Seroprevalence of Lumpy skin disease and associated risk factors in cattle in Kilolo District, Iringa Tanzania

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ABSTRACT

Lumpy skin disease (LSD) is an infectious disease of cattle caused by lumpy skin disease virus (LSDV), which is a member of the *Poxviridae* family and genus *Capripoxvirus*. The illness is marked by mild to severe symptoms, including edema, fever, lymphadenitis, widespread skin nodules, and infrequently, death. In spite of its significance, little is now understood about the magnitude and predisposing factors in Tanzania. The aim of this study was to determine the seroprevalence of LSD in cattle within Kilolo district, and to identify key predisposing factors. A cross-sectional study was conducted in 17 villages within Kilolo district from February to March 2024 to estimate the seroprevalence of LSD in cattle. A total number of 276 serum samples was obtained from 65 cattle herds and a Double Antigen ELISA (ID Screen®) (IDVet, France) was used to screen for LSDV antibodies. Logistic regression analysis was employed to assess the risk factors for LSD seropositivity. The overall animal- and herd-level seroprevalence were 18.1% (95% CI: 13.98-23.14) and 44.6% (95% CI: 7.54-15.38), respectively. The highest individual animal level seroprevalence was documented in Irindi village 39.1% (95% CI: 5.36-12.24), followed by Masalali 38.5% (95% CI: 6.25-13.50), Ng'uruhe 37.5% (95% CI: 5.65-12.66), Irole 33% (95% CI: 1.5-6.10), and Utengule 26.5% (95% CI: 3.07-8.81) with significant differences (p=0.003). The seroprevalence of LSD varied significantly (P<0.001) among the three age groups, with adults >2 years having a higher seroprevalence (29.8%, 95%CI, 52.27-64.21) than yearlings aged 1-2 years (5. 6%, 95%CI, 9.31-17.60) and calves (0.0%, 95% CI, 23.36-34.35). Sex (Female/male, OR=2.0937, 95% CI, 1.4642-3.0248), age (Yearling/Adult, OR=0.1756, 95%CI, 0.0841-0.3730), village (OR=0.8970, 95%CI, 0.8349-0.9636) and herd size (Large/small, OR=1.9464, 95%CI, 1.1597-3.2669) were significant risk factors for LSDV seropositivity in Kilolo district. Raising awareness among livestock owners and veterinary staff about the disease and its risk factors, vaccination and vector control measures should be prioritized to minimize the transmission of the disease.

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INTRODUCTION

Lumpy skin disease (LSD) is a transboundary animal disease caused by lumpy skin disease virus (LSDV), which is a member of the *Poxviridae* family genus *Capripoxvirus* (Gari *et al.*, 2012; Yousef *et al.*, 2018). The illness is marked by mild to severe symptoms, including edema, fever, lymphadenitis, widespread skin nodules, and, infrequently, death. Although water buffalos have also been documented to be infected, cattle are the primary animals affected by LSD (Al-Salihi, 2014; Kiplagat *et al.*, 2020).

The disease is primarily transmitted mechanically through biting insects such as mosquitoes, ticks, and flies, but transmission can also occur through both direct and indirect means. Various vectors play a significant role in mechanical transmission, including mosquitoes (*Culex mirificens* and *Aedes natrionus*), biting flies (*Stomoxys calcitrans*), and hard ticks (*Rhipicephalus appendiculatus, Rhipicephalus*)

decoloratus, and Amblyomma hebraeum) (Ratyotha et al., 2022).

Direct transmission occurs through contact with infected animals, including skin lesions, saliva, nasal and ocular secretions, and contaminated milk. Indirect transmission involves contact with contaminated fomites, such as contaminated vehicles, veterinary tools, or by breeding. The virus can also be spread through consumption of feeds and water that come into contact with secretions from infected animals EFSA, 2015). The main source of transmission is typically through skin contact with infected animals. The virus enters through abrasions or breaks in the skin and is further spread by biting insects that act as vectors. Additionally, vertical transmission from mother to calf is possible, either through contaminated milk or via skin lesions on the teats, potentially affecting suckling calves (Tuppurainen et al., 2017).

Lumpy skin disease was first identified in Zambia in 1929 and has since spread to various regions across Africa, the Middle East, and beyond. While LSD is widespread throughout most of Africa, it has notably not been reported in Algeria, Morocco, Tunisia, and Libya. In recent years, the disease has expanded significantly across the Middle East, with an increasing risk of further spread into Central Asia, Western Europe, and Central Europe (Tuppurainen et al., 2017). African countries have reported cases of LSD epidemiology, for instance, with animal-level prevalence ranging between 6.4% and 8.1% in Ethiopia (Gari et al., 2012, Abera et al., 2015, Hasib et al., 2021), 8.7% in Uganda (Ochowo et al., 2019), and 19.5% in Egypt (Selim et al., 2021). Herd-level prevalence of 72.3% was reported in Uganda (Ochwo et al., 2019), between 20.8% and 27.0% in Ethiopia (Gari et al., 2012, Dubie et al., 2022). The morbidity rate is estimated at 10% in endemic areas and 3 to 85% in different epizootic scenarios, while the mortality rate of LSD ranges from 1% to 3% and the percentages as high as 40% have been documented in severe outbreak conditions (Ochwo et al., 2019). The mortality rates can reach up to 40% in severe outbreak conditions due to the varying genetic susceptibilities of cattle (Ezzeldin et al., 2023).

Numerous factors, such as vector abundance, animal movement, climate conditions, and herd management practices, contribute significantly to the disease's propagation. The virus may tolerate extended periods at room temperature and in dried scabs (**OIE**, **2013**). Additionally, agroclimatic areas, which have ideal weather conditions for the growth and propagation of biting flies, are associated with where LSDV infection (**Fentie** *et al.*, **2017**; **Molla** *et al.*, **2018**; **Ochwo** *et al.*, **2019**). According to **Abera** *et al.*, (**2015**), LSD causes serious economic losses in cattle due to high morbidity. The disease is linked to decreased milk production, weight loss, abortion, skin damage, male sterility, lameness, and pneumonia in animals with upper respiratory tract nodules. The illness has a significant economic impact on the country's livestock industry because it results in lower productivity, deaths, restrictions on the international trade of live animals and animal products, and expensive control and eradication efforts (Gari *et al.*, 2011; Tuppurainen and Oura, 2012).

To stop the disease from spreading, vaccination of the affected herds, as well as those of nearby herds, is often recommended (Al-Salihi, 2014; Tuppurainen *et al.*, 2017). Since the farmers usually pay for these vaccinations out of their own pockets, the availability of vaccines, the farmers' financial capacity, and their desire to pursue vaccination are the limiting factors of the number of animals that get vaccinated (Ochwo *et al.*, 2019). As a result, fewer or no animals receive vaccinations which decrease the herd immunity. However, vaccination is mainly done by commercial farmers with huge herds while the majority of livestock owners are smallholder farmers, who do not vaccinate against LSD (Ochwo *et al.*, 2019).

Despite the significant financial losses, little is now understood about the magnitude of the occurrence and predisposing factors of LSD in Kilolo district. Therefore, this study aimed to determine the seroprevalence and identify the risk factors associated with seropositivity of the disease in cattle without vaccination history.

MATERIALS AND METHODS

Ethical Clearance

Ethical clearance for conducting this research was granted by Sokoine University of Agriculture with reference number SUA/DPRTC/R/186 issued on 08/05/2024. Also, permission for conducting this study was granted by the Kilolo District Executive Director's Office with Ref. No. FA/255/265/01J/147 issue on 03/01/2024 to ensure research is done in all selected wards and villages. All participants were informed about the study objectives, and oral consent was obtained before taking samples from their animals and administering the questionnaire.

Study area

The study was carried out in **Kilolo district** (**Fig.1**). The study area was chosen based on undocumented field observations and complaints from the farmers about the losses caused by the LSD. The district is located between -60 and 80S latitudes and 350 and 350.51'E longitudes. The district covers an approximate land area of 7,875 km² including the Udzungwa mountain range (**Kilolo district profile**). The district has a human population of 263,559, of

which 51.4% are women and 48.6% are men (NBS, 2022). Also, it has a total indigenous cattle population of 63,922 and other livestock such as 34,044 goats, 9,645 sheep, and 34,347 pigs (Kilolo district profile). Kilolo district is administratively divided into three divisions, namely Kilolo, Mazombe, and Mahenge, which are further divided into 24 wards and 110 villages (Jalango *et al.*, 2019). The district consists of three primary agroecological zones: the highland, midland, and lowland zones.



Fig.1: A map of Kilolo District Council showing the selected ward for the study 2024 (Created by authors using QGIS version 3.20.0).

The lowland zone covers the Mahenge Plains situated at 900–1200 m above sea level. Temperatures range from 15° to 29°C. Rainfall is unreliable and averages 500–600 mm annually while the highland zone covers the Udzungwa Mountains with altitudes of 1,600 to 2,700 m above sea level, annual precipitation of 1,000–1,600 mm, and temperatures below 15°C (**Jalango** *et al.*, **2019**). In all agroecological zones, the rainy season starts from January to April and May to December is the dry season.

Study design and sample size estimation

A cross-sectional study design was employed to obtain the seroprevalence of the disease in cattle in selected wards. The sample size was calculated using a formula $N=Z^{2*}p(1-p)/d^2$, as described by **Naing** *et al.*, (2006). The values used for calculating the sample size were: Z=1.96, d= 5% and p= 13.5% as LSD prevalence in cattle as reported in Eastern zone Tanzania by **Makoga** *et al.*, (2024). N=1.96²x0.135(1-0.135) / 0.05². Therefore, the calculated sample size of 179 cattle was expected to be sampled but 276 samples were included and sampled in this study to increase precision. Where N= sample size; Z= test statistic; p= expected prevalence; d= precision.

Sample collection

A multi-stage sampling approach was used that is from ward, village, herds/households (keeping cattle) and animal selection. The sampling procedure is presented in **Fig. 2**



Fig. 2: A schematic diagram of the sampling approach.

The selected animal was restrained, and blood samples were collected using a 5ml vacutainer tubes soon after disinfecting and puncturing of the jugular vein. The vacutainer tubes containing samples were labeled with an animal number, age, sex, and village name. The tubes were then kept protected from direct sunlight at room temperature 20°C until the blood clot and sera were separated by centrifugation at 3000 rpm for 10 min (Ochwo et al., 2019). The separated sera were transferred to a sterile serum container (2 ml cryovials), and labeled with animal number, age, sex and village name. The sera samples were stored in the refrigerator (2-8°C) for one month before being transported (in the cold chain) to the College of Veterinary Medicine and **Biomedical** Sciences (CVMBS) Laboratories at Sokoine University of Agriculture where they were kept in the freezer for one week before analysis.

Data collection

Questionnaires to explore LSD factors

A semi-structured questionnaire was used to collect data on potential risk factors associated with the seroprevalence of the disease. The questionnaire was designed to capture age, sex, sharing of grazing areas, introduction of new cattle, cattle movement, sharing grazing area with wild animals, season for eruption of the disease herd size and education data. Questionnaires were subjected to 65 households, the same households where sera samples were collected, and information on vaccination against LSD was collected. Before administering the questionnaire, the questions were translated into Kiswahili and pretested to different populations for quality control. The questionnaire was revised accordingly to incorporate the lessons learned from pretesting.

ELISA test for LSD antibodies

Antibodies against LSDV were detected from serum samples using a Double Antigen ELISA (ID Screen®) (IDVet, France) for the detection of antibodies against Capripoxvirus. To perform the ELISA test, 50 μ L of each test serum sample was added to an ELISA plate microwell coated with Capripox virus purified antigen followed by 50 μ L of dilution buffer 19. Positive and negative control sera were also added to the same ELISA plate. The ELISA plate was incubated at 21 °C for 90 minutes. Afterward, the wells were emptied and washed five times with wash solution. Then, 100 μ L of conjugate was added to each well, followed by a 30-

minute incubation period at 21°C. The wells were emptied once more and washed five times. Then, 100 μ L of substrate solution was added to each well, and the plate was covered and incubated for 15 minutes at 21 °C in the dark. Stop solution (100 μ L) was then added, and the optical density (OD) was read at 450 nm using an ELISA microplate reader (Biochrom Asys UVM 340, UK) (**Ochwo et al., 2019**).



Fig. 3: Images showing laboratory analysis of the samples.

Parameter	Description	score
Plate Validity Criteria	Plate was valid if:	
	Mean OD of Positive Control (OD PC) > 0.35	+
	OR Ratio of Mean OD of Positive Control to Negative Control (ODPC/ODNC) > 3	++
	Plate is invalid if neither criterion is met.	+++
S/P Percentage Calculation	The percentage of the sample optical density (OD) relative to the positive control is calculated using the formula:	++
	S/P%= OD sample-OD Nc/ ODPC-ODNCx 100	
	-OD sample: Optical density of the sample	
	- OD PC: Optical density of the positive control	
	- OD NC: Optical density of the negative control	
Classification of Samples	-S/P% < 30%: Classified as Negative	+
	$-S/P\% \ge 30\%$: Classified as Positive	++
Prevalence Calculation	Prevalence is calculated as:	+++
	Prevalence=No of positive samples/Total No of samples collected x100	

Data analysis

Data collected was cleaned, coded and entered in Microsoft Excel software, then EPI Info software version 7.2.5.0 was used for data analysis. Chi-square test was used to assess the statistical difference between proportions at the critical probability of p<0.05. To calculate animal-level seroprevalence, the number of animals testing positive for LSDV was divided by the total number of animals tested, and the number of positive herds divided by the

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number of all herds tested was used to determine herd-level seroprevalence. The herd was considered positive if at least one animal in the herd tested seropositive for lumpy skin disease. Logistic regression both univariate and multivariate was used to identify risk factors linked with the seroprevalence of LSD. Where the risk factors were the independent variables against the seroprevalence, which was the dependent variable.

Variables that were significant in the univariable analysis (p < 0.25) were included in the multivariable analysis. The multivariable regression model was fitted using a backward stepwise approach, with all independent variables entered into the model simultaneously. The entry probability was set at 0.05, and confounding was assessed by examining changes in the odds ratio, with changes greater than 25% indicating confounding. The goodness-of-fit of the model was tested using the likelihood ratio test at a 5% significance level (Archer *et al.*, 2007).

RESULTS

Seroprevalence of LSD at the individual animal and herd level

It was established that Kilolo district had an overall seroprevalence of LSD in cattle of 18.1% (95% CI: 13.98–23.14). There was a statistical significance in villages (p = 0.003) with the highest seroprevalence recorded in Irindi village 39.1% (95% CI: 5.36–12.24), followed by Masalali 38.5% (95% CI: 6.25–13.50), Ng'uruhe 37.5 (95% CI: 5.65–12.66), Irole 33% (95% CI: 1.5–6.10), and Utengule 26.5% (95% CI: 3.07-8.81) (**Table 2**). The seroprevalence of LSD varied significantly (P<0.001) among the three age groups, with adults (29.8%, 95% CI, 52.27–64.21) having a higher seroprevalence than yearlings (5.56%, 95% CI, 9.31–17.60) and calves (0%, 95% CI, 23.36-34.35). Out of 65 herds investigated, 29 had at least one animal test positive for LSDV, resulting in a significant district-level herd-level seroprevalence of 44.6% (p = 0.0025).

Variables	No of the samples tested	Positive samples	Prevalence (%)	95% CI	Chi-square	Se	P value
Sex					3.2077		0.07
Male	137	19	13.87	43.40 - 55.52		0.0296	
Female	139	31	22.30	44.48 - 56.60		0.0352	
Age					35.8346		< 0.001
Adult	161	48	29.81	52.27 - 64.21		0.0360	
Yearling	36	2	5.56	9.31 - 17.6		0.0382	
Calf	79	0	0	23.36 - 34.35		0	
Village					40.5872		0.003
Image 1	6	0	0	0.8 - 4.67		0	
Image 8	8	0	0	1.26 - 5.63		0	
Image 5	8	0	0	0.59 - 4.18		0	
Irindi	23	9	39.13	5.36 - 12.24		0.1018	
Irole	9	3	33.33	1.5 - 6.10		0.1572	
Isuka	7	0	0	0.4 - 3.67		0	
Kitumbuka	16	1	6.25	3.35 - 9.24		0.0604	
Lukani	8	0	0	0.4 - 3.67		0	
Kilala	7	1	14.29	1.03 - 5.16		0.1323	
Mahenge	12	2	16.67	2.27 - 47		0.1078	
Masalali	26	10	38.46	6.25 - 13.50		0.0954	
Mazombe	23	5	21.74	5.36 - 12.24		0.0858	
Mbigili	19	3	15.79	4.2 - 10.54		0.0839	
Mtandika	53	3	5.66	14.73 - 24.35		0.0318	
Ng'uruhe	24	9	37.50	5.65 - 12.66		0.0988	
Utengule	15	4	26.67	3.07 - 8.81		0.1143	
Vitono	12	0	0	2.27 - 7.47		0	

Table 2: Seroprevalence of LSD in different variables

Risk factors for LSD

Univariate logistic regression evaluated nine risk factors, with eight selected for multivariate analysis at p=0.25 (**Table 3**). Multivariate analysis revealed significant associations with Lumpy Skin Disease seropositivity for age (Yearling/Adult, OR=0.1756), sex (Female/male, OR=2.0937), village (OR=0.8970), and herd size

Seroprevalence of Lumpy skin disease

(OR=1.9464) at p=0.05 (**Table 4**). The Likely hood ratio test of goodness of fit of the modal produced a p-value of < 0.001 and chi-square of 166.26, this indicates that the model fits the data very well.

Risk factor	Category	Odds ratio	95% C.I.	P value
Age	Calves/Adult	0.0	0.00-0.0001	0.9173
	Yearling/Adult	0.1466-	0.0702-0.3059-	0.0000*
	constant			0.000
Sex	Female/Male	1.9024	1.3925-2.5989	0.0001*
Location (village)	-	0.9035	0.8823-0.9253	0.0000*
Communal Grazing	Yes/ No	1.0288	0.6079-1.7412	0.9157
Cattle movement	Yes/no	0.5196	0.3780-0.7142	0.0001*
Introduction of new animal	Yes/No	0.5724	0.4156-0.7882	0.0006*
Herd size	Medium/Large	1.7790	1.2322-2.5686	0.0021*
	Small /Large	1.2905	0.7151 -2.3290	0.3972
	Extra-L/Large)	0.3179*	0.0952 -1.0617*	0.0625
	Constant			0.0000
Season	Rainy/dry	2.4633	1.0626-5.7107	0.0356*
Education	N/E	0.6229	0.4259 -0.9111	0.0147*

Table 3: Univariate logistic regression analysis for risk factors associated with LSDV seropositivity

Results are significant at P<0.25

Herd sizes were categorized as follows: small (1-20 cattle), medium (21-50 cattle), large (51-100 cattle), and extra-large (101-500 cattle). Additionally, educational levels were denoted as N for no formal education and E for formal education.

Table 4: Multi	variate logistic reg	ression analysis	for risk factors	associated with LS	D seropositivity

Risk factors	Odds Ratio	95% C.I.	P-Value
Age (Yearling/Adult)	0.1756	0.0827-0.3730	0.0000*
Herd size (Large/Small)	1.9464	1.1597 -3.2669	0.0117*
Village	0.8970	0.8349-0.9636	0.0029*
Sex (Female/Male)	2.0937	1.4642-3.0248	0.0001*

*Results are significant at P<0.05

DISCUSSION

Findings from this study suggest high seroprevalence of LSD in cattle reared in Kilolo district. A seroprevalence of 18.1% for LSD in cattle signifies a significant level of past exposure, indicating that the disease is relatively common in the population studied. This highlights the importance of ongoing surveillance, effective vaccination, and robust biosecurity measures to manage and mitigate the impact of LSD.

A high seroprevalence indicates that a substantial number of cattle have been affected by LSD, which can lead to reduced overall health and

productivity in the herd. Our study found a higher seroprevalence compared to the reported in Tanga and Pwani regions of Tanzania (**Mkoga** *et al.*, **2024**). These differences between our study and the Eastern zone might be due to differences in conducive environments that support vector abundance that prefer wet and worm areas (**Molla** *et al.*, **2018**).

The reported seroprevalence in this study was found to be lower than the seroprevalence recorded in two previous studies in Egypt by **Abd Elmohsen** *et al.*, (2019) and **Selim** *et al.*, (2021). Furthermore, the seroprevalence of LSD in the current study was comparatively lower than that of Ethiopia, which was reported by **Gari** *et al.*, (2012) and **Molla** *et al.*, (2018). However, it was significantly higher than the previous study conducted in Egypt by **Elhaig** *et al.*, (2017), Western Wollega Ethiopia (Abera *et al.*, 2015), northeastern Ethiopia (Hailu *et al.*, 2014), and Uganda (Ochwo *et al.*, 2019). The possible reasons for the variation in seroprevalence in different countries could be due to vector activities, diagnostic methods used for the study, population size where the sample was taken from, period of sampling and environmental variation of the study areas that support the vector propagation (Issimov *et al.*, 2020; Selim *et al.*, 2020).

However, herd-level seroprevalence (44.6%) in Kilolo, located in the Southern Highlands zone, was higher than what was reported by **Makoga** *et al.*, (2024) in Pwani and Tanga regions. The observed difference may be attributed to vector abundance, management type, and environmental conditions favorable for the reproduction of biting arthropods for LSDV transmission.

The current herd prevalence was lower compared to reports from other countries, Uganda (**Ochwo** *et al.*, *2019*) and Central and Northwest Ethiopia (**Molla** *et al.*, *2018*). However, it was higher than in western Ethiopia (**Abera** *et al.*, *2015*). The possible reason for these differences in herd prevalence in these three countries might be due to geographical location, diagnostic tools used, animal management and vector control measures (**Selim** *et al.*, *2021*).

seropositivity of LSD The increased dramatically with age because this study revealed that adult cattle (> 2 years) had high odds (OR = 1.96) compared to young (1-2 years) and calves (<1 year). This could be explained by extended time of exposure to the environment that may contribute to increased seropositivity in older animals, hence increasing their risk of infection. Also, the lowest susceptibility in calves might be caused by maternal immunity and being kept in a separate barn away from insects where the probability of biting flies is very low. In contrast, adults that roam in search of pasture and water hence increase the chance of encountering the arthropods responsible for the disease spread (Troyo et al., 2008; Selim et al., 2021). These findings tally with two earlier studies in Ethiopia (Abera et al., 2015, Molla et al., 2018), which found that adults had greater odds of LSDV than calves. There was an association between seropositivity and sex. Female animals (OR = 2.1196) had twice the likelihood of being seropositive than male animals. This might be due to duration exposure, as farmers keep female animals for longer periods as they are expecting to get more milk and increase the herd size while selling off males at a younger age for income generation (Ochwo et al., 2019). This is in line with the study in Uganda by Ochwo et al., (2019), who found that female animals were almost two times more likely to be seropositive when compared to males. However, this goes contrary to Radostits et al., (2006) who recorded more odds of seropositivity in males than in females; the reason may be due to stress factors such as fatigue.

Assessment of the origin of cattle in the village showed to be the predisposing factor for seropositivity; this might be attributed to different climatic conditions/weather responsible for vector activities resulting in variation in the propagation of the insect population (**Molla** *et al.*, **2018**). Also, it might be a result of the lack of dip tanks in some villages, which increases the risk of vector abundance.

Herd size showed high odds of LSD seropositivity (OR= 1.9464) among animals from larger herd sizes compared to small sizes. This is because, in free-range production systems, larger herds interact in communal grazing and water points, which increase the risk of disease transmission. Similar relationships have been reported in other studies (Muema *et al.*, 2022) and (Matope *et al.*, 2010) who found increasing seropositivity with increasing the herd size. However, in some cases, large herds may have limited resources or infrastructure for disease monitoring and surveillance; this can result in delays in detecting and controlling the disease allowing seropositive conditions to persist and spread with the herd.

CONCLUSION

Lumpy skin disease is widely distributed in the Kilolo district, as evidenced by the established animalseroprevalence of 18.1% and herd-level level prevalence of 44.6%. The study identified sex, age, village, and herd size as significant risk factors for the disease. The obtained results clarify that the seroprevalence of LSD varies in different villages throughout the year within the district, and it affects both sexes and all cattle age groups (yearlings and adults). This study is significant for its contribution to the understanding of disease prevalence and its risk factors, guiding economic and agricultural strategies, informing public health and veterinary practices, influencing policy-making, advancing research, raising community awareness, and enhancing regional and global disease control efforts. Additionally, to minimize the spread of the disease, raising awareness among livestock owners and veterinary staff about the disease and its risk factors, vaccination and vector control measures should be prioritized.

Competing Interests

The author reveals no conflict of interest

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