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ORIGINAL ARTICLE

Screening for tick-borne and tick-associated viruses in ticks collected in Ghana

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Abstract
Ticks are blood-sucking arthropods that transmit many pathogens, including arboviruses. Arboviruses transmitted by ticks are generally referred to as tick-borne viruses (TBVs). TBVs are known to cause diseases in humans, pets, and livestock. There is, however, very limited information on the occurrence and distribution of TBVs in sub-Saharan Africa. This study was designed to determine the presence and distribution of ticks infesting dogs and cattle in Ghana, as well as to identify the tick-borne or tick-associated viruses they harbour. A more diverse population of ticks was found to infest cattle (three genera) relative to those infesting dogs (one genus). Six phleboviruses and an orthonairovirus were detected in tick pools screened by RT-PCR. Subsequent sequence analysis revealed two distinct phleboviruses and the previously reported Odaw virus in ticks collected from dogs and a virus (16GH-T27) most closely related to four unclassified phleboviruses in ticks collected from cattle. The virus 16GH-T27 was considered a strain of Balambala tick virus (BTV) and named BTV strain 16GH-T27. Next-generation sequencing analysis of the BTV-positive tick pool detected only the L and S segments. Phylogenetic analysis revealed that BTV clustered with viruses previously defined as M-segment-deficient phleboviruses. The orthonairovirus detected in ticks collected from cattle was confirmed to be the medically important Dugbe virus. Furthermore, we discuss the importance of understanding the presence and distribution of ticks and TBVs in disease prevention and mitigation and the implications for public health. Our findings contribute to the knowledge pool on TBVs and tick-associated viruses.

Introduction

Ticks are among the most important vectors of arboviruses, transmitting many infectious pathogens of medical and veterinary importance [1, 2]. Arbovirus-transmitting tick

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species can be broadly categorized as soft ticks belonging to the genera *Ornithodoros*, *Carios* and *Argas* and hard ticks of the genera *Ixodes*, *Haemaphysalis*, *Hyalomma*, *Amblyomma*, *Dermacentor, Rhipicephalus,* and *Boophilus* [3–5]. The ability of ticks to infest multiple host species throughout their different life cycle stages compounds the prominence and risk of ticks as vectors of arboviruses as host migration facilitates the spread of tick-borne viruses into new areas [6]. The survival of ticks in any environment, however, depends on climatic conditions such as temperature, humidity, and precipitation [7, 8]. For example, the activity of *Hyalomma marginatum* ticks, the primary vector of Crimean-Congo hemorrhagic fever virus (CCHFV), peaks in Turkey during the summer when the temperatures range from 30°C to 40°C and maximum relative humidity ranges from 20% to 50% [6]. Thus, the distribution and density of ticks are dependent on and limited by the presence of hosts as well as the prevailing climatic conditions.

Tick-borne viruses (TBV) are vertebrate-infecting viruses transmitted by ticks. There are approximately 160 known TBVs, transmitted by about 10% of known tick species [4]. TBVs are classified into eight RNA viral families (*Flaviviridae, Nairoviridae, Orthomyxoviridae, Reoviridae, Rhabdoviridae, Phenuiviridae, Peribunyaviridae,* and *Nyamiviridae*) and one DNA viral family (*Asfarviridae*) [4]. These TBVs cause at least 25 diseases in humans, pets, and livestock [9].

At least five genera of ticks have been identified in Ghana, a country located along the coast of the Gulf of Guinea in West Africa, where it is warm all year round. The five genera, all of which are medically important for TBV transmission, include *Ixodes, Amblyomma, Haemaphysalis*, *Rhipicephalus,* and *Hyalomma* [3, 10, 11]. Two medically important TBVs, CCHFV and Dugbe virus (DUGV), have been detected in ticks infesting livestock in Ghana [12, 13]; however, information on the prevalence and distribution of TBVs in Ghana is limited. Therefore, this study was designed to investigate the presence and distribution of TBVs in ticks collected from dogs and cattle across Ghana and assess the risk of TBV infection in humans and livestock.

Materials and methods

Study site and sample collection

Ticks were collected from three different vegetations spanning four regions in Ghana in August and September 2016. Accra (5°38'28.0"N 0°10'32.7"W), a coastal savannah in the Greater Accra region, is an urban area; Hohoe (7°09'32.8"N 0°28'39.6"E), a deciduous forest in the Volta region, is semi-urban; Larabanga (9°13'01.2"N 1°51'25.4"W) in the savannah region and Jirapa (10°31'53.2"N 2°41'59.8"W) in the Upper West region are rural areas with a Guinea savannah vegetation (Fig. 1) [14].

Tick samples were collected from dogs in households in Larabanga and dogs that had been brought to veterinary hospitals in Accra. Tick samples from cattle were collected in Hohoe, Larabanga, and Jirapa (Supplementary Table S1). Questing ticks were collected from cattle-grazing fields in Larabanga by dragging a flannel sheet (70 × 100 cm) as described by Kobayashi et al. [15]. Sampled ticks were classified to the species or genus level. In addition, the sex, developmental stage, and feeding status were recorded (Supplementary Table S1) [13, 16].

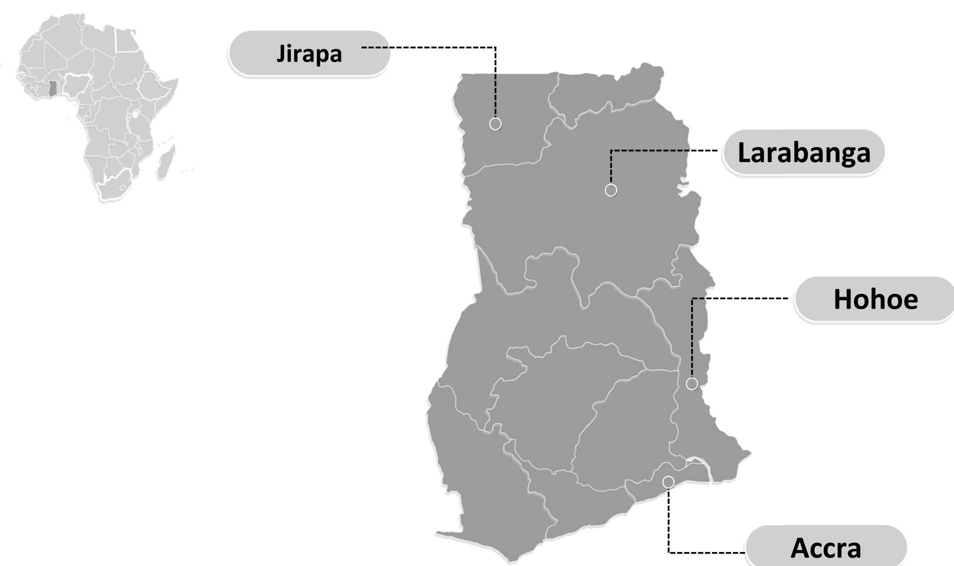

Fig. 1 Map showing sample collection sites. Map of Africa (inset) showing the location of Ghana (shaded). Map of Ghana, showing the sample collection sites (yourfreetemplate.com)

Virus isolation and detection

Virus isolation was performed using BHK-21 cells (derived from Syrian hamster kidney, Japan Health Science Research Resources Bank, Osaka, Japan) as described previously [17]. Briefly, homogenized tick pools were inoculated on a monolayer of BHK-21 cells and incubated at 37 °C and 5% CO_2 for 7 days. After two blind passages, cell culture supernatants were stored at −80 °C until used. The culture supernatant from the virus isolation process was subjected to next-generation sequencing (NGS) analysis using an Illumina MiniSeq System as described previously [14]. CLC Genomics Workbench software (CLC Bio, Aarhus, Denmark) was used to assemble reads into contigs, which were used to search the National Centre for Biotechnology Information database using the Basic Local Alignment Search Tool (BLAST)n and BLASTx.

In addition to virus isolation and NGS analysis, virus detection was performed by conventional RT-PCR. Total RNA was extracted from tick homogenates using ISOGEN II (Nippon Gene, Tokyo, Japan). Extracted RNA was screened for selected medically important tick-borne or tick-associated viruses of the genera *Alphavirus, Flavivirus, Orthonairovirus, Phlebovirus,* and *Thogotovirus,* using a PrimeScript One Step RT-PCR Kit Ver. 2 (Takara Bio, Shiga, Japan) and universal primers (Table 1). The reaction conditions for RT-PCR were as follows: 50°C for 30 min, 94°C for 2 min, and 35 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 30 s. Amplified products were purified from an agarose gel after electrophoresis, sequenced directly, and identified using BLASTn and BLASTx. The virus-positive pools were subjected to NGS analysis to determine unknown sequences [15, 18].

Phylogenetic analysis

The online version of MAFFT 7 was used to align amino acid sequences [19]. MEGA ver. 7 was used for determination of a suitable amino acid substitution model and for dendrogram construction [20].

Complete coding sequence (CDS) determination of DUGV strain 15AC-T25

NGS analysis was performed on the cell culture supernatant after six passages of DUGV strain 15AC-T25, isolated from *Amblyomma variegatum* ticks collected from cattle in Accra in 2015 [13]. The complete CDS of each genome segment of the strain was determined, and similarities to known strains of DUGV were assessed.

Results

Tick sampling and classification

Ticks were collected from dogs, cattle, and cattle-grazing fields in Ghana and classified morphologically at the genus or species level. Ticks of at least three genera were detected in this study (Supplementary Table S1). All ticks collected from dogs (in both Accra and Larabanga) were of the genus *Rhipicephalus*, while a more diverse collection of ticks was obtained from cattle across the collection

Table 1 Primers used for RT-PCR-based virus screening

Target viruses	Primer name	Primer sequence (5' - 3')	Reference
Genus *Orthonairovirus*	Nairo Forward	TCTCAAAGAAACACGTGCCGC	Lambert and Lanciotti, 2009
	Nairo Reverse	GTCCTTCCTCCACTTGWGRGCAGCCTGCTGGTA	
Tick-borne phleboviruses	TBPVL2759F	CAGCATGGIGGICTIAGAGAGAT	Matsuno et al., 2015
	TBPVL3267R	TGIAGIATSCCYTGCATCAT	
Genus *Flavivirus**	FU1	TACAACATGATGGGAAAGAGAGAGAA	Kuno et al., 1998
	cFD2	GTGTCCCAGCCGGCGGTGTCATCAGC	
	FU2	GCTGATGACACCGCCGGCTGGGACAC	
	cFD3	AGCATGTCTTCCGTGGTCATCCA	
Genus *Alphavirus*	AJUN	CTSTACGGYKRWCCTAAAT	Miller et al., 2000
	CCAP	RTAYTGSACWGCKCCRTGRTGCCA	
Genus *Orthobunyavirus* (Bunyamwera and California serogroups)	BCS82C	ATGACTGAGTTGGAGTTTCATGATGTCGC	Kuno et al., 1996
	BCS332V	TGTTCCTGTTGCCAGGAAAAT	
Genus *Thogotovirus*	THOV-uni-1-170F	AARAGRTACACTACRAGCAAGAA	Current study
	THOV-uni-1-600R	GCTGWATTGGGGRCAGAASACTTG	

*Two different primer sets (FU1 and cFD2, FU2 and cFD3) were used in detecting flaviviruses in this study.

sites (Supplementary Table S1). The ticks collected from cattle were *Amblyomma* spp. (including *A. variegatum)*, *Rhipicephalus* spp.*, Hyalomma* spp., and *Ixodidae* spp. (Supplementary Table S1). In total, 354 ticks divided into 93 pools were subjected to virus isolation, RT-PCR, and NGS analysis (Supplementary Table S1).

Virus detection and sequence analysis

In screening for tick-borne or tick-associated viruses, the second supernatant from virus isolation and the homogenate from pooled tick samples were subjected to NGS and RT-PCR analysis, respectively. No viruses were detected or isolated by the virus isolation method. RT-PCR analysis of tick homogenates revealed that, out of 93 pools, seven were positive for RNA viruses. We detected phleboviruses in six pools and orthonairoviruses in one pool (Table 2). The phleboviruses detected in pools 16GH-T12, 16GH-T13, 16GH-T14, and 16GH-T15 were confirmed to be strains of Odaw virus (ODWV), which was detected previously in *Rhipicephalus* ticks from Accra [13] (Table 2). The remaining two phlebovirus-positive pools, 16GH-T24 and 16GH-T27, had 95% nucleotide sequence identity to Balambala tick virus (BTV) detected in *H. rufipes* in Kenya and 92% nucleotide sequence identity to a tick phlebovirus detected in *H. marginatum* sampled from sheep in Turkey [21]. The viruses in 16GH-T24 and 16GH-T27 were considered to be the same, as their nucleotide sequences were 100% identical within the limits of the RT-PCR-amplified region. NGS analysis of 16GH-T27 and subsequent *de novo* assembly coupled with Sanger sequencing resulted in the detection of 5,691-nt and 1,547-nt partial-yet-continuous regions of the viral L and S segment, respectively. Phylogenetic analysis using amino acid sequences showed 16GH-T27 to form a clade with four unclassified phleboviruses, the most closely related being BTV (Fig. 2). Similarly, BTV shared the highest nucleotide and amino acid sequence identity with both the L and S segments of 16GH-T27. 16GH-T27 can therefore be considered a strain of BTV (Table 2; Fig. 2). The amino acid and nucleotide sequence identity values for the L and S segments of the four related unclassified phleboviruses are shown in Table 3 and Supplementary Table S2, respectively.

The RT-PCR-amplified product of the orthonairovirus-positive pool, 16GH-T89, was subjected to Sanger sequencing analysis. The resulting sequence shared 97% identity with the S segment of DUGV strain IbAr 1792 detected in *A. variegatum* ticks picked off cattle in Nigeria [22]. Subsequent determination of the complete genome sequence of DUGV strain 15AC-T25, consisting of a 12,201-nt L segment (GenBank accession number LC579816), a 4,849-nt M segment (GenBank accession number LC579817), and a 1,691-nt S segment (GenBank accession number LC579818), showed the S segment of 15AC-T25 and 16GH-T89 to be nearly identical. 16GH-T89 was therefore confirmed to be DUGV (Table 2). In addition, pairwise alignment analysis of 15AC-T25 by BLASTn also showed 97%–98% sequence identity to the L, M, and S segments of DUGV strain IbAr 1792 (GenBank accession numbers KU925455, KU925456, and KU925457, respectively) [22], confirming that 15AC-T25 has the typical genome organisation of DUGV.

Discussion

Ghana is a West African country slightly north of the equator with an average temperature range of 24°–30 °C [23] and 50–80% humidity. It is home to an abundance of domesticated animals, including pets and livestock [24]. Dogs and cats are the most common companion animals in Ghana; dogs also serve as a form of security in some households. The most common livestock in Ghana are cattle, goats,

Table 2 Viruses detected in this study

Virus name	Strain	Location	Source Tick species	Number, stage, and sex of pooled ticks	Host animals	GenBank accession number
Odaw virus	16GH-T12	Accra	*Rhipicephalus* sp.	13 adult males*	Dogs	LC579647
	16GH-T13	Accra	*Rhipicephalus* sp.	14 adult males*	Dogs	LC589706
	16GH-T14	Accra	*Rhipicephalus* sp.	10 adult females*	Dogs	LC589707
	16GH-T15	Accra	*Rhipicephalus* sp.	8 adult females*	Dogs	LC589708
Balambala tick virus	16GH-T24	Jirapa	*Amblyomma* sp.	3 adult females*, 1 adult female**	Cattle	LC579820
	16GH-T27	Jirapa	*Hyalomma* sp.	9 adult males*	Cattle	LC579819 (L segment) LC589978 (S segment)
Dugbe virus	16GH-T89	Hohoe	*Rhipicephalus* sp.	5 adult females**	Cattle	LC579815

* Non-engorged

** Engorged (partially or fully engorged)

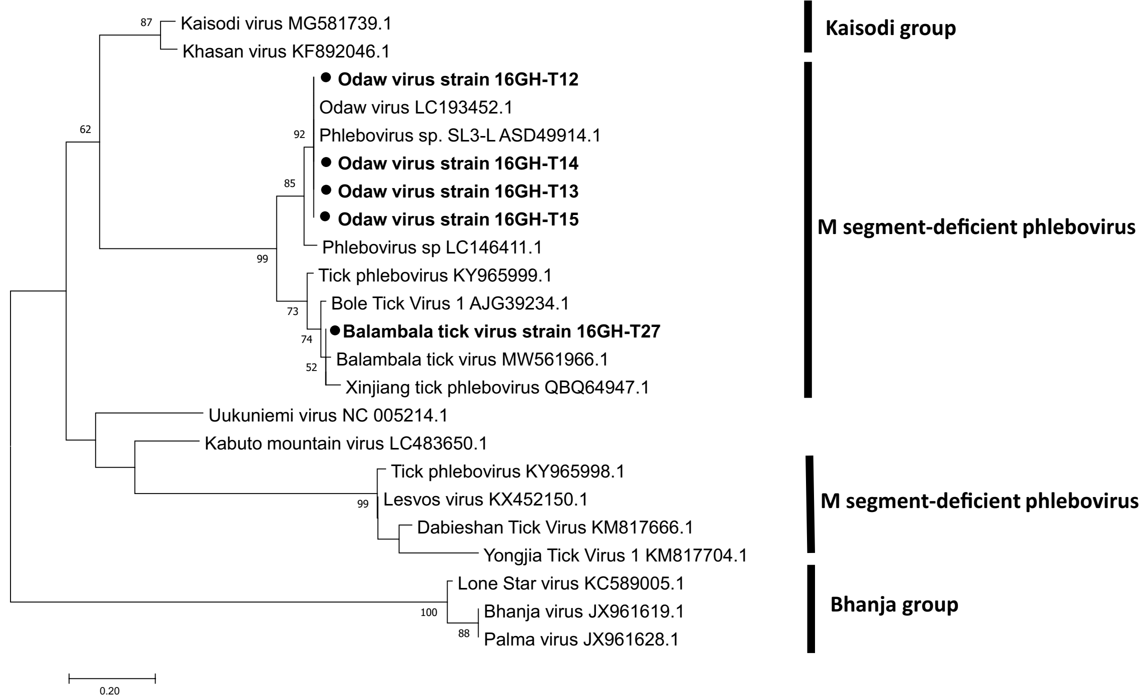

Fig. 2 Phylogenetic analysis of Balambala tick virus (BTV) strain 16GH-T27. An outgroup-rooted maximum-likelihood tree was constructed, using the LG + G substitution model, based on complete RdRp amino acid sequences with GenBank accession numbers LC579819 (BTV strain 16GH-T27) and LC579820, LC589706, LC589707, and LC589708 (ODWV strains 16GH-T12, 16GH-T13, 16GH-T14, and 16GH-T15, respectively). The Bhanja virus group was used as the outgroup. Bootstrap support values from 1000 bootstrap replicates are indicated on the branches. Viruses detected/isolated in this study are preceded by a bullet (●) and shown in boldface type. The scale bar represents the number of substitutions per site.

Table 3 L segment nucleotide and amino acid sequence identity values for the four related unclassified phleboviruses

Virus name	Accession number	Reference virus	Accession number	Region analyzed	Nucleotide sequence identity (%)	Amino acid sequence identity (%)
Balambala tick virus strain 16GH-T27*	LC579819	Balambala tick virus	MW561966.1	Complete CDS	93.13	97.65
		Bole tick virus 1	AJG39234.1	Complete CDS	71.20	77.95
		Tick phlebovirus	KY965999.1	Partial CDS	78.00	81.18
		Xinjiang tick phlebovirus	QBQ64947.1	Complete CDS	72.90	81.45
Balambala tick virus	MW561966.1	Bole tick virus 1	AJG39234.1	Complete CDS	73.98	79.38
		Tick phlebovirus	KY965999.1	Partial CDS	78.52	78.74
		Xinjiang tick phlebovirus	QBQ64947.1	Complete CDS	74.42	82.11
Bole tick virus 1	AJG39234.1	Tick phlebovirus	KY965999.1	Partial CDS	74.51	90.00
		Xinjiang tick phlebovirus	QBQ64947.1	Complete CDS	74.30	81.15
Tick phlebovirus	KY965999.1	Xinjiang tick phlebovirus	QBQ64947.1	Complete CDS	75.88	90.59

*Virus detected in this study

sheep, pigs, and poultry [25]. Livestock animals in the cities are reared in intensive or semi-intensive systems, while rural areas employ extensive farming models [24]. As the name suggests, the intensive model restricts livestock movement, whereas semi-intensive and extensive models, which are less capital-intensive, allow animals varying degrees of freedom

to move and interact with the community and ecosystem [24]. This interaction between humans and animals in a climate conducive to tick survival and breeding increases the likelihood of transmission of tick-borne infections of public health and veterinary importance. This study was therefore designed to identify and characterize tick-borne or tick-associated viruses harboured by ticks infesting domesticated animals in different regions of Ghana. A total of 354 nymph or adult ticks collected from four different geographic locations in Ghana were subjected to virus isolation, RT-PCR, and NGS analysis.

Ticks of at least three genera were collected across Ghana; however, members of only one genus (*Rhipicephalus*) were found on dogs, consistent with a previous report by Kobayashi et al. [13]. Furthermore, tick samples were collected from dogs in Accra and Jirapa, but only those from Accra were positive for an RNA virus, ODWV. The detection of ODWV-positive ticks in Accra is consistent with the findings of Kobayashi et al. [13]. However, in this study, ODWV was detected in pools of non-engorged ticks, whereas, previously, it was detected only in pools containing engorged ticks [13]. These results confirm that ODWV is a tick-associated virus that is widely distributed in *Rhipicephalus* ticks infesting dogs in Accra; however, whether ODWV is infectious to dogs and/or humans is yet to be determined.

Among the cattle-infesting ticks, three pools, each representing a genus, were positive for an RNA virus. Two pools were positive for BTV, and the third was positive for DUGV. BTV was detected in *Amblyomma* and *Hyalomma* ticks from Jirapa, while DUGV was detected in *Rhipicephalus* ticks from Hohoe.

BTV, like ODWV, is a phlebovirus belonging to the group defined as the M-segment-deficient phleboviruses (MdPVs) [15]. To our knowledge, this is the first report on the evolutionary history of any BTV strain that confirms its classification as a member of the MdPV group. These MdPVs can further be classified into three distinct groups based primarily on their phylogeny [15]. The host ticks of the MdPVs also appear to be distinct, with only group II, which includes BTV and ODWV, detected in ticks of multiple genera: *Dermacentor, Hyalomma*, and *Rhipicephalus* [15, 26–30]. Owing to the apparent relationship between MdPVs and their host ticks, they are postulated to have coevolved with their hosts through vertical transmission. Nevertheless, the possibility of horizontal transmission has not been ruled out. In this study, BTV was detected in *Hyalomma* and *Amblyomma* ticks, representing the first report of a group II MdPV in ticks of the genus *Amblyomma*. Furthermore, the detection of BTV in both *Amblyomma* and *Hyalomma* ticks infesting cattle in Jirapa may be an indication of possible horizontal transmission between ticks of different genera infesting the same host, possibly through co-feeding. Labuda et al. [31] reported that migration of cells, such as monocytes and macrophages, from tick feeding sites facilitates the transmission of viruses between infected and uninfected ticks. The potential for horizontal transmission through co-feeding raises concerns about the evolutionary and public health implications of coinfection of an MdPV and related phleboviruses (with an M segment). It has been postulated that phlebovirus L and N proteins recognize the untranslated region of the M segment of a related virus as a functional promoter for transcription and replication, producing viable reassortant progeny [32].

DUGV is an orthonairovirus of public health importance that has been reported to cause thrombocytopenia and febrile illnesses in humans [33]. Although there are no reports of pathology in livestock animals or pets, the public health implications of the widespread distribution of DUGV in Africa, and possibly Ghana, must be evaluated [33, 34]. DUGV was originally isolated from *A. variegatum*, the main vector. Other tick species, *Hyalomma truncatum, Boophilus decoloratus,* and *Rhipicephalus appendiculatus*, are also regarded as major vectors of DUGV [35]. In this study, DUGV was detected in *Rhipicephalus* ticks collected in Hohoe. Kobayashi et al. [13] isolated DUGV from *A. variegatum* collected in Accra, demonstrating that DUGV is distributed throughout southern Ghana and may be transmitted by multiple vectors.

The results of this study suggest that the close interaction between people and livestock/pets in Ghana may increase the transmission potential of TBVs in the country. Thus, screening for TBVs (such as DUGV and CCHFV) when diagnosing febrile illnesses is important, particularly in malaria-negative patients. A public health adjustment of this nature may help reduce the number of (and issues surrounding) undiagnosed febrile cases in Ghana [36]. Furthermore, the results of this study highlight the need to comprehensively examine the actual disease burden of TBVs in Ghana, which will facilitate the development of health policies to include TBV-screening in the diagnoses of febrile illnesses.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00705-021-05296-4.

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Author contributions Conceptualization: MA-B, DK, HI. Methodology: MA-B, DK, ANF, SK, AA, EA, DP, MO, HE. Formal analysis and investigation: MA-B, DK, HI. Writing—original draft preparation: MA-B. Writing—review and editing: MA-B, DK, HI. Funding acquisition: HI. Resources: HI. Supervision: JHKB, SD, NO, KS, SI, HI.

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Availability of data and materials The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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