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Transmission Assessment Survey-3 for lymphatic filariasis in eastern Nepal: a school-based cross-sectional survey with programmatic follow-up of antigen-positive children

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Abstract

Background

Transmission Assessment Surveys (TAS) determine whether lymphatic filariasis (LF) infection has been reduced below programme-defined thresholds following mass drug administration (MDA). This study assessed lymphatic filariasis antigenemia among Grade 1 and Grade 2 schoolchildren in three districts of eastern Nepal during the third Transmission Assessment Survey (TAS-3) and summarized routine programme follow-up information from families of antigen-positive children.

Methods

A school-based cross-sectional third Transmission Assessment Survey was conducted in two Evaluation Units (EUs): Ilam and Panchthar districts constituted Evaluation Unit 1, and Tehrathum district constituted Evaluation Unit 2. Schools and sample sizes were selected using the World Health Organization Survey Sample Builder. Finger-prick blood samples were tested using Filariasis Test Strips, and the number of antigen-positive children was compared with the Evaluation Unit-specific critical cutoff. As part of post-survey programme follow-up, parents or guardians of antigen-positive children were contacted by telephone to document information relevant to case and programme follow-up, including residence and mobility, reported previous participation of family or household members in mass drug administration, potential mosquito-exposure conditions, treatment, and remaining information or follow-up needs.

Results

Of 3,277 eligible enrolled children, 3,001 were present and tested. One inconclusive result was excluded, leaving 3,000 valid results. Thirteen children were antigen-positive. All positive children were identified in Evaluation Unit 1, where 13 of 1,587 children were positive (0.82%; 95% CI: 0.44%–1.40%), exceeding the critical cutoff of six; Evaluation Unit 1 therefore did not pass the survey. No positive children were detected among 1,413 children in Evaluation Unit 2, which passed against a cutoff of four. Positive children attended six schools, including seven children at one school in Ilam Municipality Ward 9. Programme follow-up information was available from parents or guardians of 11 positive children. Follow-up identified long-term residence in many affected communities alongside short-term mobility, commonly reported mosquito exposure and nearby environmental conditions potentially conducive to mosquito breeding, reported gaps in previous MDA participation among family or household members, variable knowledge of lymphatic filariasis, initial concerns following notification of the positive result, and continuing information needs after counselling and treatment.

Conclusions

TAS-3 produced contrasting programme outcomes across the two EUs. Evaluation Unit 1 exceeded the TAS-3 critical cutoff and requires further household, community, entomological, and programme investigation

before any decision on re-MDA. Evaluation Unit 2 met the TAS-3 criterion, supporting continued surveillance and progression toward programme documentation for national elimination validation. Routine post-survey follow-up of antigen-positive children provided additional programme context regarding residence and mobility, potential mosquito-exposure circumstances, treatment, and remaining communication and follow-up needs.

Introduction

Lymphatic filariasis (LF) is a mosquito-borne neglected tropical disease caused primarily by the filarial parasites *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*.[1] The parasites are transmitted by several mosquito genera, including *Anopheles*, *Culex*, *Aedes*, and *Mansonia*. Although many infections remain asymptomatic, chronic infection can damage the lymphatic system and lead to lymphoedema, elephantiasis, hydrocoele, recurrent inflammatory episodes, disability, social stigma, reduced social participation, and loss of economic productivity.[2,3] In response to the global burden of LF, the World Health Organization (WHO) established the Global Programme to Eliminate Lymphatic Filariasis by 2030.[4] The programme is based on two complementary strategies: interrupting transmission through repeated mass drug administration (MDA) to eligible populations in endemic areas and providing morbidity management and disability-prevention services to people affected by chronic manifestations of the disease. In 2025, 34 countries were considered to still require MDA. By the end of 2025, 40 countries had reached the surveillance phase and no longer required MDA nationally, including 23 countries that had achieved elimination of lymphatic filariasis as a public health problem and were under post-validation surveillance.[5] These achievements demonstrate substantial global progress, although continued surveillance remains necessary in countries and areas where MDA has been stopped.

The national LF elimination programme of Nepal initially aimed to eliminate LF as a public health problem by 2020. As this target was not achieved, the program was subsequently aligned with the WHO Neglected Tropical Diseases Roadmap, which sets a global target for LF elimination by 2030.[6] Of Nepal's 77 districts, 64 were classified as endemic for LF at the beginning of the programme, placing approximately 27.7 million people, representing 95.1% of the national population, at risk of infection.[7] *Wuchereria bancrofti*, transmitted primarily by *Culex quinquefasciatus*, is the only filarial parasite–vector combination reported to cause lymphatic filariasis in Nepal.[8] The Epidemiology and Disease Control Division under the Ministry of Health and Population has implemented the national LF elimination programme since 2003. The programme initially used annual MDA with a two-drug combination of diethylcarbamazine and albendazole. Repeated MDA aims to reduce microfilariae in infected individuals and thereby decrease the likelihood that mosquitoes will acquire and transmit infection. However, before MDA can be stopped[9], programmes must demonstrate that infection has been reduced below thresholds considered unlikely to sustain transmission. Despite substantial national progress, residual transmission remains a concern in some areas. In January 2026, additional MDA was implemented in selected municipalities of Banke, Rautahat, Sarlahi, and Jhapa districts because of continued transmission, highlighting the need for sustained subnational surveillance and targeted responses as Nepal progresses toward LF elimination.[10]

As an initial step in assessing the impact of MDA, WHO recommends conducting surveys at sentinel and spot-check sites within an Evaluation Unit. When infection prevalence is below 2% for circulating filarial antigen or below 1% for microfilariae at each assessed site, the Evaluation Unit may become eligible to proceed to the first Transmission Assessment Survey.[9] The Transmission Assessment Survey is a standardized population-based cluster survey used to determine whether LF infection has been reduced below a programme-defined critical threshold. TAS is generally conducted among children aged six to seven years or among Grade 1 and Grade 2 students in areas where school enrolment is sufficiently high.[9] Children in this age group are expected to have been born during or after MDA and to have lived most or all of their lives under reduced transmission pressure. Therefore, the detection of circulating filarial antigen in these children may indicate relatively recent exposure or continuing transmission.

The first Transmission Assessment Survey (TAS-1) is conducted after programme eligibility requirements have been met to determine whether mass drug administration can be stopped.[9] Subsequent surveys are conducted during post-MDA surveillance to assess whether infection remains below the programme-defined critical threshold and to confirm the absence of recrudescence of transmission. Tehrathum completed TAS-2 in 2021, while Ilam and Panchthar completed TAS-2 in 2023, after which the respective Evaluation Units became eligible for TAS-3 according to programme timelines. Under the WHO Monitoring and Evaluation framework[9], TAS-3 is generally conducted at least two-year post the successful result of TAS-2 in evaluation units. For the present survey, Ilam and Panchthar districts were combined as Evaluation Unit 1, while Tehrathum district constituted Evaluation Unit 2. This grouping reflected geographical proximity, programme history, epidemiological context, school-based sampling requirements, and operational feasibility. For each Evaluation Unit, the number of antigen-positive children is compared with a statistically determined critical cutoff. Therefore, the programme decision is based on the number of positive children relative to the Evaluation Unit-specific cutoff rather than on pooled prevalence across multiple Evaluation Units. Although all three districts had reported no antigen-positive children during previous TAS-1 and TAS-2 surveys, historical MDA coverage varied across districts and years. Continued surveillance was therefore necessary to determine whether infection levels had remained below the programme-defined threshold and to detect any recrudescence of transmission following the cessation of MDA.

In this context, this study aimed to determine whether the number of lymphatic filariasis antigen-positive Grade 1 and Grade 2 students remained below the critical cutoff in the two Evaluation: EU-1, comprising Ilam and Panchthar districts, and EU-2, comprising Tehrathum district. Secondary objectives were to describe the demographic and geographical distribution of antigen-positive children, assess survey coverage and diagnostic-quality indicators, document treatment and household follow-up, and summarize the follow-up information concerning residence and mobility, possible mosquito-exposure circumstances, and post-diagnosis information and follow-up needs among families of antigen-positive children.

Methods

Study design and period

A school-based cross-sectional third Transmission Assessment Survey (TAS-3) was conducted as part of post-MDA surveillance of Nepal's National Lymphatic Filariasis Elimination Programme. The survey was

implemented from June to July 2026. In accordance with programme procedures following identification of antigen-positive individuals, the positive children and their families underwent post-survey programme follow-up. Parents or guardians were subsequently contacted by telephone to document information relevant to case management and programme investigation, including residence and mobility history, household and environmental mosquito exposure, treatment, and remaining information and follow-up needs.

Study setting: The survey was conducted in Ilam, Panchthar, and Tehrathum districts of Koshi Province in eastern Nepal.[Figure1] Two Evaluation Units were established for survey design and interpretation: 1) Evaluation Unit 1 included Ilam and Panchthar districts. 2) Evaluation Unit 2 included Tehrathum district. Ilam and Panchthar were combined as Evaluation Unit 1 because they were neighbouring districts with comparable lymphatic filariasis programme histories and epidemiological contexts, and their grouping was considered appropriate for school-based sampling and operational implementation.[9] Tehrathum district constituted Evaluation Unit 2. Separate sample sizes, selected schools, critical cutoffs, and programme decisions were applied to each Evaluation Unit; therefore, the TAS-3 results were interpreted separately for the two Evaluation Units.

TAS-3 eligibility and previous programme history

Ilam, Panchthar, and Tehrathum districts became eligible for TAS-3 after successfully completing the required preceding programme milestones, including pre-TAS or repeat pre-TAS, TAS-1, and TAS-2. Tehrathum completed TAS-1 in 2017 and TAS-2 in 2021, whereas Ilam and Panchthar completed TAS-1 in 2019 and TAS-2 in 2023. Ilam and Panchthar required repeat pre-TAS assessments in 2018 before progressing to TAS-1, while Tehrathum proceeded to TAS-1 following its pre-TAS assessment in 2016. Historical programme results, including antigen prevalence during pre-TAS and repeat pre-TAS, TAS-1 and TAS-2 results, and annual mass drug administration coverage, are summarized in Tables 1 and 2. For the present TAS-3 survey, Ilam and Panchthar were grouped as Evaluation Unit 1 (EU1), while Tehrathum constituted Evaluation Unit 2 (EU2).

Table 1
Historical pre-TAS, TAS-1, and TAS-2 results in districts included in TAS-3

District	Current population	Pre-TAS/ (Ag %)	Re-pre-TAS result (Ag %)	Year	TAS-1 result	Year	TAS-2 result	Year
Tehrathum	88,731	1.33 / 0.98 (2016)	-	-	0 / 16	2017	0 / 18	2021
Ilam	2,79,534	1.65/3.30 (2016)	1.32/0.33	2018	1 / 18	2019	0 / 18	2023
Panchthar	1,72,400	0.66/2.84 (2016)	0.32/0.32	2018		2019		2023

Source

Lymphatic Filariasis Elimination Program Annual Report 2017/18 and unpublished internal records of the National Lymphatic Filariasis Elimination Programme, Epidemiology and Disease Control Division, Ministry of

Health and Population, Nepal.

Department of Health Services. Annual Health Report 2079/80. Kathmandu: Ministry of Health and Population, Government of Nepal.[11]

Table 2
Baseline antigen prevalence and reported annual MDA coverage in districts included in TAS-3

District	Baseline ICT prevalence (%)	2012	2013	2014	2015	2016	2017	2018	2019
Tehrathum	1.06	48.99	60.85	65.29	65.88	66.27	69.88	—	—
Ilam	0.80	61.25	69.82	70.77	78.01	78.10	79.83	79.64	60.77
Panchthar	0.30	59.11	65.73	66.59	69.61	70.59	72.74	64.84	66.51

Source

Lymphatic Filariasis Elimination Program Annual Report 2017/18 and unpublished internal records of the National Lymphatic Filariasis Elimination Programme, Epidemiology and Disease Control Division, Ministry of Health and Population, Nepal.

Department of Health Services. Annual Health Report 2076/77. Kathmandu, Nepal: Ministry of Health and Population, Government of Nepal.[12]

Survey population

The survey population consisted of students enrolled in Grade 1 or Grade 2 in selected schools. A school-based design was used because net primary-school enrolment in the survey districts exceeded 75%.[9] All Grade 1 and Grade 2 students present on the day of the survey were eligible for testing. Because eligibility was determined by school grade, participating children included some students outside the usual six-to-seven-year age range. Children who declined testing were not enrolled. Children with inconclusive final test results were excluded from the valid sample used for TAS interpretation.

Sampling technique and sample-size determination

The survey followed the World Health Organization methodology for Transmission Assessment Surveys.[9] Districts were included based on their eligibility for TAS-3. A school-based sampling design was used because net primary-school enrolment in the survey area exceeded 75%. The sampling frame consisted of an updated list of all schools with grade one and grade two students in each Evaluation Unit. The lists were obtained from the Ministry of Education, Science and Technology through the Center for Education and Human Resource Development, Sanothimi, Bhaktapur. Within each Evaluation Unit, schools were arranged in geographical order and entered into the WHO Survey Sample Builder for TAS and soil-transmitted helminthiasis surveys. The Survey Sample Builder was applied separately to each Evaluation Unit to determine the required sample size, number of schools to be selected, critical cutoff, and sampling interval. Schools were selected by matching the random numbers generated by the software to the geographically

ordered school lists. All Grade 1 and Grade 2 students present on the survey day in the selected schools were invited to participate. For areas where *Anopheles*, *Culex*, or *Mansonia* mosquitoes are the principal vectors of *Wuchereria bancrofti*, the TAS design uses a critical threshold corresponding to an antigen prevalence of less than 1%.[9] The design provides at least a 75% probability of passing when the true antigen prevalence is 0.25% and no more than approximately a 5% probability of passing when the true prevalence is 1% or higher. Sample sizes were calculated separately for the two Evaluation Units and included a 15% allowance for absenteeism. Ten additional schools were selected in each Evaluation Unit for use if the required sample size could not be achieved in the initially selected schools. The calculated sample sizes, critical cutoffs, and numbers of selected schools are presented in Table 3.

Table 3
Sampling frame, calculated sample size, critical cutoff, and selected schools by Evaluation Unit

Evaluation Unit	Total schools	Total eligible students	Calculated sample size	Critical cutoff	Selected schools	Within-school sampling
EU1: Ilam and Panchthar	931	15,602	1,548	6	109	All eligible children tested
EU2: Tehrathum	248	3,518	1,368	4	114	All eligible children tested

Note: *The calculated sample sizes included a 15% allowance for absenteeism. Ten additional schools were selected in each Evaluation Unit to address possible sample shortfalls.*

School selection

School lists were obtained from the Ministry of Education, Science and Technology and the Center for Education and Human Resource Development. The sampling frame included government, private, religious, Madrasa, Gumba, and other eligible primary schools. Schools were arranged in geographical order within each Evaluation Unit. Random numbers generated by the Survey Sample Builder were matched with the ordered school lists to identify selected schools. All Grade 1 and Grade 2 students present in each selected school were offered testing. When the target sample was not achieved after completion of the initially selected schools, the survey team proceeded sequentially to additional schools. Seven additional schools were visited in Evaluation Unit 1 and 10 additional schools were visited in Evaluation Unit 2. All eligible children in the final school were tested even when the required sample was reached before the last child in that school.

Training and field preparation

The Vector Borne Disease Research and Training Centre (VBDRTC) conducted a two-day training on 23–24 June 2026 for supervisors, counsellors, laboratory personnel, enumerators, and other field team members. The training covered the TAS protocol, participant counselling, finger-prick blood collection, use and interpretation of Filariasis Test Strips, data recording in paper forms and KoboToolbox, ethical conduct, and field procedures. Practical role-play exercises were also conducted. Mean knowledge scores increased from

17.15 out of 24 before training to 21.97 out of 24 after training. District-level coordination and orientation meetings were held on 26 June 2026 in Ilam, Panchthar, and Tehrathum districts. The meetings were attended by representatives of the District Health Offices, municipal and rural-municipal health and education sections, selected school principals or teachers, local health facilities, EDCD, VBDRTC, WHO, and partner organisations. Participants reviewed school selection, field-team deployment, student mobilisation, diagnostic and data-collection procedures, treatment arrangements for antigen-positive children and eligible household members, and the responsibilities of each stakeholder.

School-level coordination and participant recruitment

Before the survey visit, enumerators contacted the principal or head teacher of each selected school to agree on the testing date and coordinate survey activities. School authorities were requested to inform parents or guardians in advance about the survey purpose, testing procedures, potential benefits, and the minor discomfort associated with finger-prick blood collection. On the survey day, the field team met with the principal or head teacher and relevant school representatives. The survey objectives, procedures, potential benefits, possible discomfort associated with finger-prick blood collection, and the responsibilities of school personnel were explained. Meeting minutes were prepared to document school-level authorization. Teachers then facilitated the participation of eligible Grade 1 and Grade 2 students according to the programme procedure. Each eligible child received an age-appropriate explanation of the survey and testing procedure, and verbal assent was obtained before blood collection. Children who were unwilling to participate or became distressed were not tested.

Data-collection tools: Two survey forms were used. 1) Form 1 was a school check-in form completed directly in KoboToolbox using mobile devices. It collected: Evaluation Unit; district; municipality or rural municipality; school identification and school code; geographical coordinates; school enrolment; Grade 1 and Grade 2 attendance; school authorization; and reported reasons for absence. 2) Form 2 recorded individual-level sociodemographic and diagnostic information. It was initially completed on paper during fieldwork and later entered into KoboToolbox. The school and child datasets were linked using Evaluation Unit, school code, and sample code.

Blood collection and diagnostic testing

Finger-prick blood was collected according to the survey standard operating procedure and tested using the Abott Bioline Filariasis Test Strip[9] for qualitative detection of *Wuchereria bancrofti* antigen. Test kits were stored at 2–37°C, protected from freezing and prolonged exposure to extreme heat, and used before the manufacturer's expiration date. Before field deployment, two test strips from each lot were tested using a positive control; lots that did not produce the expected positive result were not used. Before testing, kit components were allowed to reach ambient temperature. The selected finger was cleaned with an alcohol swab and allowed to dry, after which the internal side of the finger was punctured with a sterile, single-use lancet. The lancet was immediately discarded into a sharps container. A 75-µL finger-prick blood sample was collected using the fixed-volume micropipette supplied with the test kit and applied to the lower half of the sample pad. Each test strip was labelled with a unique identification code comprising the Evaluation Unit, school code, and sequential student number. Test results were read exactly at 10 minutes of sample

application under bright, unfiltered light. Results were classified as negative, positive, invalid, inconclusive, or refused according to the TAS testing algorithm. When the first test was invalid, a second test was performed, and the valid repeat result was considered final. When both tests were invalid, or when repeat testing was refused or not completed after an invalid first test, the final result was classified as inconclusive and excluded from the valid TAS denominator.

Survey outcomes: The primary outcome was the TAS 3 result in each Evaluation Unit, determined by comparing the number of antigen-positive children with the Evaluation Unit-specific critical cutoff. Evaluation Unit 1 passed only if the number of positive children was equal to or below six. Evaluation Unit 2 passed only if the number was equal to or below four. Secondary outcomes included: overall antigen positivity; antigen positivity by age group and sex; number and location of schools with positive children; attendance and testing coverage; invalid and inconclusive test results; household residence duration among positive children; and treatment and household follow-up.

Data management

School-level data were uploaded directly to KoboToolbox, while individual data were entered after fieldwork from the completed paper forms. The data-monitoring team reviewed uploaded records and communicated identified errors or inconsistencies to district supervisors for verification and correction. Data was cleaned and cross-checked in Microsoft Excel against paper forms. Final datasets were exported to STATA version 16 for analysis. Geographic coordinates were mapped using QGIS 4.2.1.

Statistical analysis: Categorical variables were summarized using frequencies and percentages. The valid diagnostic denominator included only children with final positive or negative results. Inconclusive results were excluded from antigen-positivity calculations and TAS interpretation. Antigen positivity was calculated as: Number of antigen-positive children ÷ number of children with valid results × 100. Exact 95% confidence intervals were calculated for the overall positivity and descriptive estimates by age, sex, and Evaluation Unit. Attendance was calculated as the number of eligible children present divided by the number of eligible children enrolled in Grade 1 and Grade 2. Testing coverage was calculated as the number tested divided by the number of eligible children present. Because the TAS decision was based on the number of positive children relative to the critical cutoff, pooled positivity across two Evaluation Units was not used to determine the programme outcome. Geographic coordinates of all surveyed schools and schools with antigen-positive children were recorded during fieldwork. Kernel density estimation (KDE) was performed in QGIS (version 4.2.1.) to visualize the spatial concentration of antigen-positive schools. The analysis was based on the geographic locations of schools reporting one or more antigen-positive children, while all surveyed schools were displayed to illustrate the spatial distribution of the sampling frame. KDE was used solely as an exploratory visualization to identify areas with relatively higher concentrations of antigen-positive schools.

Programme follow-up of antigen-positive children

All 13 antigen-positive children were eligible for post-survey programme follow-up. Families were contacted using information available through the TAS and local health system, and parents or guardians of 11 children were successfully reached. The follow-up was undertaken to support the national lymphatic filariasis programme's assessment and management of antigen-positive cases and to identify information relevant to

further programme investigation. Information collected during follow-up included duration and place of residence, movement or temporary stays outside the current locality, household composition, reported previous participation of family or household members in lymphatic filariasis MDA, including reported reasons for non-participation where applicable, knowledge of lymphatic filariasis, reported mosquito exposure and household environmental conditions, use of mosquito-protection measures, known family or community history of lymphatic filariasis, treatment following the positive result, short-term experiences after treatment, and remaining questions or follow-up needs. Telephone follow-up was conducted in Nepali during July 2026. Participants were informed of the purpose of the programme follow-up, that participation was voluntary, that information would be handled confidentially, and that they could decline to answer individual questions or discontinue the call. Personally, identifying information was removed from the dataset used for analysis. Follow-up information was organized into predefined programme-relevant variables and summarized descriptively using frequencies and percentages. Percentages for household and caregiver characteristics were calculated using the 11 families for whom follow-up information was available. The follow-up data were used to provide additional programme context for the TAS findings and were not used in determining the TAS pass/fail outcome.

Results

Survey coverage and sample adequacy

Of 3,679 students expected from EMIS-based school lists, 3,277 (89.07%) were confirmed as eligible Grade 1–2 enrolees during school check-in, of whom 3,001 (91.6%) were present and tested on the survey day (Table 4). Attendance was similar across EUs (92.0% in EU1, 91.1% in EU2). At the school level, among the 114 schools that reported a primary reason for student absence, migration was identified as the primary reason in 34 schools (29.8%). Testing coverage among eligible, present children was 100% in both EUs. Both EUs exceeded their planned WHO SSB sample size: EU1 achieved 1,587 valid samples against a target of 1,548 (102.5%), and EU2 achieved 1,413 against a target of 1,368 (103.3%).

Table 4. School enrollment, attendance, and testing coverage by Evaluation Unit

Evaluation Unit	Districts	Expected students	Eligible admitted	Eligible present	Attendance rate	Testing coverage
EU1	Ilam, Panchthar	1,880	1,726	1,588	92.0%	100%
EU2	Tehrathum	1,799	1,551	1,413	91.1%	100%
Total	—	3,679	3,277	3,001	91.5%	100%

Geographic distribution of surveyed schools

A total of 240 schools were surveyed across the two Evaluation Units. EU1 included 116 schools, comprising 61 schools in Ilam and 55 schools in Panchthar, whereas EU2 included 124 schools in Tehrathum. The

surveyed schools were distributed across the municipalities and rural municipalities of all three districts, providing broad geographic coverage of the survey area (Figure 2).

Demographic profile

The 3,001 tested children were nearly evenly distributed by sex, with 1,454 (48.4%) females and 1,547 (51.6%) males. The age distribution differed between the two Evaluation Units. In EU1, 1,046 of 1,588 children (65.9%) were aged 6–7 years, while 542 (34.1%) were outside the 6–7-year age range. In EU2, 812 of 1,413 children (57.5%) were aged 6–7 years, while 601 (42.5%) were outside this age range. Thus, the proportion of children outside the 6–7-year age range was higher in EU2 than in EU1, reflecting the grade-based rather than strictly age-based sampling design.

Table 5. Demographic characteristics of children tested during TAS-3 by Evaluation Unit

Characteristic	EU1 (n = 1,588), n (%)	EU2 (n = 1,413), n (%)	Total (N = 3,001), n (%)
Sex			
Female	772 (48.61)	682 (48.27)	1,454 (48.45)
Male	816 (51.39)	731 (51.73)	1,547 (51.55)
Age group			
6 years	498 (31.36)	367 (25.97)	865 (28.82)
7 years	548 (34.50)	445 (31.49)	993 (33.08)
Outside the 6–7-year age range	542 (34.13)	601 (42.53)	1,143 (38.08)

TAS 3 results and pass/fail determination

In EU1, 1,588 children were tested; one child had an inconclusive final result and was excluded from the analytic denominator, leaving 1,587 valid results. Thirteen children were antigen-positive, corresponding to an antigen positivity of 0.82% (95% CI: 0.44–1.40%). The 13 positive results exceeded the EU1 critical cutoff of six; therefore, EU1 did not pass TAS-3. In EU2, all 1,413 tested children had valid results, and no antigen-positive children were detected. Because the number of positive children was below the critical cutoff of four, EU2 passed TAS-3. TAS-3 outcomes were therefore interpreted separately for the two Evaluation Units according to their respective critical cutoffs.

Table 6. Valid sample, positivity rate, and TAS 3 pass/fail status by Evaluation Unit

Evaluation Unit	Tested	Inconclusive	Valid sample	Positive	Antigen positivity, %	Critical cutoff	TAS 3 result
EU1	1,588	1	1,587	13	0.82%	6	Did not pass
EU2	1,413	0	1,413	0	0.00%	4	Passed
Total	3,001	1	3,000	13	-	—	—

Applying WHO decision rules, EU1's 13 observed positive cases exceeded its critical cutoff of 6, indicating that EU1 did not pass TAS 3; EU2's zero positive cases remained below its cutoff of 4, and EU2 passed TAS 3. Because the TAS outcome was determined by comparison with the critical cutoff rather than by the pooled positivity rate, the two EUs required separate interpretation, despite the low pooled positivity rate (0.43%) when considered in isolation.

Prevalence by age and sex

Descriptive antigen-positivity estimates were similar across age and sex groups.(Table 7). Overall, prevalence was 0.43% (8/1,857) among children aged 6–7 years versus 0.44% (5/1,143) among children outside that age range, and 0.41% (6/1,454) among females versus 0.45% (7/1,546) among males. Within EU1, prevalence was somewhat higher among children outside the 6–7-year range (0.92%) than within it (0.77%), and slightly higher among males (0.86%) than females (0.78%), though confidence intervals overlapped substantially in all comparisons.

Table 7. Prevalence of LF antigenemia by age group and sex, overall and within EU1

Stratum	Positive (n)	Valid sample (n)	Prevalence % (95% CI)
Overall — 6–7 years	8	1,857	0.43 (0.19–0.85)
Overall — outside 6–7 years	5	1,143	0.44 (0.14–1.02)
Overall — female	6	1,454	0.41 (0.15–0.90)
Overall — male	7	1,546	0.45 (0.18–0.93)
EU1 — 6–7 years	8	1,045	0.77 (0.33–1.50)
EU1 — outside 6–7 years	5	542	0.92 (0.30–2.14)
EU1 — female	6	772	0.78 (0.29–1.68)
EU1 — male	7	815	0.86 (0.35–1.76)

Geographic distribution of positive cases

All 13 antigen-positive children were identified in six schools in EU1, including 11 children in Ilam district and two in Panchthar district. **Figure 3** shows the spatial distribution of all surveyed schools together with the

kernel density estimation (KDE) surface based on schools with one or more antigen-positive children. Positive schools were confined to EU1 (Ilam and Panchthar), whereas no antigen-positive children were detected in EU2 (Tehrathum). The greatest concentration of positive schools was observed in Ilam Municipality, where one school in Ward 9 accounted for seven antigen-positive children and another school in Ward 5 accounted for two. The remaining four positive schools each recorded one antigen-positive child and were located in other localities, including Sandakpur, Falgunanda, and Miklajung. Given the small number of positive schools, the KDE surface was interpreted as an exploratory visualization of the spatial concentration of positive schools rather than as evidence of a statistically defined transmission cluster. Information on household or family residence duration was available for all 13 antigen-positive children. Families had lived in their current locality for 1 to 35 years (mean 13.7 years; median 10 years). Nine of the 13 families (69.2%) had resided in the current locality for more than five years, whereas four (30.8%) had lived there for five years or less. These findings indicate that many antigen-positive children belonged to families with relatively long-term residence in their current communities.

Test performance and quality indicators

Nine of 3,001 first-round tests (0.30%) were invalid; 8 were successfully retested and returned negative results, and 1 child in EU1 declined retesting, yielding the single inconclusive result excluded from the analytic sample. No second-round tests were invalid. Reasons for first-test invalidity were distributed among human error (insufficient blood volume; 4/9, 44.4%), test-strip sample migration issues (4/9, 44.4%), and an operational fault (broken sample pad; 1/9, 11.1%).

Programme follow-up of antigen-positive children and their families

Programme follow-up information was available for 11 of the 13 antigen-positive children (84.6%), including nine families from Ilam and two from Panchthar (Table 8). Reported mosquito-exposure conditions were common: seven caregivers (63.6%) reported high mosquito density around the household, eight (72.7%) described nearby environmental conditions potentially conducive to mosquito breeding, and nine households (81.8%) reported limitations in mosquito-protection practices. No caregiver reported a known household or community history of lymphatic filariasis, elephantiasis, hydrocele, or chronic swelling. Reported previous MDA participation among family or household members varied. Five families (45.5%) reported non-participation in previous MDA rounds due to pregnancy, while two families (18.2%) reported non-delivery or unavailability of MDA medicines. Seven caregivers (63.6%) identified mosquito bites as the route of LF transmission, whereas three (27.3%) reported little or no previous knowledge of LF. All 13 antigen-positive children received the programme-recommended treatment, as the team visited their homes to provide the medications directly. Short-term symptoms following treatment were reported in four of the 11 followed-up households (36.4%), with no persistent or severe events reported. Seven families (63.6%) had remaining questions or information needs concerning repeat testing, household testing, future antigen positivity, or further clinical assessment.

Table 8. Programme follow-up characteristics of antigen-positive children and their families

Domain	Programme follow-up characteristic	n/N	%
Follow-up participation	Families with programme follow-up information available	11/13	84.6
	Families from Ilam	9/11	81.8
	Families from Panchthar	2/11	18.2
Residence and mobility	Children reported to have moved from another locality within Ilam	3/11	27.3
Mosquito exposure and household environment	Caregivers reporting high mosquito density around the household	7/11	63.6
	Nearby environmental conditions potentially conducive to mosquito breeding*	8/11	72.7
	Limitations in household mosquito-protection practices†	9/11	81.8
	Livestock or domestic animals kept near the household	5/11	45.5
	Known household or community history of LF, elephantiasis, hydrocele, or chronic swelling	0/11	0.0
Previous MDA participation	Missed previous MDA because of pregnancy	5/11	45.5
	Reported non-delivery or unavailability of MDA medicines	2/11	18.2
	Reason for previous non-participation unknown	2/11	18.2
	Family recalled participation in only one previous MDA round	1/11	9.1
	Family reported previous participation by all household members	1/11	9.1
Knowledge and communication	Caregivers identifying mosquito bites as the route of LF transmission	7/11	63.6
	Caregivers reporting little or no previous knowledge of LF	3/11	27.3
	Families reporting remaining information or reassurance needs after treatment	7/11	63.6
Treatment and follow-up	Antigen-positive children receiving programme-recommended treatment	13/13	100.0
	Households reporting short-term symptoms after treatment‡	4/11	36.4
	Persistent or severe treatment-related events reported	0/11	0.0

Abbreviations: *LF, lymphatic filariasis; MDA, mass drug administration.*

** Environmental conditions included stagnant water, drainage channels, ponds, rice fields, irrigation canals, waste, jungle, or dense vegetation near the household.*

† Limitations in mosquito-protection practices included no mosquito protection, unscreened doors or windows, seasonal rather than year-round bed-net use, or regular bed-net use beginning only after the child tested positive.

‡ Reported short-term symptoms included vomiting, dizziness, or headache and reportedly resolved within approximately one day.

Note: *Percentages are based on the 11 families for whom programme follow-up information was available unless otherwise specified. Some categories are not mutually exclusive and therefore do not sum to 100%.*

Discussion

This TAS-3 demonstrated markedly different programme outcomes in the two Evaluation Units of eastern Nepal. EU1, comprising Ilam and Panchthar districts, recorded 13 antigen-positive children, exceeding the critical cutoff of six, and therefore did not pass TAS-3. In contrast, no antigen-positive children were identified in EU2, comprising Tehrathum district, which passed the survey. Although pooled antigen positivity across both Evaluation Units was only 0.43%, this aggregate estimate masks the programme-relevant difference between the two areas. TAS decisions are made independently for each Evaluation Unit according to the number of positive children relative to the prespecified critical cutoff, rather than according to pooled prevalence across Evaluation Units.

The identification of antigen-positive children in EU1 after no antigen-positive children were reported during TAS-1 and TAS-2 is epidemiologically important and raises concern about possible local recrudescence of LF transmission after cessation of MDA. This interpretation is supported cautiously by the programme follow-up findings: many affected children had long-term residence in their current communities, while caregivers frequently reported high mosquito density, nearby environmental conditions potentially conducive to mosquito breeding, and limitations in household mosquito-protection practices. Similar post-MDA studies in Nepal have demonstrated persistent or residual infection within geographically localized hotspots despite previously favourable programme indicators, highlighting the potential for transmission to persist or re-emerge at a focal level.[13] Recrudescence after apparently successful earlier TAS has also been documented in American Samoa, where TAS-3 subsequently identified antigen-positive children and further investigation confirmed ongoing transmission.[14] These findings support additional investigation of the affected localities in EU1, including molecular xenomonitoring of vector mosquitoes, which may complement human antigen surveillance in detecting low-level residual transmission. Nevertheless, the present findings should be interpreted as suggestive of possible local recrudescence rather than definitive evidence of ongoing transmission.

The focal distribution observed in EU1 indicates substantial spatial heterogeneity in antigen-positive cases within the Evaluation Unit. All 13 antigen-positive children were identified in six schools, including seven children attending one school in Ilam Municipality Ward 9. This concentration suggests that low infection levels at the broader Evaluation Unit level may coexist with localized areas requiring further investigation. Previous post-MDA investigations[13] in Nepal have similarly shown that residual infection may persist in geographically localized hotspots even when broader TAS indicators remain below programme thresholds.

However, the observed pattern in the present study should be interpreted cautiously because school location does not necessarily represent the location where infection was acquired, and the spatial analysis was based on only six antigen-positive schools. Further household and community investigation, including residential geocoding, testing of household members and nearby residents, and entomological assessment, would help determine whether the observed concentration represents localized ongoing transmission.

The present findings also resemble experiences reported from other LF elimination settings. American Samoa[14] passed TAS-1 and TAS-2 but subsequently failed TAS-3 in 2016, when nine antigen-positive children were identified across five schools. Follow-up investigation of the households of these children found that 12 of 58 household members were antigen-positive, and at least one additional antigen-positive person was detected in five of the nine index households. In Haiti[15], follow-up around eight children detected during TAS-2 identified an antigen prevalence of 1.5% among 1,927 household and neighbourhood participants, with evidence that additional infections occurred close to the index children. These studies underscore the importance of treating antigen-positive children and their household members to prevent renewed community transmission. In the present study, the 13 antigen-positive children attended six schools, including seven who attended one school in Ilam Municipality Ward 9, indicating substantial spatial heterogeneity within EU1. Available residential information provided additional geographic context. Of the seven children attending the school in Ward 9, five also resided in Ward 9, while one resided in Ward 5 and one in Ward 12. Across all 13 antigen-positive children, reported residences were distributed across Ilam Municipality Wards 3, 4, 5, 9, and 12, as well as Phalgunanda Rural Municipality Ward 5, Tumbewa Rural Municipality Ward 5, and Sandakpur Rural Municipality Ward 3. The concentration of five antigen-positive children residing in Ilam Municipality Ward 9 supports the presence of a focal geographic pattern, although positive cases were not confined to a single ward. Further household testing and investigation of nearby communities would help determine the extent of residual transmission around these locations. Because the kernel density surface was generated from only six antigen-positive schools, it should be interpreted as an exploratory visualization rather than as a formal spatial-cluster analysis. Post-survey programme follow-up provided additional household-level context for interpreting this spatial distribution. Among the 11 families reached, caregivers reported both long-term residence in the current locality and some movement or temporary stays outside the community. Seven of 11 caregivers (63.6%) reported high mosquito density around the household, eight (72.7%) described nearby environmental conditions potentially conducive to mosquito breeding, and nine households (81.8%) reported limitations in mosquito-protection practices. These findings indicate possible opportunities for mosquito exposure but cannot establish when or where infection was acquired.

The clustering observed in EU1 directly supports the investigation pathway in the updated WHO monitoring and evaluation guidance.[9] For TAS-2 or TAS-3 results above the threshold, WHO recommends reporting and reviewing the results with WHO, examining the geographic distribution of positive individuals, reviewing previous survey findings, analysing subnational MDA coverage and vector-control information, and convening an expert review to determine the appropriate response. The guidance does not prescribe an identical intervention for every TAS-3 failure; rather, it emphasizes a contextualized decision informed by the distribution of infection and the quality of earlier programme implementation. Programme follow-up information also provided additional context regarding previous MDA participation. Five of the 11 followed-

up families (45.5%) reported missed participation associated with pregnancy, during earlier campaigns. Two families (18.2%) reported non-delivery or unavailability of medicines, while two (18.2%) could not identify the reason for previous non-participation. These findings suggest that administrative coverage estimates may conceal differences between programme ineligibility, incomplete campaign reach, medicine availability, and actual treatment uptake.

Reported historical MDA coverage in Ilam and Panchthar was variable, with some annual estimates close to or below the commonly used programme target of 65%. Such variation may indicate gaps in population-level treatment coverage that could have contributed to persistence of residual infection. Further investigation should therefore consider historical MDA performance alongside population mobility and local vector-control practices. A multi-country analysis of Evaluation Units identified several ecological and epidemiological factors associated with TAS failure, including higher baseline infection prevalence and greater population density, while lower elevation was associated with an increased likelihood of failure. [16] These associations do not imply that any single ecological factor explains the TAS-3 outcome in EU1. Rather, the focal distribution of antigen-positive children, reported mosquito exposure and environmental conditions potentially conducive to vector breeding, historical MDA performance, population distribution, and mobility should be considered together when interpreting the observed pattern.

The absence of antigen-positive children in EU2 is reassuring and indicates that Tehrathum met the TAS-3 decision criterion. However, passing TAS-3 should not be interpreted as definitive evidence that transmission has been completely interrupted. Evidence from Sri Lanka has shown that complementary post-MDA surveillance methods, including anti-filarial antibody testing among schoolchildren and molecular xenomonitoring of mosquito populations, can identify focal residual transmission even in areas where TAS criteria have been met.[17–20] In some settings, geographically localized signals detected through school-based antibody surveillance have corresponded with areas of molecular xenomonitoring positivity, suggesting that these approaches may provide additional sensitivity for identifying residual transmission. These findings support continued post-TAS surveillance and the use of complementary surveillance tools, particularly in areas where there is previous evidence or suspicion of persistent or focal transmission.

Several aspects of survey implementation strengthen confidence in the principal findings. Both Evaluation Units exceeded their required sample sizes, attendance exceeded 90%, all eligible children present were tested, and the initial invalid-test rate was only 0.30%. Eight of the nine initially invalid tests produced valid negative results after repeat testing, leaving only one inconclusive result. The broad geographical distribution of the 240 surveyed schools also increased the likelihood of detecting geographically dispersed infection. Immediate treatment of all antigen-positive children and provision of treatment to eligible household members were important programme responses.

However, presumptive treatment of household members does not generate information on the magnitude or distribution of infection around the positive children. Studies in American Samoa[14,21] and Haiti[15] indicate that testing household members and nearby residents can efficiently identify additional antigen-positive and microfilaremic individuals. In the programme follow-up, none of the 11 caregivers reported a known household or community member with lymphatic filariasis, elephantiasis, hydrocele, or chronic swelling. However, household members had generally not been systematically tested. The absence of a reported

family history should therefore not be interpreted as evidence that household or neighbourhood infection is absent. A structured investigation in EU1 should include testing of household members and nearby residents, assessment of microfilaremia, residential geocoding, and entomological surveillance where feasible. These investigations would help determine whether the detected infections are isolated or represent a wider community reservoir and would inform the appropriate scale of further intervention.

This study has several limitations. The school-based design excluded children who were absent or not enrolled, and their infection status may have differed from that of participants, particularly given the reported role of migration. Filariasis Test Strips detect circulating antigen but do not determine microfilaremia, infectiousness, or the timing and location of infection; and molecular xenomonitoring was not conducted. Spatial analysis relied on school rather than household coordinates and included only six antigen-positive schools, so the KDE findings should be considered exploratory. Finally, programme follow-up information was available for 11 of the 13 antigen-positive children and was collected primarily to support programme assessment and case follow-up rather than as a dedicated research assessment. The small number of families limits the interpretation and generalizability of these descriptive findings. Information was based on caregiver reports and was not independently verified and may therefore have been affected by recall or reporting bias.

Conclusion

TAS-3 produced contrasting outcomes in eastern Nepal. EU1, comprising Ilam and Panchthar districts, recorded 13 antigen-positive children and exceeded the critical cutoff, whereas EU2, comprising Tehrathum district, recorded no antigen-positive children and passed the survey. The positive children in EU1 were distributed across six schools, with the largest concentration observed among children attending and residing in Ilam Municipality Ward 9. Programme follow-up provided additional context on residence and mobility, reported mosquito exposure and household environmental conditions, treatment, and remaining information needs among affected families. These findings indicate focal persistence of LF infection within EU1 and support further targeted investigation of the affected localities, while continued surveillance remains appropriate in EU2.

Declarations

Ethics approval and consent to participate:

The TAS-3 was conducted by the Vector Borne Disease Research and Training Centre (VBDRTC) as part of routine monitoring and surveillance activities of Nepal’s National Lymphatic Filariasis Elimination Programme. Activities undertaken to monitor progress toward national lymphatic filariasis elimination targets had been exempted from ethical review by the Nepal Health Research Council (reference no. 1530 NHRC). Prior to the survey, school principals or head teachers were informed about the survey objectives and procedures, and authorization to conduct the survey was obtained. Eligible children were informed about the objectives and procedures of the survey, and verbal consent was obtained before collecting information and blood samples.. Children who declined participation or showed unwillingness or distress were not tested.

Participant information and test results were recorded using unique identification codes and maintained confidentially.

Availability of data and materials

The datasets generated and analysed during the current survey are available from the corresponding author on reasonable request and subject to approval by the relevant programme authorities.

Competing interests

The authors declare that they have no competing interests.

Consent for publication: Not applicable.

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Author Contribution

BPS finalise the survey protocol and took the overall leadership to accomplish the TAS survey, Conceptualise the draft manuscriptLP contributed for the data analysis and drafted the zero draft manuscriptLRB contributed orientation to enumerators supervise the enumerators during field workJC contributed orientation to enumerators and supervise the enumerators during field workHJ contributed orientation to enumerators and supervise the enumerators during field workPP contributed to draft the Survey ProtocolRS contributed orientation to enumerators and supervise the enumerators during field workGD reviewed the zero draft manuscript RKM contributed the orientation to enumerators and reviewed the zero draft manuscript SRP contributed the orientation to enumerators and reviewed the zero draft manuscript AB contributed the orientation to enumerators and reviewed the zero draft manuscript DKS contributed the orientation to enumerators and reviewed the zero draft manuscript

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Figures

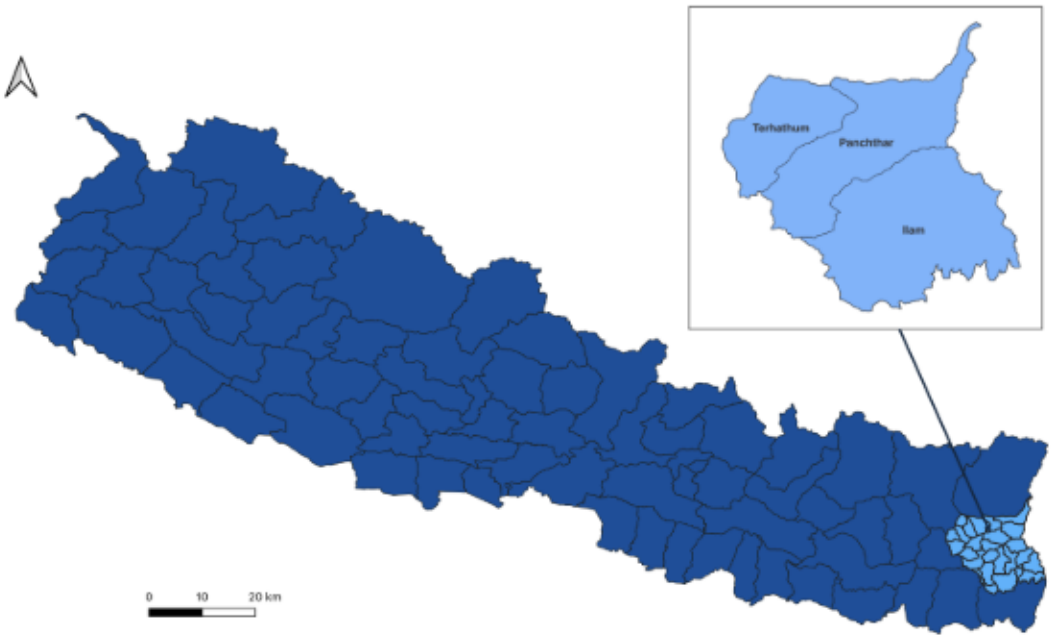

Figure 1

Survey Area

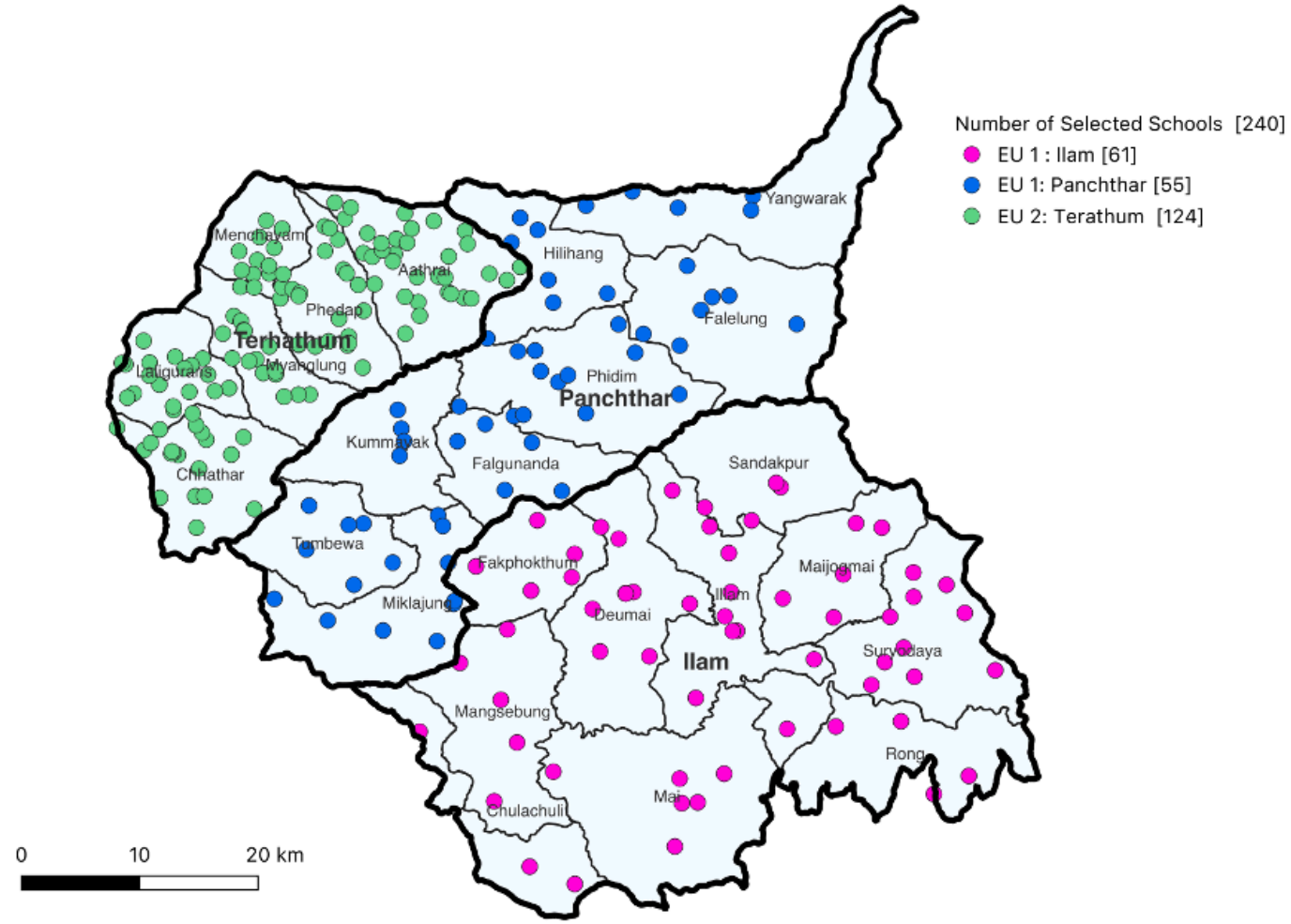

Figure 2

Geographic distribution of the 240 schools surveyed during TAS-3 in Ilam, Panchthar, and Tehrathum districts, eastern Nepal. Pink symbols indicate surveyed schools in Ilam, blue symbols indicate surveyed schools in Panchthar, and green symbols indicate surveyed schools in Tehrathum. Ilam and Panchthar constituted Evaluation Unit 1, whereas Tehrathum constituted Evaluation Unit 2.



Figure 3

Geographical distribution of positive cases using Kernel Density