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Seroprevalence and molecular analysis of yellow fever virus in mosquitoes at Namanga and Mutukula borders in Tanzania

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ABSTRACT

Objectives: Yellow fever (YF) is a major public health concern, particularly in Africa and South America. This study aimed to detect YF in human and mosquito samples to understand transmission dynamics in the Tanzania–Uganda and Tanzania–Kenya cross-border areas.

Methods: Blood samples were collected from individuals aged ≥9 months for serological testing. Mosquitoes were captured and tested for YF virus RNA. Logistic regression models were used to predict seroprevalence and associated risk factors.

Results: The overall YF seroprevalence was 12.5%, with higher rates among older individuals (7.0%) and female participants (immunoglobulin [Ig] G 4.4%, IgM 6.0%). Notably, YF virus RNA was detected in three out of 46 pools of 192 mosquitoes. The odds of testing positive for YF IgG were lower among those with primary education compared with college education (AOR = 0.27, CI: 0.08-0.88) and increased with those experiencing muscle pain (AOR = 4.5, CI: 1.08-18.78) while the odds of testing positive to YF IgM increased with being female (AOR = 4.7, CI: 1.5-14.7), traveling to YF endemic areas (AOR = 5.2, CI: 1.35-44.75), exposure to Aedes mosquitoes (AOR = 3.7, CI: 1.27-10.84) and exhibiting bruising (AOR = 13.5, CI: 1.23-145.72)

Conclusions: Although Tanzania has not experienced YF outbreaks, evidence of YF exposure at the studied borders highlights the need for strengthening cross-border surveillance, vector control, and vaccination efforts. Further research is needed to evaluate the country's overall YF risks.

Introduction

Yellow fever (YF) is an acute viral illness caused by the YF virus (YFV), a member of the *Flaviviridae* family. It is transmitted through the bites of infected mosquitoes, predominantly the *Aedes aegypti* species [1]. YF poses a significant public health threat in tropical regions of Africa and South America, characterized by sporadic outbreaks and a potential for large-scale epidemics [2–4]. The disease manifests as a spectrum of symptoms, ranging from mild febrile illness to severe hepatitis and hemorrhagic fever. Severe cases can lead to organ failure and death, making YF a serious concern in affected regions.

Tanzania, located in East Africa, is classified by the World Health Organization (WHO) as having low risk of YF, given the absence of reported YF cases in human or non-human primates [5,6]. The country's diverse ecological and geographical features provide

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a suitable environment for the primary vector of the YF, the *A. aegypti* mosquito. Tanzania shares borders with countries that have reported YF outbreaks, including Kenya and Uganda [7,8]. This geographical proximity increases the likelihood of cross-border transmission and necessitates vigilant surveillance and control efforts to mitigate the risk of outbreaks within Tanzania.

Understanding the seroprevalence of YF antibodies in the population and detecting the presence of the YFV in mosquitoes near specific border areas such as Mutukula and Namanga are crucial for assessing the potential for YF transmission in these regions. Seroprevalence studies can reveal the proportion of individuals with previous exposure to the virus, informing vaccination strategies and highlighting vulnerable populations. Meanwhile, virus detection in mosquitoes is instrumental in evaluating the risk of local transmission and guiding vector control initiatives. The WHO emphasizes the need for conducting a national YF risk assessment to estimate the risk of exposure and determine the control measures based on risk status [9,10].

Previous research has shed light on the risk factors associated with YF transmission. Proximity to forests, where the virus circulates among non-human primates and mosquitoes, increases the risk of exposure [11]. Additionally, urbanization and population movements can influence the spread of the disease, as urban areas with high population densities and inadequate sanitation can create optimal conditions for mosquito breeding and disease transmission. Effective control measures and targeted vaccination campaigns are imperative to reduce the burden of YF and prevent outbreaks. Tanzania's approach to addressing YF primarily centers on entry screening, which includes verification of international vaccination certificates for travelers arriving from areas classified by the WHO as YF risk zones or those who have transited through such areas for $\geq$12 hours, as well as the implementation of integrated vector management [12]. Furthermore, syndromic screening is being implemented at airports, ports, and borders by checking the body temperatures of international arrivals and observing for signs and symptoms of the disease.

Despite these measures, Tanzania's mobility and connectivity with neighboring countries endemic to YF and experiencing repeated outbreaks highlight the need for a closer examination of alternative successful approaches. Detection of YF in humans and mosquitoes at cross-border areas will enhance existing knowledge and support efforts to strengthen screening or surveillance systems at the Mutukula and Namanga border areas in Tanzania. It will also inform revision of vaccination policies and strategies, including the potential inclusion of YF vaccination among cross-border communities with increased risk of YF.

Materials and methods

Study area

This study was conducted at the Mutukula border area of the Kagera Region, situated between Tanzania and Uganda, and the Namanga border of the Arusha Region, located between Tanzania and Kenya. These borders were selected because of their critical geographical locations, high-volume international traffic with YF-endemic neighboring countries, the diverse ecological and climatic conditions, and the implementation of the East African Community Protocol, which allows cross-border communities to interact freely within the perimeter of 10 km on both sides. The recent population projection for the Namanga and Mutukula wards is 38,872 [13]. Geographically, the Mutukula border, being part of the Missenyi District, boasts a diverse landscape, marked by mountain ranges, swampy valley bottoms, and expansive wetlands. The altitude in this region spans from 900 to 1600 m above sea level. Rainfall follows a bimodal pattern, peaking from March to May and September to December. The vegetation consists of lush forests in certain areas and widespread savannah bushes in others. The local economy relies on subsistence agriculture, mainly banana crops, fishing in Lake Victoria, and livestock keeping. Similarly, the Namanga border, being part of the Longido District, is characterized by a semi-arid climate, with short rains from October to December and long rains from March to May. The district is traversed by the Great Rift Valley, shaping its diverse ecological landscape encompassing forests and water bodies. Altitudes range from 1251 to 2434 m above sea level, contributing to its unique climate and vegetation. Local economic activities primarily revolve around agriculture, tourism, and livestock keeping, integral to the district's socioeconomic fabric. Both the Namanga and Mutukula borders have numerous porous points; thus, some border community members and travelers cross the border without being screened by health authorities.

Specific hamlets were considered during the selection process on the basis of their proximity and the ecology of the area, including forests, water bodies, population density, and the occurrence of mosquito vectors. In this study, the selected hamlets were Kabwoba, Mgambaizi, Chadimba, Kasharu, Kateebe, and Byamtemba in Mutukula, and Buguruni, Mtaa wa saba, Kisongo, Lesoti, Skarda, and Eworendeke in Namanga.

Study design and population

A cross-sectional study was conducted between June and July 2023, integrating serological and entomological aspects, targeting both health facilities and households in the study area. The inclusion criteria included individuals aged $\geq$9 months. Individuals aged $<$9 months were excluded because of potential maternally derived immunity against YF. Foreigners were also excluded, as their antibodies might reflect exposure in their countries of origin rather than the study area. Moreover, vaccinated individuals were excluded from the study if they provided proof of being vaccinated (valid vaccination certificates).

Sample size estimation and sampling technique

Given that the prevalence of fevers in the cross-border zones was unknown during the time of the study, a 50% prevalence was assumed to provide the maximum variance in the binomial distribution [14]. A desired absolute precision of 5% and a 95% confidence level were used, yielding a minimum estimated sample size of 384 individuals. After accounting for a 17.2% contingency for non-responses, refusals, or absences, the final sample size was set at 450 participants.

To determine the sample size for each study location from the overall sample of 450, we used probability-proportional-to-size (PPS) sampling. With populations of 26,532 in Mutukula and 12,340 in Namanga [15], the total population was 38,872. Consequently, the sample size for Mutukula was 306, calculated as (26,532/38,872) $\times$ 450, and for Namanga, it was 144, calculated as (12,340/38,872) $\times$ 450. The sample was evenly divided (50:50) between households and health facilities [16]. Taking into account an average household size of four people in a study location [15], the exact number of households needed from each hamlet was determined using PPS sampling based on the hamlet sizes obtained from village registers. Systematic sampling was then applied, with the sampling interval calculated by dividing the total number of households in a hamlet by the required sample size. The first household was selected randomly, and subsequent households were selected using the sampling interval. All eligible members aged $\geq$9 months who consented were included until the target sample was reached. For health facilities, the required number of patients was determined using PPS sampling based on aver-

age monthly patient counts obtained from health facility medical records. All consenting patients who met the recruitment criteria, including being a resident of a study location for ≥1 year, were included during the study period.

Data collection

At both health facilities and households, data were collected using a pre-tested semi-structured questionnaire and face-to-face interviews conducted in Kiswahili (the national language). Information gathered included socio-demographic characteristics, duration of stay in the study area, history of residency/travel in other countries, and history of vaccination against YFV. Body temperature was recorded using a digital clinical thermometer. Data quality assessment was conducted throughout the sampling period by examining the data collected on a daily basis. Before data collection, tools were pre-tested and validated. Risk factor–related questions were drawn from the WHO guide on YF risk assessment in endemic countries of 2014 [9].

Blood sample collection and handling

Whole blood samples of 5 ml were collected through venipuncture from adults and children aged ≥10 years, whereas 2-ml whole blood samples were collected from children aged between 9 months and 10 years for serological testing. All procedures for sample collection were performed in accordance with the WHO protocol and the existing standard operating procedure at the Tanzania National Public Health Laboratory. Whole blood samples were transported to the district laboratory for centrifugation. The resulting serum was kept in ice packs during transport to the National Public Health Laboratory of Tanzania, where it was stored at −70°C until examination.

Mosquito sample collection and handling

For the entomological investigation, adult and immature stages of mosquitoes were collected in the study area. Adult mosquitoes were trapped using carbon dioxide attractant baits (Biogents AG, Regensburg, Germany) from 6:00 a.m. to 6:00 p.m. outside buildings within 15 m in cool and shaded areas. These mosquitoes were transferred to laboratories at the borders for sorting, identification, recording, and packaging. The sampling procedure used for collecting larvae and pupae is as described by Ngingo et al. [17] and the WHO operational guide for assessing the productivity of *A. aegypti* mosquito breeding [18]. Forty randomly selected households in each study location were inspected in and around houses for the presence of immature stages. Larvae and pupae were collected from water-holding containers, ditches, puddles, discarded tires, and other potential mosquito breeding habitats using plastic dippers and Pasteur pipettes, and then transferred to labeled water-filled Whirl-Pak plastic bags (Thomas Scientific, Swedesboro, NJ, USA) and transported to the laboratory at the border. The larvae were reared, fed on Whiskas® cat food, and maintained at 26 ± 2°C with 82 ± 10% relative humidity. Pupae were transferred to emergence cups in netting cages until adults emerged. The adult mosquitoes were then euthanized at −20°C for 15 minutes and identified morphologically with a stereo-microscope. A total of 543 *A. aegypti* mosquitoes were collected from all households and pooled into labeled cryovial tubes according to household of origin, and then frozen in liquid nitrogen. These samples were transported in triple packages by a government courier company (Tanzania Posts Corporation) through Express Mail Service (EMS) to the National Public Health Laboratory in Dar es Salaam, Tanzania, for storage and testing.

Laboratory procedures

Serological testing using ELISA

Human serum samples were tested for immunoglobulin (Ig) G and IgM antibodies against YF using commercial enzyme-linked immunosorbent assay kits (Sunlong Biotech, Co., Ltd, Hangzhou, China), following the manufacturer's instructions. Briefly, 50 µl of serum was dispensed to a 96-well plate pre-coated with the YF virus specific antigen, with samples diluted at a rate of 1:5. Positive and negative controls were included, and one well was left as a blank control. The plate was incubated at 37°C for 30 minutes, washed five times with buffer solution, and then 50 µl of horseradish peroxidase conjugate reagent was added to each well (except the blank). After another 30-minute incubation at 37°C, the plates were washed again five times, and 50 µl of tetramethylbenzidine substrates A and B was added. The plate was gently shaken, covered, and incubated at 37°C for 15 minutes, avoiding light exposure. The horseradish peroxidase catalyzed tetramethylbenzidine to produce a blue color, which turned yellow after adding the acidic stop solution. The intensity of the yellow color was proportional to the amount of YFV-IgM/IgG bound to the plate. The optical density absorbance was measured spectrophotometrically at 450 nm in a microplate reader. The presence of YFV IgM/IgG was determined on the basis of cutoff values established per the manufacturer's guidelines, with effectiveness criteria met when both intra-assay and inter-assay coefficients of variability were <15%.

Detection of YFV RNA in mosquitoes

Out of 543 adult *A. aegypti* mosquitoes collected through several techniques, 192 were female and analyzed for YFV. In this case, 46 female *A. aegypti* mosquito pools, containing 192 mosquitoes (1-4 per pool), were processed for YFV identification using reverse transcription-polymerase chain reaction (RT-PCR). Each homogeneous pool, in terms of collection method, sites, date, and time, was homogenized, vortexed, and centrifuged at 13,000 rpm for 5 minutes to remove tissue debris. The supernatant was collected for RNA extraction using the QIAamp Viral RNA Kit (Qiagen, Hilden, Germany), with RNA eluted in 60 µl of elution buffer, aliquoted into three 20-µl portions, and stored at −80ºC. The one-step RT-PCR was performed using RealStar Yellow Fever Virus RT-PCR Kits 1.0 (altona Diagnostics, Hamburg, Germany) in accordance with the manufacturer's instructions on a Bio-Rad CFX96 thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA). The reaction mixture contained 20 µl of the prepared mix and 10 µl of RNA template. The RT-PCR process included reverse transcription at 55°C for 20 minutes, initial denaturation at 95°C for 2 minutes, followed by 45 cycles of denaturation at 95°C for 15 seconds, annealing at 55°C for 45 seconds, and elongation at 72°C for 15 seconds. Successful runs showed a sigmoid curve in the HEX channel for the negative control and in the FAM channel (and possibly HEX channel) for the positive control. YFV-specific RNA detection was confirmed by a sigmoid curve in the FAM channel of the tested sample.

Data analysis

Data were captured using a standard questionnaire, cleaned at point of collection, and recorded into the KoboToolbox database for safe storage before statistical analysis. The data were extracted into Excel software and exported to Epi Info software (Centers for Disease Control and Prevention, Atlanta, GA) for analysis. Continuous variables, such as age and body temperature, were expressed as median and interquartile range. The dichotomous variable was analyzed using the chi-square test, and logistic regression was conducted to predict risk factors for YF and control for confounding variables. The level of significance was set at *P*-value <0.05.

Table 1
Socio-demographic and clinical characteristics of study participants by border.

Characteristics	Total	Mutukula border		Namanga border	
Sex	**N = 450**	**Frequency**	**%**	**Frequency**	**%**
Male	172	130	28.89	42	9.33
Female	278	102	22.67	176	39.11
Age (years)	**N = 450**				
0-15	70	54	12.00	16	3.56
16-30	175	118	26.22	57	12.67
31-45	133	89	19.78	44	9.78
46-60	57	38	8.44	19	4.22
≥61	15	8	1.78	7	1.56
Education level	**N = 450**				
None	59	32	7.11	27	6.00
Primary	289	224	49.78	65	14.44
Secondary	79	42	9.33	37	8.22
College	24	7	1.56	17	3.78
Main occupation	**N = 450**				
Employed	109	79	17.56	59	13.11
Business	22	7	1.56	15	3.33
Crop farming	161	151	33.56	10	2.22
Livestock	38	3	0.67	35	7.78
Students	68	50	11.11	18	4.00
Others	24	15	3.33	9	2.00
Yellow fever vaccination status	**N = 450**				
Vaccinated	6	0	0	6	1.33
Not vaccinated	444	306	68.92	138	31.08
Clinical characteristics	**N = 450**				
Bleeding	10	7	1.5	3	0.7
Body malaise	30	24	5.4	6	1.3
Body rashes	20	14	3.1	6	1.3
Bruising	5	5	1.1	0	0
Reported fever	152	123	27.3	29	6.5
Headache	84	58	2.9	26	5.8
Muscle pain	46	35	7.8	11	2.4
Nausea	22	20	4.5	2	0.4
Red eyes	10	5	1.1	5	1.1
Stomachache	34	24	5.4	10	2.2
Vomiting	19	18	4.0	1	0.2
Jaundice	8	6	1.4	2	0.4
Body temperature					
≤37.4°C	361	248	68.7	113	11.5
37.5°C-38.0°C	65	47	10.4	18	4.0
>38°C	16	7	1.6	9	2.0

Ethical considerations

The study objectives, benefits, and risks were explained in Kiswahili (the national language) to all participants before inclusion. Written informed consent was sought and obtained from all adult participants and parents or legal guardians of children. Furthermore, assent was obtained from participants aged <18 years. Heads of houses were asked for consent and permission before setting the traps in their premises. This study received ethical approval from the Tanzania Medical Research Coordinating Committee of the National Institute for Medical Research (NIMR/HQ/R.8a/Vol IX/4325).

Results

Socio-demographic characteristics

A total of 450 participants took part in the study, with 306 (68%) from Mutukula and 144 (32%) from Namanga, achieving a 100% participation rate. Most participants were female (278, 61.78%), with a mean age of 30 years (SD ± 16.9). Most participants were aged between 16 and 30 years (175, 38.89%), followed by those aged ≥31 years (133, 29.56%). Most had primary education (289, 64.22%), while 59 participants (13.11%) had no formal education, 79 (17.56%) had completed secondary education, and 24 (5.33%) had attended post-secondary education (college). Crop farming was the predominant occupation (161, 33.56%), followed by formal employment (109, 30.67%). Livestock keeping was more common in Namanga (35, 7.78%) than in Mutukula (3, 0.67%). The median temperature of participants was 36.9°C (interquartile range 0.4), with 18% having a temperature >37.5°C. Fever was the most reported symptom (152, 33.8%), followed by headache (84, 18.7%), muscle pain (46, 10.2%), stomachache (34, 7.6%), and body malaise (30, 6.7%). Other symptoms included bleeding, rashes, red eyes, vomiting, and jaundice (Table 1).

Serological testing

Table 2 summarizes the seroprevalence of IgG and IgM antibodies against YF by socio-demographic characteristics, study area, and sampling location. Overall, the YF antibodies (IgG and IgM) were present in 56 (12.5%) of 450 study participants. The prevalence varied among age groups, with higher rates observed among older individuals aged between 16 and 45 years (9.7%). Notably, female participants exhibited higher seroprevalence than male participants for both IgG (4.4%) and IgM (6.0%) antibodies. Participants with primary education had significantly higher seroprevalence (7.2%) compared with those with no formal education (2.6%), secondary education (1.3%), and college education (1.4%) (P <0.001). Seroprevalence was higher at the Mutukula border (8.3%) than the Namanga border (4.2%). Regarding sampling location, households exhibited a higher seroprevalence (6.6%) than health facilities (5.9%) (Table 2).

Table 2
Seroprevalence of IgG and IgM antibodies against YF by socio-demographic characteristics, study area, and sampling location.

Variable		YF		Overall (%)
		IgG, $P = 5.7$ (%)	IgM, $P = 6.8$ (%)	(IgG + IgM)
Age (years)	0-15	0.6	0.2	0.8
	16-30	1.5	2.8	4.3
	31-45	2.7	2.7	5.4
	46-60	0.7	0.9	1.6
	≥60	0.2	0.2	0.4
Sex	Female	4.4	6.0	10.4
	Male	1.3	0.8	2.1
Education level	None	1.1	1.5	2.6
	Primary	3.5	3.7	7.2
	Secondary	0	1.3	1.3
	College	1.1	0.3	1.4
Borders	Mutukula	3.5	4.8	8.3
	Namanga	2.2	2.0	4.2
Sampling location	Household	3.3	3.3	6.6
	Facility	2.4	3.5	5.9
Vaccination for YF	Vaccinated	0	0	0
	Not vaccinated	5.5	7.0	12.5

Ig, immunoglobulin; YF, yellow fever.

Table 3
Prevalence of clinical characteristics by YF status (IgM + IgG).

Clinical characteristics		YF status (IgM + IgG)		
		YF-positive	YF-negative	Chi-squared *P*-value
Stomachache	Yes	8 (9.1%)	26 (7.3%)	
	No	80 (90.9%)	329(92.7%)	0.111
Headache	Yes	18 (20.2%)	64 (17.8)	
	No	71 (79.8%)	295 (82.2%)	0.137
Body rashes	Yes	6 (6.7%)	14 (3.9%)	
	No	83 (93.3%)	343 (96.1%)	0.746
Bleeding	Yes	3 (3.4%)	7 (2.0%)	
	No	86 (96.6%)	352 (98.0%)	0.169
Body malaise	Yes	6 (6.8%)	24 (6.7%)	
	No	82 (93.2%)	332 (93.3%)	**0.000**[a]
Bruising	Yes	4 (4.6%)	1 (0.28%)	
	No	84 (95.4%)	355 (99.7%)	**0.005**[a]
Fever	Yes	33 (37.1%)	119 (33%)	
	No	56 (62.9%0	242 (67%)	0.372
Muscle pain	Yes	9 (10.2%)	37 (10.3%)	
	No	79 (89.8%)	322 (89.7%)	**0.000**[a]
Nausea	Yes	4 (4.5%)	18 (5.0%)	
	No	85 (95.5%)	340 (95.0%)	**0.000**[a]
Red eyes	Yes	2 (2.3%)	8 (2.3%)	
	No	86 (97.7%)	348 (97.8%)	**0.000**[a]
Vomiting	Yes	3 (3.4%)	16 (4.5%)	
	No	86 (96.6%)	340 (95.5%)	**0.030**[a]
Jaundice	Yes	2 (2.3%)	6 (1.7%)	
	No	86 (97.7%)	348 (98.3%)	**0.000**[a]

Ig, immunoglobulin; YF, yellow fever.
[a] $P<0.05$.

Cross-tabulation of clinical characteristics by YF serostatus (IgM +IgG)

Table 3 summarizes the analysis of the clinical characteristics by IgM and IgG serostatus. Symptoms such as body malaise, bruising, muscle pain, nausea, red eyes, vomiting, and jaundice were significantly associated with the serological status for YF ($P<0.05$).

Multivariate analysis of YF seropositivity by socio-demographic, clinical, and exposure risk factors

YF seropositivity increased with age, peaking in the 45- to 60-year age group for IgM (adjusted odds ratio [AOR] = 7.76; confidence interval [CI] = 0.8-75.5), although the result was not statistically significant. Participants without formal education were three

Table 4
Multivariate logistic regression model to predict yellow fever seropositivity (IgG and IgM) by socio-demographic and clinical characteristics.

Predictor variables	Levels	Dependent variable (IgM)				Dependent variable (IgG)			
		AOR	Lower 95% CI%	Upper 95% CI	*P*-value	AOR	Lower 95% CI	Upper 95% CI	*P*-value
Age group	**Ref = (0 – 15) years**								
	(15 – 30)	4.83	0.60	39.27	0.14	0.94	0.22	3.94	0.93
	(30 – 45)	5.98	0.75	47.86	0.09	1.58	0.41	6.06	0.51
	(45 – 60)	7.76	0.80	75.54	0.08	1.43	0.26	7.74	0.68
	>60	7.29	0.40	134.00	0.18	1.96	0.18	21.56	0.58
Sex	**Ref = Male**								
	Female	4.69	1.49	14.71	**0.01**[a]	2.36	0.86	6.46	0.09
Education	**Ref = College**								
	None	3.22	0.36	29.03	0.30	0.33	0.08	1.38	0.13
	Primary	1.96	0.24	15.93	0.53	0.27	0.08	0.88	**0.03**[a]
	Secondary	2.20	0.24	19.99	0.48	0.00	0.00	>1.0E12	0.97
Occupation	**Ref = Business**								
	Farmer	191252.38	0.00	>1.0E12	0.96	2342013.31	0.00	>1.0E12	0.98
	Livestock	235872.83	0.00	>1.0E12	0.96	3728344.02	0.00	>1.0E12	0.98
	Other	177811.48	0.00	>1.0E12	0.96	3271429.73	0.00	>1.0E12	0.98
Body temperature (°C)	**Ref = 37.4**								
	37.5-38	0.62	0.18	2.13	0.45	0.22	0.03	1.64	0.14
	>38	0.00	0.00	>1.0E12	0.97	0.84	0.10	6.75	0.87
Clinical signs	**Ref = None**								
	Bleeding	0.58	0.02	15.38	0.74	0.94	0.06	15.19	0.96
	Body malaise	0.41	0.04	4.20	0.45	0.09	0.01	1.56	0.10
	Body rashes	2.75	0.48	15.74	0.26	1.08	0.15	7.89	0.94
	Bruising	**13.51**	**1.23**	**148.72**	**0.03**[a]	4.61	0.29	73.37	0.28
	Headache	1.14	0.36	3.63	0.83	1.26	0.39	4.05	0.70
	Muscle pain	1.15	0.20	6.68	0.88	**4.51**	**1.08**	**18.78**	**0.04**[a]
	Nausea	0.00	0.00	>1.0E12	0.97	0.00	0.00	>1.0E12	0.96
	Red eyes	2.33	0.28	19.51	0.43	5.09	0.74	35.09	0.10
	Stomachache	2.11	0.64	6.93	0.22	2.16	0.63	7.33	0.22
	Jaundice	1.44	0.15	13.97	0.76	1.80	0.17	19.11	0.62
	Vomiting	0.00	0.00	>1.0E12	0.97	0.00	0.00	>1.0E12	0.98

AOR, adjusted odds ratio; CI, confidence interval; Ig, immunoglobulin; Ref, reference.
[a] P<0.05.

times more likely to be IgM-positive for YF compared with those with post-secondary (college) education, but this finding was not statistically significant. Similarly, participants with primary education had significantly lower odds of testing positive for IgG antibodies against YF (AOR = 0.27; CI = 0.08-0.88) compared with those with post-secondary (college) education. Female participants had significantly higher odds of IgM seropositivity than male participants (AOR = 4.7; CI = 1.5-14.7). Symptoms such as muscle pain (AOR = 4.5; CI = 1.08-18.78) and bruising (AOR = 13.5; CI = 1.23-148.72) were significantly associated with seropositivity (Table 4). Regarding risk factors, travel to YF risk areas (AOR = 5.2; CI = 1.35-44.75) and presence of *A. aegypti* mosquitoes within the household environment (AOR = 3.7; CI = 1.27-10.84) increased IgM seropositivity. Living at the border for >10 years without travel history to YF-endemic areas reduced IgG seropositivity (AOR = 0.2; CI = 0.07-0.70) (Table 5).

Detection of YF virus in mosquitoes

A total of 192 female *A. aegypti* mosquitoes, including both adult and larval stages, were captured and grouped into 46 pools. Remarkably, YFV was detected in three pools (specifically, pools 1, 8, and 43). Among these, two pools comprised adult mosquitoes, while one pool from the larval breeding sites also tested positive for the YFV (Figure 1).

Discussion

YF remains a public health concern with risk of international spread, posing a potential threat to global health security. This study aimed to detect YF in humans and mosquitoes at Tanzanian border areas connected to YF-endemic countries. The seroprevalence of YF (12.5%) was higher than that observed in 2021 by Rugarabamu et al. (1.4%) [16], who collected samples in different ecological zones throughout the country. The odds of YF seropositivity were significantly (fivefold) higher among participants with a history of travel to YF-endemic neighboring countries compared with those without such history. This finding underscores the role of cross-border mobility and connectivity in shaping transmission dynamics across border areas and highlights potential risk factors [19]. Additionally, notable discrepancies were observed between this study's findings and those from other regions with varying environmental and population profiles, reinforcing the need for context-specific prevention and control strategies [20]. This emphasizes the importance of tailoring interventions on the basis of the country's unique characteristics, including vector populations, climate conditions, and patterns of international travel and cross-border mobility.

The gender-based disparity in YF seroprevalence, with female participants showing a higher rate, is a significant finding. This aligns with similar gender-based variations observed in tropical regions for YF and other flaviviruses [21]. The reasons behind this disparity could be multifaceted, including differences in exposure due to behavioral factors, variations in immunity, or hormonal influences on the immune response [22]. Addressing this gender difference in vulnerability to YF is crucial for designing targeted prevention strategies.

Education level emerged as a significant determinant of YF seroprevalence, with existing research indicating that higher education is typically associated with better health outcomes and lower disease prevalence [23]. Interestingly, while individuals with primary education exhibited higher seroprevalence rates of YF antibodies, they also had lower odds of testing IgG-positive for the virus compared with those with post-secondary (college) education. This might be due to cross-border interactions for schooling purposes in the neighboring YF-endemic countries. This finding aligns with the results of this study, which found that par-

Table 5
Multivariate logistic regression to predict yellow fever seropositivity status by exposure risk factors.

Predictor variables	Levels	Dependent variable (IgM)				Dependent variable (IgG)			
		AOR	Lower 95 CI%	Upper 95% CI	*P*-value	AOR	Lower 95% CI	Upper 95% CI	*P*-value
History of travel to endemic areas	**Ref = No**								
	Yes	**5.15**	**1.35**	**44.75**	**0.03**[a]	1.96	0.56	6.85	0.29
Presence of *Aedes aegypti* mosquitoes at place of stay	**Ref =No**								
	Yes	**3.71**	**1.27**	**10.84**	**0.02**[a]	1.59	0.61	4.13	0.34
Reported use of mosquito repellent	**Ref = No**								
	Yes	0.78	0.30	2.02	0.60	1.33	0.52	3.40	0.55
History of being vaccinated against yellow fever	**Ref = No**								
	Yes	0.00	0.00	>1.0E12	0.98	0.00	0.00	>1.0E12	0.97
History of frequent visits to forests	**Ref = No**								
	Yes	1.04	0.33	3.31	0.95	2.09	0.72	6.05	0.17
Reported use of mosquito nets	**Ref = No**								
	Yes	0.59	0.26	1.33	0.20	0.61	0.26	1.46	0.27
Duration of stay (years) at the border	**Ref = ≤1 year**								
	>1-6	1.12	0.29	4.33	0.87	0.41	0.11	1.56	0.19
	>6-10	1.61	0.40	6.45	0.50	0.28	0.06	1.33	0.11
	>10	0.43	0.12	1.52	0.19	**0.22**	**0.07**	**0.70**	**0.01**[a]
Location where sample was collected	**Ref = Household**								
	Health facility	0.65	0.28	1.48	0.31	0.45	0.18	1.15	0.09

AOR, adjusted odds ratio; CI, confidence interval; Ig, immunoglobulin; Ref, reference.
[a] P<0.05.

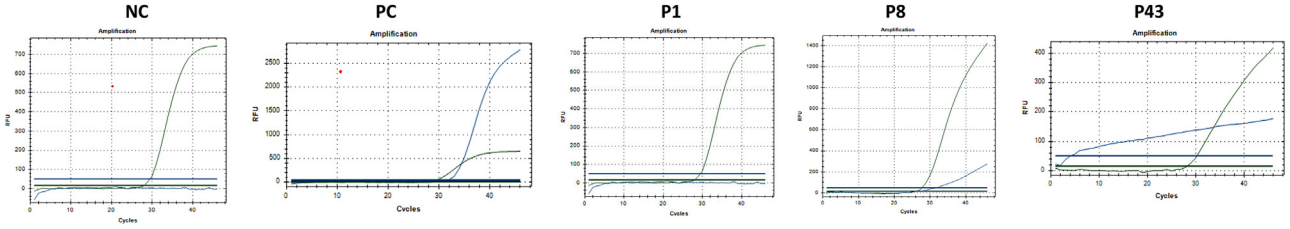

Figure 1. Amplification and curves of reverse transcription-polymerase chain reaction for yellow fever detection in mosquitoes. NC, negative control; PC, positive control; P1, adult mosquito pool 1; P8, adult mosquito pool 8; P43, Mosquito Larvae Pool 43; RFU, Relative fluorescence unit

ticipants with a history of travel to YF-endemic countries had five times higher odds of being YF seropositive compared with those without such history. This finding challenges the conventional assumption that higher education invariably correlates with improved health outcomes and reduced disease prevalence. It necessitates targeted health education and awareness campaigns for all individuals, specifically addressing the unique needs of those with lower education levels. Moreover, the seroprevalence patterns observed among individuals with primary education are similar to those observed in studies on dengue fever [24], reinforcing the need for targeted health initiatives to improve preventive practices across all educational groups.

Notably, the presence of mosquitoes in surroundings and a history of travel to YF-endemic countries significantly increased the odds of YF IgM seropositivity in our study, consistent with findings from other studies linking YF to international travel and cross-border mobility [23–25]. Detecting YFV in mosquitoes highlights the critical role of vector control in minimizing the risk of YF transmission [26]. These findings reinforce the importance of targeted mosquito control strategies, guided by continuous surveillance to curb YF transmission.

Tanzania's lack of reported YF outbreaks, alongside the reported presence of IgM-positive cases, YFV in mosquitoes, and significantly higher odds of some YF-related symptoms (muscle pain, bruising, vomiting, red eyes, nausea, body malaise, and jaundice), raises concerns about potential undetected or silent transmission, especially in cross-border areas. This highlights the challenges in YF detection due to symptom similarities with other febrile illnesses and the absence of specific diagnostic tests [27]. It underscores the need for comprehensive multi-pathogen cross-border surveillance, robust diagnostic capabilities, and further research to understand the dynamics of YF transmission in the region.

The higher seroprevalence in Mutukula compared with Namanga may be attributed to various factors, including geographical location, proximity, and connectivity to Uganda, a country with frequent reports of YF epidemics [28]. The possibility of cross-border transmission highlights the need for coordinated efforts and collaboration between neighboring countries to effectively control and prevent YF [29]. This suggests that YF control efforts in border regions should be tailored.

This study acknowledges certain limitations, including the ability of study participants to recall mosquito exposure and travel frequency to YF-endemic areas. However, this study provides valuable insights into the prevalence and potential transmission dynamics of YF in cross-border areas. The presence of YF antibodies and YFV in mosquitoes, despite the absence of reported outbreaks, highlights the necessity for robust surveillance and diagnostic capabilities, with the main focus on border areas. The gender disparity in seroprevalence and the higher prevalence in specific border regions underscore the necessity for targeted interventions. International travel to YF-endemic countries and mosquito abundance emerged as potential risk factors, emphasizing the need to implement vector control programs consistent with international health regulations and to enhance health screening at points of entry.

Conclusion and generalizability

This study highlights the presence of YF antibodies in humans and virus circulation in mosquitoes, indicating a potential risk of undetected transmission in the cross-border buffer zones of Mutukula and Namanga in Tanzania. Presence of mosquito vectors and history of travel to endemic countries emerged as potential risk factors for YF transmission. Gender disparities in seroprevalence and variations in educational levels indicate the need for targeted

awareness campaigns. Strengthening effective YF prevention and control strategies, cross-border collaboration, and ongoing research in other regions at risk are highly recommended. These results, although not generalizable to the entire country, highlight the need for a comprehensive national YF risk assessment, as recommended by WHO. The results of this study should guide targeted interventions, including the possible introduction of YF vaccination as part of routine immunization, especially in areas with elevated YF risk.

Declarations of competing interest

The authors have no competing interests to declare.

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Ethics approval

Ethical approval was provided by the National Institute for Medical Research Committee.

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Author contributions

R.K. drafted the manuscript. R.K., J.K., S.R., P.M.M., K.M., E.G.K., and E.M. jointly reviewed and updated the draft manuscript. All authors checked and approved the final report.

Availability of data and materials

The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

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