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Epidemiological dynamics of influenza B virus across multiple seasons in Kenya and Uganda inferred from sequence data, 2010–2021

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Abstract

Background Influenza B virus (IBV) contributes significantly to morbidity and mortality during Influenza seasons annually. However, IBV genomic surveillance occurs unevenly across the globe, particularly within the African region, obscuring its epidemiology. This study aims to elucidate the epidemiological dynamics of IBV in Kenya and Uganda between 2010 and 2022.

Methods In this study, 83 near complete IBV genomes circulating in Kenya and Uganda between 2010 and 2022 were generated through Oxford Nanopore Technologies sequencing (ONT). Publicly available IBV datasets were incorporated to evaluate the public context of these genomes. Further evolutionary dynamics analysis investigated the antigenic mutation, reassortment and glycosylation patterns of IBVs circulating in Kenya and Uganda within this period.

Results Alternating IBV lineage predominance and clade turnover was observed consistent with global patterns. No B/Yamagata strains were detected at the study sites after 2019. Multiple B/Victoria clade/subclades (V1A, V1A.3, V1A.3a, V1A.3a.2) and B/Yamagata clades (Y2 and Y3) were identified with no inter-lineage reassortments observed. Over time, the clades/subclades appeared to diversify through the accumulation of amino acid changes along the hemagglutinin (HA) segment backbone, especially within the known antigenic sites. Local outbreak strains appeared to be putatively introduced from both within and outside Africa.

Conclusions The congruence of local and global strains in circulating lineages and amino acid changes suggests potential effectiveness of vaccines recommended for the Northern and Southern Hemispheres in East Africa.

Keywords IBV, B/Victoria, B/Yamagata

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Introduction

Three influenza types (A, B and C) can cause human infection and disease. Influenza B viruses (IBV) account for about 30% of the total influenza disease burden, which is estimated at ~290,000 to 650,000 deaths and 3–5 million severe cases annually [5, 41, 52, 61]. Previous studies in Kenya and Uganda, indicated significant influenza disease burden throughout childhood (0–14 years) and among vulnerable populations e.g., pregnant women and immunocompromised individuals [7, 11, 34, 37, 51, 53, 57]. Influenza disease and perhaps transmission is predominantly seasonal in temperate climatic regions [4]. Contrastingly, year-round influenza activity is observed in tropical and subtropical regions although the types (or subtypes) within may exhibit seasonal occurrence [3]. Understanding of the origin(s), phylogenetic relatedness and evolution of circulating influenza strains in tropical populations, including sub-Saharan Africa remains limited [25].

Two IBV lineages (B/Yamagata and B/Victoria) have co-circulated with variable predominance since early 1980s [42]. Strains within these lineages are classified into clades and subclades following hemagglutinin (HA) sequence phylogenetic analysis [10]. A brief decline in influenza activity was observed between 2020 to 2021 during the coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [35, 63]. This has been hypothesized to have been caused by the non-pharmaceutical interventions (NPIs) that were put in place to slow the spread of the epidemic e.g., lockdowns, social distancing and improved personal hygiene measures [18, 23]. Remarkably, no B/Yamagata cases have been identified and confirmed by sequencing since March of 2020, leading to reports of potential extinction of this lineage [23, 39]. Previous studies observed that there is constant turnover of genetically distinct clades in both lineages over time [25, 55, 56]. This turnover has been attributed to factors such as population immunity, vaccination and stochastic processes that underlie strain introduction, transmission and extinction [33, 58].

Influenza vaccines are reformulated periodically (~1–2 years) based on circulating strains [60]. Vaccines used in tropical and subtropical regions are guided by the Northern or Southern Hemisphere recommendations due to limited local strain surveillance [60]. Trivalent vaccine formulations (TIV) having one IBV lineage plus a H1N1 and H3N2 strain and quadrivalent vaccines (QIV) [20] having both IBV lineages plus a H1N1 and H3N2 strain are available [14]. Mismatch between circulating and recommended strains in the vaccines has been periodically observed [2, 16, 41]. There is an ongoing current debate whether B/Yamagata strains should continue to be included in annual influenza vaccines regimens [22, 27].

Notably, Kenya and Uganda do not have a national influenza policy yet. However, the Kenya National Immunisation Technical Advisory Group (KENITAG) in 2016 provisionally recommended vaccination of children aged 6–23 months [8, 12].

Influenza surveillance and genomic analysis is instrumental in revealing circulating subtypes, clades and subclades, and processes such as antigenic drift, reassortment and glycosylation that potentially impact their epidemiological success [33]. This information is invaluable to assess predominant strains in circulation immune escape capacity of emergent strains and predict potential vaccine effectiveness of recommended vaccines during resurgence of IBV in communities. In this study, we investigated the epidemiological dynamics of the circulating IBV strains over eight years in Kenya and three years in Uganda, their global context and evolution using genomic sequence data.

Materials and methods

Study area and design

The specimens analysed in this study were collected during three studies: (a) the Health Facility (HF) surveillance study which is an ongoing outpatient acute respiratory illness (ARI) surveillance study within Kilifi Health and Demographic Surveillance System (KHDSS) that has been running since December 2020 (Kenya), (b) the Kilifi County Hospital (KCH) paediatric pneumonia admissions study which has been ongoing since 2007 (Kenya) and, (c) the Children's Oxygen Administration Strategies Trial (COAST) study [28] and COAST-Nutrition trial conducted between 2017 and 2020 (Uganda and Kenya).

In the HF surveillance, ~15 samples per week per facility were collected from patients across all age groups presenting with ARI at five outpatient facilities, namely, KCH, Mavueni, Pingilikani, Mtondia and Matsangoni (Fig. 1a). The inpatient KCH samples were collected from all eligible consented hospitalised children aged ≤5 years with syndromic severe or very severe pneumonia [36]. The COAST study samples were obtained from children aged 28 days to 12 years admitted to hospital with respiratory distress complicated by hypoxia at the Mulago National Referral Hospital, Jinja, Mbale, and Soroti Regional Referral Hospitals in Uganda and the KCH and Coast Province General Hospital (CPGH) in Kenya (Fig. 1b). The participant inclusion criteria for these studies are described in detail in previous reports [28, 32, 34, 36].

Sample collection and IBV laboratory detection

Nasopharyngeal (NP) and oropharyngeal (OP) swabs were transferred to viral transport media (VTM) and transported to KEMRI-Wellcome Trust Research Programme (KWTRP) in cool boxes with ice packs. The samples were stored at –80 °C before retrieval for diagnostics

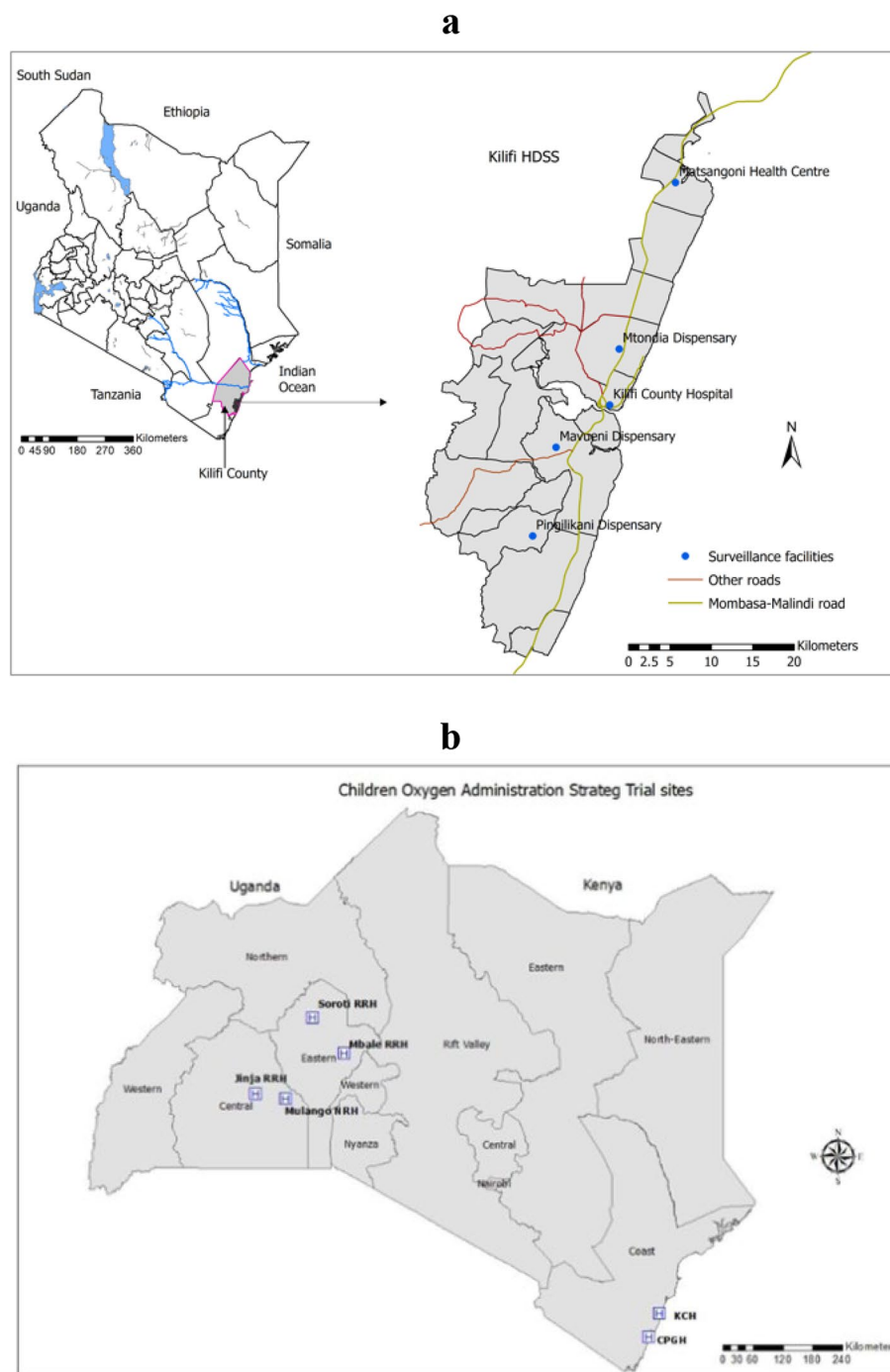


Fig. 1 Geographical location of study sites in Kenya and Uganda. **a** A map of Kenya showing the Kilifi Health and Demographic Surveillance System area, the location of the Health Facilities that participated in the study and Kilifi County Hospital **b** Map showing the location of Children's Oxygen Administration Strategies Trial (COAST) hospitals in Uganda and Kenya

and sequencing. A triplex real-time reverse transcription polymerase chain reaction (RT-PCR) diagnostic assay was used to screen samples for influenza A, B, and C [36].

RNA extraction and multi segment PCR

Viral RNA was extracted from 151 IBV positive samples using the QIAamp Viral RNA Mini Kit according to the

manufacturer's instructions (Qiagen, Hilden, Germany). The universal IBV-GA2 primer set [65] was used to perform reverse transcription and amplification of the 8 IBV segments in one reaction using the SuperScriptIII One-Step RT-PCR system kit with Platinum Taq DNA High Fidelity polymerase (Invitrogen, Carlsbad, CA, USA). Multi-segment PCR products were stained with

the RedSafe Nucleic Acid Staining solution (iNtRON Biotechnology Inc., Seoul, South Korea) on a 1.5% agarose gel and amplicons were visualised through a UV trans-illuminator.

Next generation sequencing and assembly

PCR amplicons were purified using $\times 1$ Agencourt AMPure XP beads to remove remaining PCR reagents (Beckman Coulter Inc., Brea, CA, USA) then quantified using Quant-iT dsDNA High-Sensitivity Assay (Invitrogen). Samples with > 40 ng/ μ l were classified as grade 1 and underwent normalisation by dilution with 5:7 μ l RNase free water to sample ratio. The Oxford Nanopore Technologies (ONT) ligation sequencing kit and the ONT Native Barcoding Expansion kit EXP-NBD196 (Oxford Nanopore Technologies, Oxford, UK) were used to generate barcoded libraries according to the optimised ARTIC protocol [24]. Pooled libraries were sequenced on a FLO-MIN106 R9.4.1 flow cell following the manufacturer's instructions (ONT). Sequence base-calling and demultiplexing of FAST5 files was performed by the ONT Guppy v6.3.9 software then ONT adapters were trimmed by porechop v0.2.4. Reference-based assembly was carried out by Iterative Refinement Meta-Assembler (IRMA) v1.0.3 FLU-MinION module, suited for the highly variable nature of influenza virus due to the iterative refinement method for the reference sequences [48]. IRMA default settings: median read Q-score filter of 30, the minimum read length of 125, the frequency threshold for insertion and deletion refinement of 0.25 and 0.60, respectively, Smith–Waterman mismatch penalty of 5, and gap opening penalty of 10 were applied for this dataset.

Global and reference dataset collation

A reference dataset comprising 9 IBV clade and sub-clade representative sequences (Table 1) downloaded from Global Initiative for Sharing All Influenza Data (GISAID) was used to assign clades to the sequences. A second global dataset of all complete IBV sequences in the database from 1st January 1987 to 16th May 2023 of the two lineages was obtained separately. The dataset was cleaned by removing duplicated sequences, missing collection dates and Influenza A sequences (supplementary Fig. 1). The cleaned dataset was subset using a criterion randomly selecting up to 10 sequences per month, year and continent. In addition, 108 sequences generated in 2016 from the KHDSS study surveillance in Kilifi with GISAID accession numbers EPI_ISL_336258 and EPI_ISL_336282–EPI_ISL_336395 were downloaded from the database [33].

Phylogenetic analysis

Near complete ($> 70\%$) assembled segments were aligned with the reference and global datasets by MAFFT v7.515

[21]. The aligned sequences were manually curated by removing leading and trailing gaps and visualised in Aliview v1.28 [26]. Per segment maximum likelihood phylogenies were inferred using IQTREE v2.2.6 with a clustering reliability evaluated by using 1000 bootstrap iterations [31]. The best fitting nucleotide (nt) substitution model was determined by Modelfinder [19] and resulting ML phylogenies visualised with ggtree R package v3.6.2 [64]. Clade and lineage assignment was done according to ECDC guidelines [10] as described in (Table 1). Per segment phylogeny comparisons with HA were done to detect reassortants in dendextend v1.17.1 R package [13]. Whole genomes were concatenated by Sequence matrix v1.7.6 [54] and analysed to statistically detect reassortant strains using the Graph-incompatibility-based Assortment Finder (GiRaF) tool v1.01 [30].

Mutation and prediction of N-glycosylation sites analysis

Nextclade tool v2.14.1 [1] was used to identify amino acid changes along the HA backbone of the Kilifi, Uganda and global strains. These changes were plotted using the trackViewer R package v1.36.2 [38]. Potential N-linked glycosylation sites on the HA were identified using NetN-Glyc v1.0 tool with a threshold of 0.5 applied to signify glycosylated sites [15].

Global context and import/export analysis

This analysis was carried out on the HA segments of each lineage separately. Maximum likelihood phylogenies of aligned global sequences and HA segments of Kilifi and Uganda genomes were generated using IQTREE v2.2. We examined the tree structures that emerged and assessed them for temporal molecular clock signals using the clock feature of TreeTime v0.2.4 [44]. Time-scaled phylogenies were then inferred by TreeTime implementing a strict clock model based on the temporal signal observed. Flagged outlier sequences were removed by the Ape R package v5.7–1 [40] and the regenerated phylogenies visualised by the ggtree R package v3.6.2. Ancestral state reconstruction to estimate the imports and export events into and from Kilifi and Uganda locations was undertaken using the migration TreeTime function and the number of state changes was determined [50]. The outputs were plotted using the ggplot2 package v3.4.3 in R [59].

Results

IBV detections in Kenya (Kilifi) and Uganda

A total of 18,037 samples were tested for IBV in the KCH, KHDSS and COAST studies whereby 173 (1.0%) tested positive. The number of IBV cases were variable from month to month in the years having detections (Fig. 2a). Every year of the study samples sequenced, except 2018 and 2020 for Uganda and 2018 for Kenya. Sequencing

Table 1 IBV lineage, clade and subclade characterisation according to ECDC classification

Lineage	Clade	Subclade	Number of strains detected in Kenya*	Number of strains located in Uganda*	% of strains detected in other countries**	Reference virus	Defining mutations
Victoria	V1A	-	112	32	United states (47%), Japan (4%), Lao (3%), Singapore (3%), Kenya (3%)	B/Brisbane/60/2008	I146V, I117V, N129D,
Victoria	V1B	-	-	-	China (64%), Hong Kong (21%), Norway (14%)	B/Odessa/3886/2010	L58P
Victoria	V1A.1	-	1	-	United states (65%), Guatemala (4%), Brazil (3%), El Salvador (3%), Jamaica (2%)	B/Colorado/06/2017	D129G, I180V, R151K (HA2)
Victoria	V1A.2	-	-	-	United states (42%), Hong Kong (29%), China (8%), Japan (5%), Korea (5%)	B/HongKong/269/2017	I180T, K209N
Victoria	V1A.3	-	72	3	United States (62%), Russia (8%), China (5%), Japan (2%), Singapore (2%)	B/Washington/02/2019	K136E often with G133R,
Victoria		V1A.3a	-	2	Bangladesh (37%), Niger (11%), Singapore (11%), United States (10%), Bhutan (7%)	B/Washington/02/2019 (N150K subgroup)	N150K, G184E, N197D (loss of a glycosylation site), R279K
Victoria		V1A.3a.1	-	-	China (96%), Russia (2%), Côte D'Ivoire (0.4%), Brazil (0.3%), Bhutan (0.1%)	B/Washington/02/2019 (N150K subgroup)	V117I, V220M
Victoria		V1A.3a.2	71	1	Spain (18%), France (14%), United States (11%), China (10%), Russia (7%)	B/Austria/1359417/2021	A127T, P144L, K203R
Yamagata	Y1	-	-	-	United States (21%), China (17%), Taiwan (4%), Hong Kong (3%), South Africa (3%)	B/Florida/4/2006	S150I, N165Y, N202S, G229D
Yamagata	Y2	-	12	2	Lao (11%), Kenya (9%), Norway (8%), Congo (7%), Hong Kong (5%)	B/Massachusetts/02/2012	R48K, P108A, T181A
Yamagata	Y3	-	20	5	United States (59%), Chile (4%), Japan (3%), Bangladesh (2%), France (2%)	B/Wisconsin/1/2010–B/Phuket/3073/2013	L172Q, M251V

*Number of strains detected both locally and globally in Kenya/Uganda

**% calculated from complete IBV strains in GISAID from 1 st January, 1987 to 16th May

was attempted for 151 samples (87.0%) that had sufficient sample volume available (> 140 µl) and these yielded 83 (55.0%) complete or near complete genomes (> 70.0% coverage). Of these, 7 (8.0%) clustered with B/Yamagata lineage reference strains and 76 (91.0%) clustered with B/Victoria lineage reference strains (Fig. 2b).

IBV lineages, clades and subclades circulating in Kilifi and Uganda

IBVs are classified into lineages and subsequent clades and subclades by the characteristic amino acid changes on the HA segment (Fig. 3a). Sequencing yielded near complete genomes from 3 years in Uganda and from 9 years in Kilifi (Fig. 3b). Notably, B/Victoria lineage predominated in 6 years in Kilifi and in all 3 surveillance

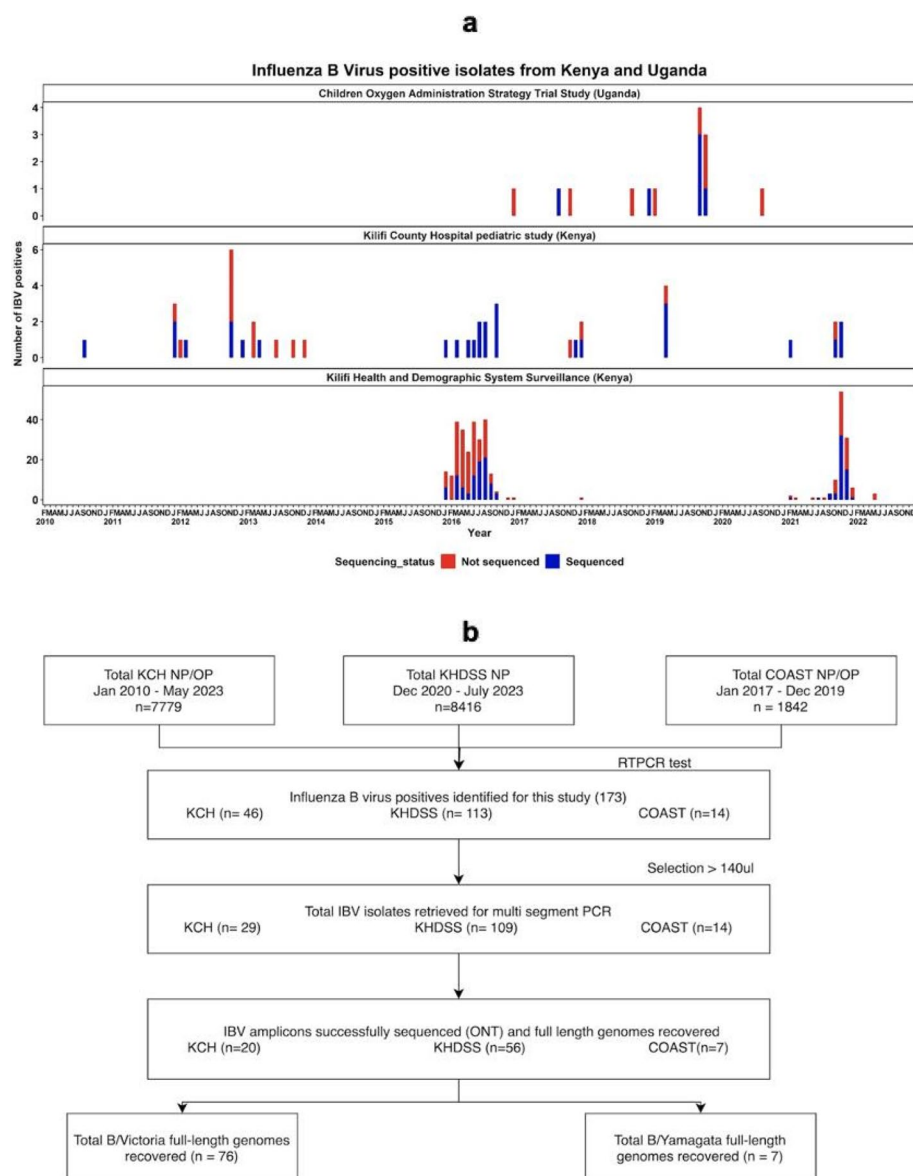


Fig. 2 Source, temporal distribution and sample laboratory processing of IBV positives during the surveillance period. **a** Bar graph showing the number of IBV detections per study per month across the surveillance period. Bars are coloured by the proportions of isolates sequenced or not sequenced. **b** Sample flow chart showing the number of samples processed and whole genome sequencing yield from the three studies

years in Uganda. Co-circulation with B/Yamagata strains was observed in 2016 in Kilifi and in 2019 in Uganda. Lineage predominance in Kilifi and Uganda was consistent with the global trends inferred from the complete global dataset from the GISAID database (Supplementary Fig. 2) except for the 2017 season in Uganda where only one sample was available. The lack of detected B/Yamagata strains among Kilifi and Uganda strains after 2019 is consistent with WHO and global reports of no confirmed B/Yamagata cases among samples collected from March 2020 onwards [60].

The B/Victoria lineage strains in Kilifi were classified into clade V1A and further into subclade V1A.3 and

V1A.3a.2 while Uganda strains clustered with subclade V1A.3 and its subclade V1A.3a reference strains (Supplementary Fig. 3). Notably, there was an observed clade shift to the V1A.3a.2 subclade as from 2020 which was one of the peak years of the COVID-19 pandemic characterised by low influenza detections, with the clade being predominant globally to date (May 2023) (Fig. 3b). B/Yamagata lineage strains circulating in Kilifi fell into clades 2 and 3 whereas only clade 3 viruses were detected in Uganda. As observed globally, clade 2 strains in Kilifi were observed until 2016 when clade 3 strains became predominant (Fig. 3b).

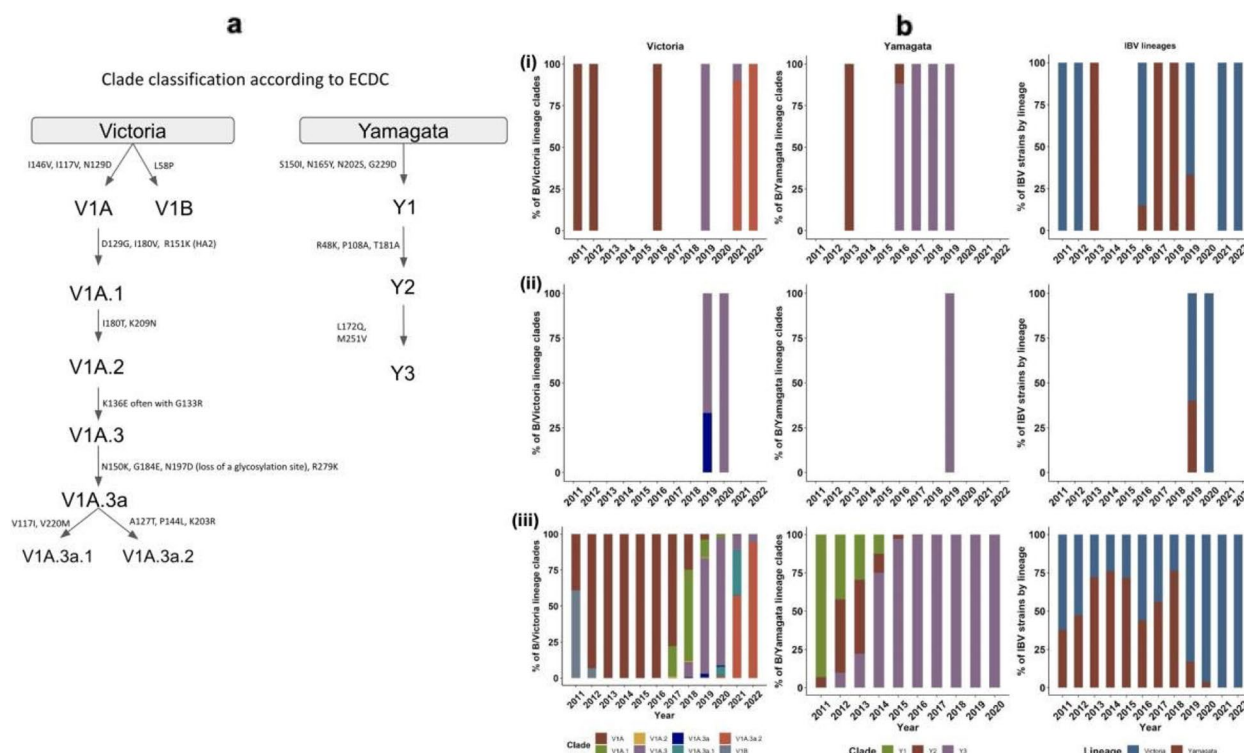


Fig. 3 Identification and temporal distribution of circulating lineage, clade and subclades in Kilifi and Uganda. **a** Cartoon representation of IBV clade and subclade classification according to ECDC annotated with clade defining mutations. **b** Temporal patterns of IBV lineages, clades and subclades among subtyped sequences observed in Kilifi and Uganda during the surveillance period in comparison with global trends. Lineages, clades and subclades were assigned according to ECDC guidelines (Table 1). Panels (i), (ii), (iii) represent clade and subclade distribution of B/Victoria strains (left), B/Yamagata strains (centre) and lineage distribution (right) of Kilifi, Uganda and global strains respectively

Phylogenetic analysis of IBV gene segments

The eight IBV gene segments from the Kilifi and Uganda strains were analysed for genomic clustering with known clade and subclade representative viruses (Table 1). Consistent phylogenetic clustering patterns was observed between B/Victoria and B/Yamagata strains. (Supplementary Fig. 2a). In addition, no visual evidence of reassortment events (a line connection from a B/Victoria strain to B/Yamagata strain) was observed between the segment phylogeny pairs (Supplementary Fig. 2b). These findings were validated statistically using the GiRaF reassortment tool showing absence of inter- and intra-lineage reassortment events in Uganda and Kilifi strains. Five distinct phylogenetic clusters, defined by clustering of strains with clade and subclade reference strains with high bootstrap support (>70.0%), were identified in the Kilifi strains and three observed in Uganda strains for both lineages (Supplementary Fig. 3).

B/Yamagata clade 2 strains clustered with the B/Massachusetts/02/2021 strain bearing the clade defining mutations. Clade 3 strains were B/Phuket/3073/2013-like with additional N116K, K298E and E312K mutations previously detected in this group [10]. The B/Victoria strains from 2011 and 2012 grouped with the B/Brisbane/60/2008 reference strain with an additional I146V

mutation (supplementary Fig. 3). Kilifi and Uganda B/Victoria lineage strains from 2019 to 2022 were deletion variants bearing the 3-aa deletion 162–164 in the HA segment. The clade 1A.3 strains clustered with the B/Washington/02/2019 reference strain but were grouped into strains containing the subclade defining mutations (Table 1) which comprised three Ugandan strains and one Kilifi strain and a subcluster comprising six Kilifi strains with K75E, E128K, T155A, G230N mutations which have been previously detected in Netherlands and other Kenyan strains [9, 10]. The subclade 1A.3a split into the 1A.3a.1 and 1A.3a.2 subclades with Kilifi strains from the latter clustering with the B/Austria/1359417/2021 having subclade defining mutations. In addition, a sub-cluster with T182A, T221A, N126S and S286F mutations within the subclade were identified (supplementary Fig. 3).

Glycosylation analysis of the HA segment

A total of eleven N-linked glycosylation potential sites were identified in the B/Victoria strains, seven on the HA1 domain at positions 25, 59, 145, 166, 233, 304 and 333 and four in the HA2 domain at positions 507, 533, 546 and 578 (Supplementary Fig. 4). A loss in the glycosylation site at position 197 of the HA1 domain was

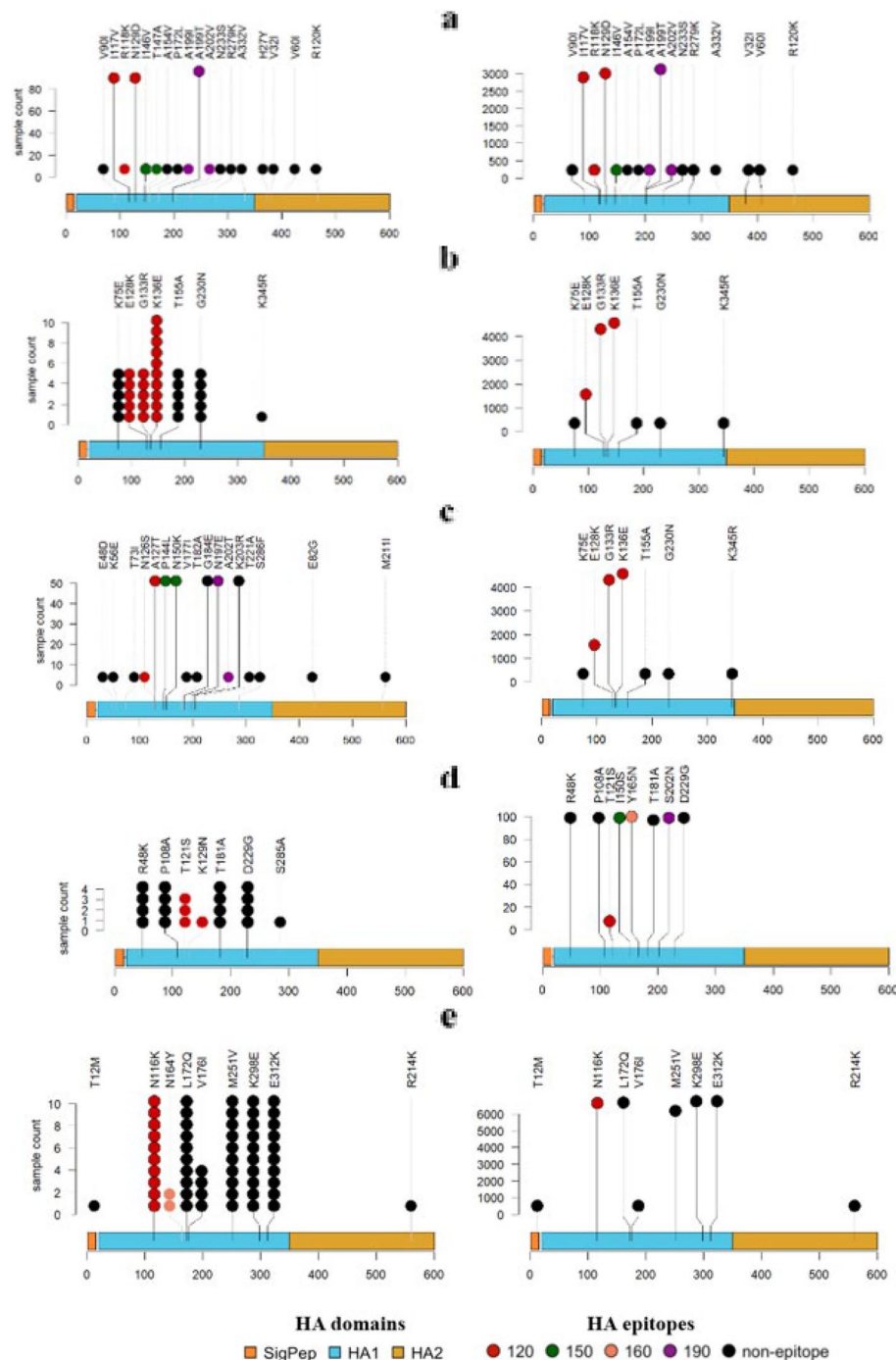


Fig. 4 Distribution of amino acid changes along the IBV HA segment. Lollipop pairs labelled a-e represent the amino acid changes identified in the V1A, V1A.3, V1A.3a.2, Y2 and Y3 clades respectively. Lollipop plots on the left side of each pair show the amino acid changes identified along the HA segment of Kilifi and Uganda strains whereas lollipop plots on the right represent the number of amino acid changes also identified among the global strains. Epitope and non-epitope amino acid changes are colour coded (see key). The height of the plot represents the number of samples containing the amino acid change. Lollipop branches in grey represent amino acid changes identified in three sequences or less. The plots were generated using the Trackviewer package in R

observed in subclade 1A.3a and 1A.3a.2 strains (Supplementary Fig. 4). Gain or loss at this site affects the receptor-binding properties and modulates the antigenicity of the virus [45]. Kilifi and Uganda B/Yamagata strains

possessed 7 potential N- linked glycosylation sites in the HA1 domain at positions 25, 59, 145, 167, 196, 303 and 332 and at positions 506, 532, 545 and 577 in the HA2 domain (Supplementary Fig. 4).

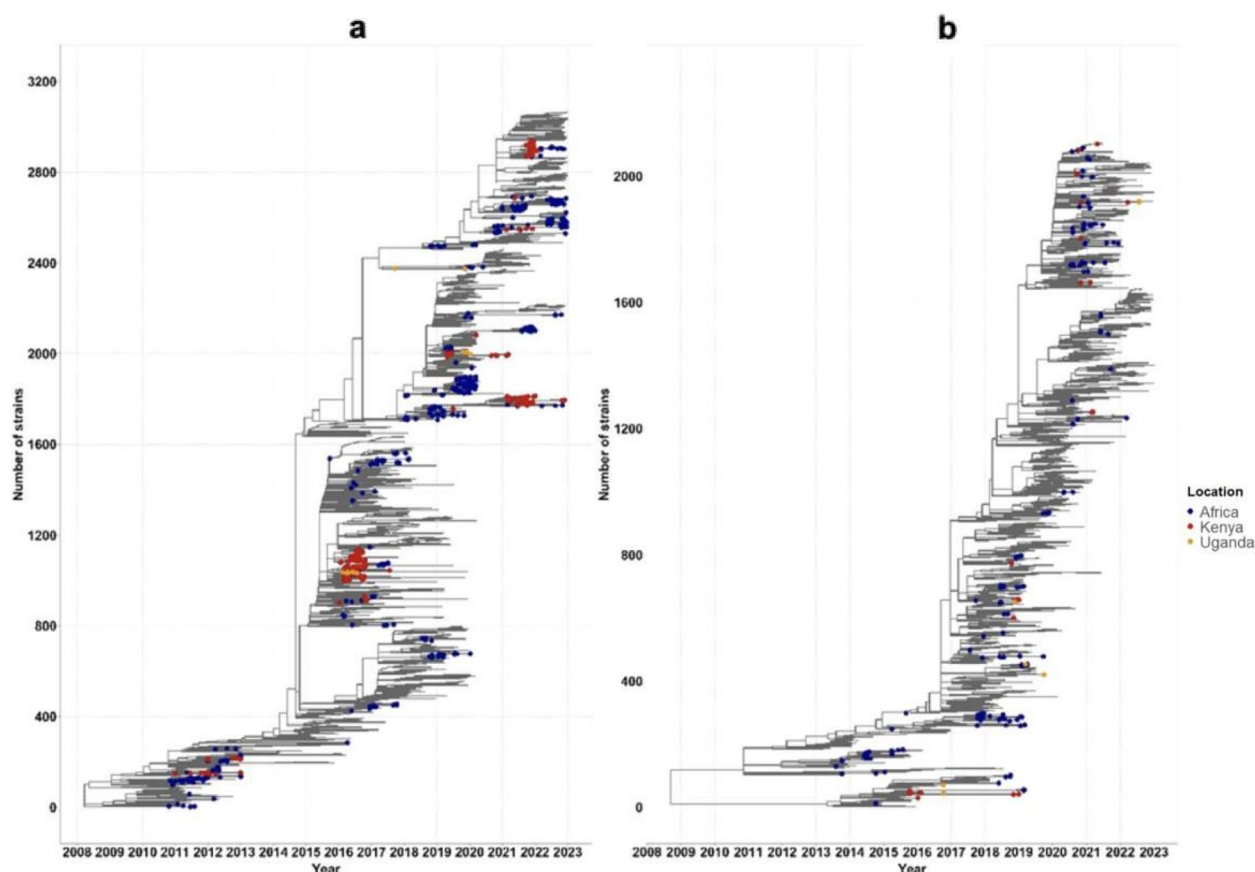


Fig. 5 Global phylogenetic context of Kenya, Uganda and global IBV strains. **a, b** represents time-resolved phylogenies of global, Kilifi and Uganda IBV strains of B/Victoria and B/Yamagata lineages respectively inferred using TreeTime. Unlabelled tips represent strains from other continents

Antigenic mutations along the HA backbone

IBV contains four main antigenic epitopes located in the HA1 domain comprising the 120 loop (116–137), 150 loop (141–150), 160 loop (162–167) and the 190 helix (194–202) [58]. Antigenic mutations in the B/Victoria strains majorly accumulated in the 120 and 190 loops (Fig. 4). Comparison with global strains identified two unique amino acid changes in V1A clade (T147A, H27Y), two in the B/Yamagata clade 2 viruses (K129N, S285A) and one (N164Y) in clade 3 strains. However, these unique changes were observed in less than three local strains. A majority of epitope and non-epitope amino acid changes detected locally in both lineages were also detected in global strains, suggesting similar clade and subclade antigenic profiles.

Global context of Kenya and Uganda IBV strains

The global phylogenetic context of IBV lineages was examined using an approximate maximum likelihood (ML) approach in TreeTime. The global dataset included 2994 B/Victoria and 2088 B/Yamagata strains subsampled from six continents between 2010 and 2022. For both lineages, Kilifi (Kenya) and Uganda strains clustered with strains from both Africa and the rest of the world

(Fig. 5). Multiple distinct clusters of Kilifi and Uganda strains were identified across the years (Fig. 5), suggesting multiple putative IBV introductions into the countries followed by onward local transmission. Ancestral state reconstruction of these phylogenies identified 7 exports, 28 import and 68 inter-facility events among Kilifi B/Victoria strains while Uganda strains exhibited 2 inter-facility events and 3 imports, 2 originating from Kenya (supplementary Fig. 5). B/Yamagata Kilifi strains had 8 imports and 2 inter-facility events while 2 import events were detected in Uganda strains (Supplementary Fig. 5). These events suggest putatively sustained local and regional spread and repeated viral introductions into the region over time.

Discussion

IBV epidemiology and evolution in East Africa is understudied, despite its significant disease burden in the region [25, 33, 46, 47, 52]. We analysed circulating IBV strains over nine years in Kilifi, Kenya, and four years in Uganda. Both IBV lineages were detected with alternating lineage predominance and co-circulation in some years. The lineage and clade predominance patterns were highly consistent with global trends. No B/Yamagata

strains were detected from 2019 onward, contrary to B/Victoria strains. A clade shift within B/Victoria lineage to subclade V1A.3a.2 was observed in 2021 in Kilifi. The IBV strains detected were non-reassortants having additional amino acid changes and showing a loss in a potential N-glycosylation site.

Lineage patterns in Kilifi between 2012 and 2016 mirrored those observed in a previous study in Kenya [12]. The B/Victoria lineage predominated most years in both Kenya and Uganda compared to the B/Yamagata lineage. However, the clade dominance was less predictable, and infections appeared to be sporadic, punctuated with periods of more intense transmission. This complicates the optimal selection of vaccine strains. No B/Yamagata strains were detected since 2019, and this is consistent with global reports suggesting the possible extinction of the lineage [23, 39]. Although the extinction of other B/Yamagata clades such as the B/Yamanashi/166/98 which was in circulation until 2002, has been documented [25], the potential resurgence of seemingly extinct influenza virus lineages remains a concern in the future, as was the case in the reemergence of A (H1N1) in 1977 [43].

Amino acid changes in IBV HA and NA surface proteins guide the selection of suitable vaccine strains. The detection of subclades whose additional amino acid changes were first detected in Kilifi strains and subsequently identified in the Netherlands highlights the importance of global IBV genomic surveillance for vaccine strain reformulation. The highly conserved NA gene encodes a sialidase enzyme that catalyses virion release from the host cell and has been a target for NA inhibitors [17, 29]. Over time, mutations conferring resistance to these inhibitors have been identified [49] however, these were not identified in the Kilifi and Uganda strains. The absence of oseltamivir-zanamivir resistance amino acid changes in Kilifi and Uganda strains indicates the effectiveness of these interventions and might be due to the infrequent use of these drugs in local communities.

N-linked glycosylation plays a crucial role in protein folding, pH stability and modulation of virus antigenicity in the HA stalk and near RBS regions [62]. The loss of the 196/197 glycosylation site observed here has previously been attributed to egg adaptation and has an effect on binding to α -2,3-linked sialic acid receptor, hence affecting virus antigenicity [6].

This study has several limitations. First, our sample size is small creating significant uncertainty in some of our inferences. There were some years with low number or no IBV positive isolates to sequence. The parent study periods only partially overlapped, with the surveillance in Uganda happening in few seasons. Thus, the long-term transmission patterns of the virus in Kenya and Uganda could not be concluded. The Kenya surveillance only sampled one of the 47 counties in the country. Second,

the limited number of positive IBV isolates from Uganda impacts the generalizability of lineage predominance, intra- and inter-country viral flow within a season. Third, while antigenic similarity suggests potential vaccine effectiveness, the absence of reliable influenza vaccine uptake and coverage data in Kenya and Uganda limits the ability to assess its real-world public health impact.

Conclusions

Genomic surveillance of Influenza viruses identifies circulating strains informing effective selection of vaccines which is the mainstay for Influenza prevention. The results of this surveillance study of IBV lineages in East Africa revealed strains circulating with an alternating predominance, with a progressive turnover of clades undergoing continuous evolution through antigenic drift and glycosylation mechanisms. The observed congruence with global patterns shows that these strains are quite like global strains, hence suitability of global interventions such as vaccines for local use. These outputs underscore the importance of continuous genomic surveillance of Influenza viruses for optimal prevention approaches.

Abbreviations

IBV	Influenza B virus
GISAID	Global Initiative for Sharing All Influenza Data
ONT	Oxford Nanopore Technologies
WHO	World Health Organization
COVID-19	Coronavirus diseases 2019
SARS-CoV-2	Severe acute respiratory syndrome Coronavirus 2
HA	Hemagglutinin
NA	Neuraminidase
ML	Maximum likelihood
KENITAG	Kenya National Immunisation Technical Advisory Group
KHDSS	Kilifi Health and Demographic Surveillance Study
KCH	Kilifi County Hospital
COAST	Children's Oxygen Administration Strategies Trial
CPGH	Coast Province General Hospital
KEMRI	Kenya Medical Research Institute
KWTRP	KEMRI-Wellcome Trust Research Programme
ECDC	European Centre for Disease Prevention and Control

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12879-025-11442-z>.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.

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Authors' contributions

C.N.A and K.M obtained the funding, conceptualized and designed the project. B.K.N, G.G, D.C.O and E.I designed and implemented the methodology. J. N, S.K and P.O.O conducted the field study across the surveillance facilities. Laboratory analysis including viral genome purification, amplification, viral sequencing and interpretation of laboratory data was conducted by M.M, R.C, T.M and B.K.N. B.K.N, D.C.O, G.G, C.N.A and A.L were involved in the bioinformatics analysis. E.K conducted data management, retrieval and archiving. B.K.N drafted the article and all authors reviewed the article.

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Data availability

High throughput sequence datasets for IBV segments are available in GenBank under accession numbers OR240875-OR240981 and OR267522-OR268202.

Declarations

Ethics approval and consent to participate

Ethical approvals for the study protocols and procedures were obtained from the Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Unit (SERU), Nairobi, Kenya under study protocol numbers 3433 (KCH), 3103 (KHDSS) (KEMRI/SERU/CGMRC-C/0053/3300 and amendment C 215/4109), Makerere University REC (2016–030 and the amendment 2020–155) in Uganda and Imperial College London Research Ethics Committee (15IC3100). Participants or their parents/caregivers (if under 18 years of age) provided written informed consent to take part in the study. All research conducted in this study adhered to and was in compliance with the Helsinki Declaration.

Consent for publication

Not applicable for this study.

Competing interests

The authors declare no competing interests.

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