses, such as Bhanja virus and Dugbe virus, are present in surrounding countries (Moore et al., 1975; Matsuno et al., 2013). The present tick survey was conducted in three study sites in and near to Accra, the capital city of Ghana, to determine the geographical distribution and infection risk of tick-borne viruses in the Greater Accra Region in Ghana.

* Corresponding author.
E-mail address: hisawa@nih.go.jp (H. Isawa).
[1] Research Fellow of Japan Society for the Promotion of Science.

http://dx.doi.org/10.1016/j.ttbdis.2017.04.010
Received 7 December 2016; Received in revised form 7 March 2017; Accepted 14 April 2017
1877-959X/ © 2017 Elsevier GmbH. All rights reserved.

Please cite this article as: Kobayashi, D., Ticks and Tick-borne Diseases (2017), http://dx.doi.org/10.1016/j.ttbdis.2017.04.010

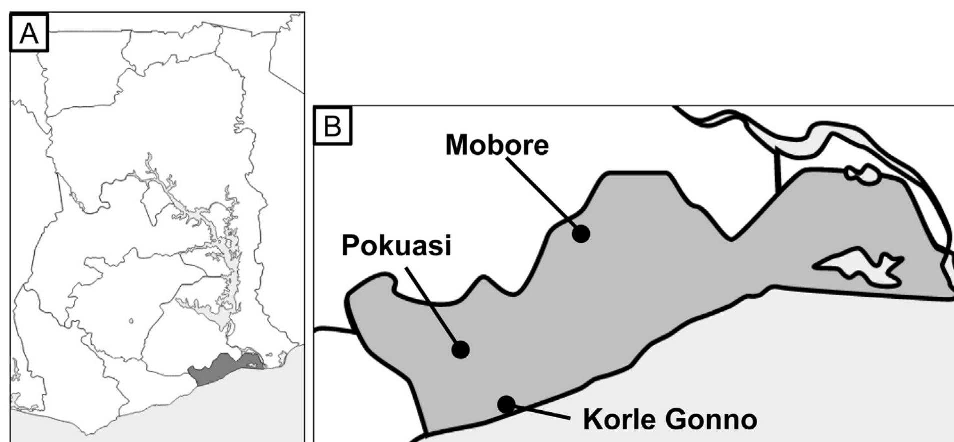

Fig. 1. Geographical location of the tick collection sites in Ghana. (A) The Greater Accra Region, which is the tick collection area in this study, is shown in gray. (B) Enlarged figure of the Greater Accra Region. Each tick collection site (Mobore, Pokuasi, and Korle Gonno) is indicated by a black dot.

2. Materials and methods

2.1. Study sites, tick collections and identifications

Tick collections were performed in the following three study sites; Mobore (N5°82′, W0°02′), Pokuasi (N5°69′, W0°28′), and Korle Gonno (N5°53′, W0°23′) districts within the Greater Accra Region, Ghana (Fig. 1) in September 2015. Mobore is a rural site in coastal scrub outside the city of Accra, where many domestic animals (cattle or goats) are reared. There are some remaining bushes for the animals to be grazed, and they are often put out to graze in the bush. Korle Gonno is a long-established residential district of urban Accra, and crammed with human residences. Thus, the district has very few domestic animals and sparse bushes. Pokuasi is one of the cities located outside the boundary of urban Accra, and there are some bushes around the residences. In this study site, we came across goats and sheep occasionally, but no pastured cattle. Therefore, Pokuasi is intermediate between the other two sites being more developed than Mobore but still outside the city boundary. It was unclear whether any tick control for domestic animals were practiced at each study site (Awumbila, 1996).

In these three study sites, ticks infesting domestic animals (dogs and cattle) were collected. The collected ticks were identified to genus and, where possible, species level according to the identification keys of Walker et al. (2003), separated according to instar and sex and stored at −80 °C until virus detection and isolation commenced.

2.2. Virus detection and isolation

Collected ticks were separated into a maximum of 17 individuals per pool (Supplementary Table S1) and homogenized using the disposal tissue homogenizer (BioMasher II, Nippi Inc., Tokyo, Japan) in 500 μl of Eagle's minimum essential medium (MEM, Sigma–Aldrich, St. Louis, MO) supplemented with 2% heat-inactivated fetal bovine serum (Sigma–Aldrich), 2% non-essential amino acids (Sigma–Aldrich), 200 U/ml penicillin (Life Technologies, Carlsbad, CA), 200 μg/ml streptomycin (Life Technologies), 5 μg/ml fungizone (Life Technologies). Subsequently, each homogenate was passed through a sterile 0.45 μm filter (Merck Millipore, Darmstadt, Germany) and total RNA was extracted from 200 μl of the resultant filtrates using ISOGEN II reagent (Nippon Gene, Tokyo, Japan) for reverse transcription-polymerase chain reaction (RT-PCR)-based virus detection. The RT-PCR for detection of nairoviruses (genus Nairovirus, family Bunyaviridae) and phleboviruses (genus Phlebovirus, family Bunyaviridae) was carried out using the PrimeScript One Step RT-PCR Kit Ver. 2 (Takara, Shiga, Japan) with universal primers to amplify the partial N protein gene in the nairovirus S segment (Nairo forward and Nairo reverse; Lambert and Lanciotti, 2009) and the partial L protein gene in the phlebovirus L segment (TBPVL2759F and TBPVL3267R; Matsuno et al., 2015), respectively. In addition, for the purpose of phylogenetic analysis of nairoviruses, we also used another primer set (6942+ and 7385−; Honig et al., 2004) to amplify the genomic region including the conserved motifs in the L protein genes of nairoviruses. All primer sets were used under a common RT-PCR thermal condition: RT at 50 °C for 30 min; termination of RT at 94 °C for 2 min; and 35 cycles for PCR at 94 °C for 30 s, 53 °C for 30 s, and 72 °C for 30 s. The amplified products were confirmed by agarose gel electrophoresis and directly sequenced.

Virus isolation using cell cultures was performed as previously described (Ejiri et al., 2015). In brief, the filtrates were inoculated into monolayers of BHK-21 (Syrian hamster kidney) cells (Japan Health Science Research Resources Bank, Osaka, Japan) and incubated at 37 °C in 5% CO_2 conditions for 7 days. The development of cytopathic effects (CPE) was monitored by comparing with mock-inoculated controls. After three additional blind passages, the supernatants were harvested and cryopreserved at −80 °C until further analysis.

2.3. Next generation sequencing of the cell culture supernatants

To confirm and identify the isolated viruses, next generation sequencing (NGS) was performed on the cell culture supernatants after the blind passages. However, the supernatant from the pool 15AC-25, from which DUGV was isolated, was omitted from this NGS analysis. The basic approach of NGS has been described previously (Kobayashi et al., 2016). In brief, the supernatants of 4 to 8 individual pools were mixed together, and then 4 units of TURBO DNase (Life Technologies), 4 units of Baseline ZERO DNase (Epicentre, Madison, WI), and 0.4 μg of RNase A (Wako Pure Chemical Industries, Osaka, Japan) were added to 390 μl of each mixed supernatant. After incubation at 37 °C for 1 hr, total RNA was extracted and the cDNA synthesis and amplification were performed using SeqPlex RNA Amplification kit (Sigma-Aldrich). The amplified cDNAs were subjected to NGS using the Ion PGM system (Thermo Fisher Scientific, Waltham, MA). The resultant data was analyzed by the CLC genomics workbench software (CLC bio, Aarhus, Denmark).

2.4. Phylogenetic analysis

Phylogenetic analyses of phleboviruses and nairoviruses were performed based on the amino acid (aa) or nucleotide (nt) sequences derived from the RT-PCR amplicons using the primers described above. Multiple alignments with aa or nt sequences from related viruses were conducted using the Clustal W method. The phylogenetic analysis was carried out using the Neighbor-Joining method (Saitou and Nei, 1987).

Download English Version:

https://daneshyari.com/en/article/5546318

Download Persian Version:

https://daneshyari.com/article/5546318

Daneshyari.com