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Rodent Density and *Leptospira* sp. Detection by qPCR in Pangkep, South Sulawesi

Running title: qPCR Detection of Leptospira sp. in Pangkep Rats

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Abstract

Background: Rodent reservoirs sustain leptospirosis transmission, but molecular surveillance data from South Sulawesi remain limited. **Objective:** To characterize rodent density, species composition, ectoparasites, and renal qPCR positivity for *Leptospira* sp. in three surveillance areas of Pangkajene and Kepulauan. **Methods:** A descriptive field-laboratory surveillance study was conducted in Bowong Cindea, Biringkassi, and Jollo during 2-6 September 2024. A total of 150 rodent traps were deployed across 75 households for three consecutive nights. Captured rats were identified morphometrically, examined for ectoparasites, and kidney samples were tested by real-time PCR. Proportions were summarized with Wilson 95% confidence intervals. **Results:** Twenty-one rats and six *Suncus murinus* were captured. The operational success-trap index was 8% in Bowong Cindea, 10% in Biringkassi, and 24% in Jollo, all above the 1% benchmark applied in 2024 surveillance. *Rattus tanezumi* comprised 57.1% and *Rattus norvegicus* 42.9% of rats. *Leptospira* sp. was detected by qPCR in 7/21 rats (33.3%; 95% CI 17.2-54.6), with positive results in every surveillance area. **Conclusion:** High rodent density and widespread qPCR positivity indicate sustained reservoir pressure and support integrated rodent control, environmental sanitation, human case detection, and repeated One Health surveillance.

Keywords: *Leptospira*; leptospirosis; qPCR; rodents; environmental health

Research Highlights

- All three surveillance areas exceeded the 1% operational rodent-density benchmark used in 2024, with Jollo reaching 24%.
- Renal *Leptospira* sp. was detected by qPCR in 33.3% of rats and occurred in both *Rattus tanezumi* and *Rattus norvegicus*.
- Rodent abundance and molecular positivity did not rank identically across sites, supporting combined ecological and molecular surveillance rather than reliance on density alone.

1 Introduction

Leptospirosis is a neglected bacterial zoonosis with a disproportionate burden in tropical and subtropical settings. A global systematic review estimated approximately 1.03 million cases and 58,900 deaths annually,

with substantial burden in South and Southeast Asia [1]. Human infection generally follows direct contact with infected animal urine or indirect contact with contaminated water or soil. Environmental exposure is amplified by flooding, heavy rainfall, poor sanitation, and close contact with rodent-infested settings [2]. These features make leptospirosis an archetypal One Health problem in which animal reservoirs, environmental contamination, and human exposure are tightly interconnected.

Rodents are particularly important maintenance hosts because *Leptospira* can colonize renal tubules and be shed in urine without causing obvious disease in the host. Molecular surveys across Southeast Asia have demonstrated marked geographic and habitat-related heterogeneity in rodent infection [3]. In Malaysian Borneo, rodent-borne *Leptospira* was strongly associated with urban environments [4], whereas Indonesian studies have documented substantial variation between settings. In Bogor, *Leptospira* was isolated from 31.3% of captured *Rattus norvegicus* [5]; in Bantul and Gunungkidul, PCR positivity was lower in *Rattus tanezumi* (4.8%) and *Rattus norvegicus* (12.5%) [6]. More recently, a molecular survey in Surabaya reported much higher PCR positivity among urban *R. norvegicus* [7]. This heterogeneity underscores the need for locally resolved surveillance rather than extrapolation from other cities or provinces.

Pangkajene and Kepulauan (Pangkep), South Sulawesi, contains densely inhabited mainland areas interspersed with agricultural, drainage, and coastal environments that can support frequent human-rodent-environment contact. The governmental surveillance report underlying this manuscript documented leptospirosis activity and an outbreak response in Pangkep during 2023, which prompted follow-up reservoir surveillance in 2024 [15]. The operational public-health question was not only whether rats were abundant, but whether captured animals carried detectable *Leptospira* in the kidney and therefore represented a plausible source of environmental contamination.

Routine vector and reservoir surveillance in Indonesia also uses standardized environmental-health indicators to guide action. At the time of this surveillance, Ministry of Health guidance and the environmental-health standard used a rodent success-trap benchmark and zero tolerance for *Leptospira*-positive rats as programmatic indicators [10-12]. However, a density index alone cannot identify infected reservoirs, while molecular positivity without ecological context cannot quantify how commonly humans may encounter rodents. Integrating trapping metrics, species identification, ectoparasite examination, and molecular testing provides a more informative surveillance profile.

This study therefore aimed to characterize rodent density, species composition, ectoparasites, and renal qPCR positivity for *Leptospira* sp. in three surveillance areas within the Bowong Cindea Primary Health Center catchment of Pangkep. A secondary objective was to examine whether site-level rodent density and molecular positivity showed concordant patterns, thereby informing priorities for environmental-health and One Health interventions.

2 Materials and Methods

2.1 Study design and setting

This was a descriptive cross-sectional field and laboratory surveillance study conducted in the Bowong Cindea Primary Health Center catchment, Bungoro District, Pangkajene and Kepulauan Regency, South Sulawesi, Indonesia. Field trapping was undertaken from 2 to 6 September 2024 in three surveillance areas: Bowong Cindea, Biringkassi, and Jollo. Kidney samples were subsequently examined at the Vector and Disease-Bearing Animal Laboratory of Balai Laboratorium Kesehatan Masyarakat Makassar on 10-11 September 2024. The study was designed as operational public-health surveillance rather than an etiologic risk-factor study [15].

The three areas were selected for programmatic surveillance within a locality that had experienced leptospirosis activity. Geographic coordinates of trap placements were recorded using GPS and mapped in ArcGIS as part of the field surveillance workflow [15]. The georeferenced trap/sample distribution is presented in Figure 1. Because the source report did not document a probability-based procedure for selecting participating households, the household component is interpreted as an operational convenience sample rather than a population-representative household survey.

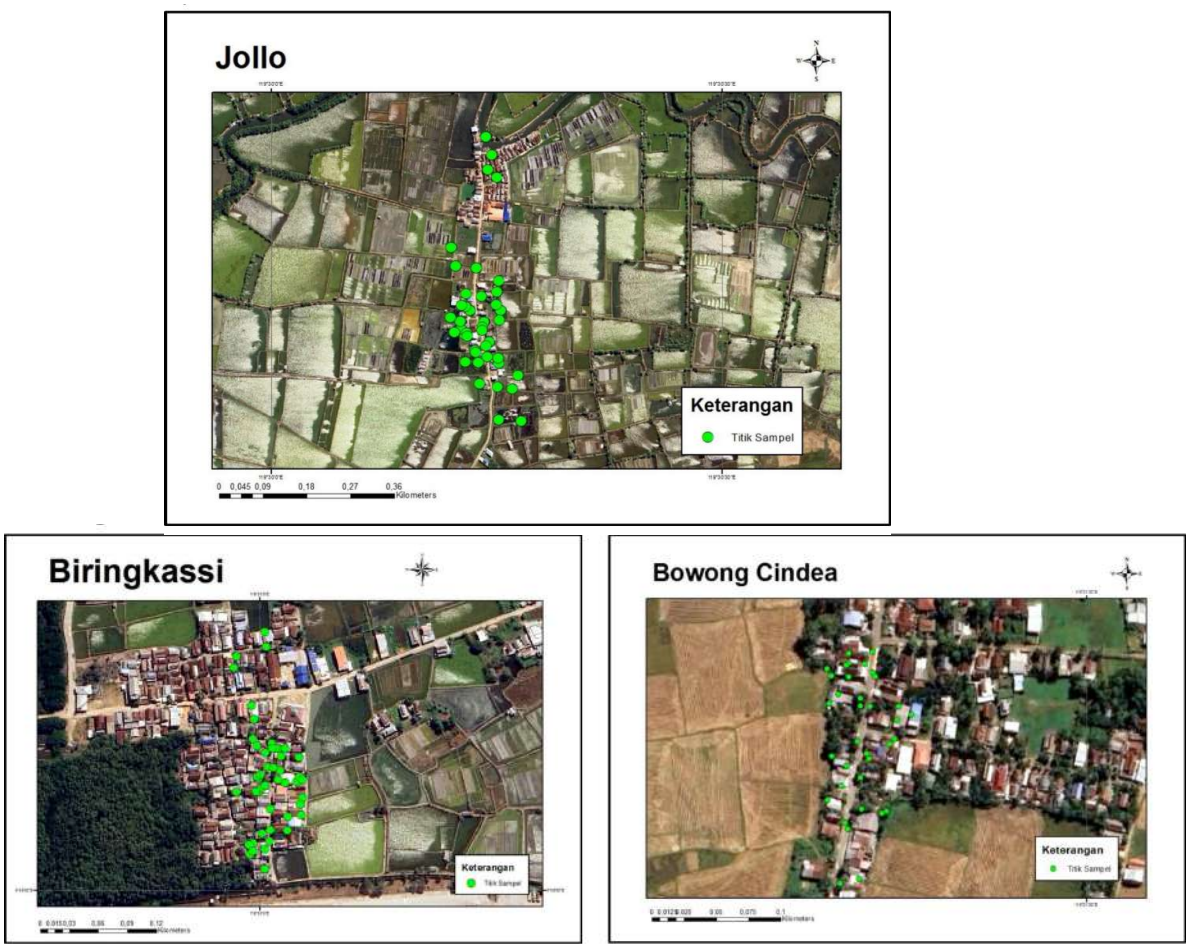

Figure 1. Georeferenced trap/sample locations in Jollo (A), Biringkassi (B), and Bowong Cindea (C), Bungoro District, Pangkajene and Kepulauan, September 2024. Green points in the original maps denote sample/trap locations ("Titik Sampel"). Source: 2024 surveillance report [15].

2.2 Population, sampling, and sample size

The operational sampling frame comprised 25 participating households in each surveillance area. Two traps were placed per household, one indoors and one outdoors, yielding 50 deployed traps per area and 150 traps overall. Traps were set in the late afternoon with salted fish bait and checked the following morning for three consecutive nights. After captured animals were removed, traps were cleaned and returned to their original positions. The surveillance report defines the study sample as all rats captured during this procedure [15].

No formal sample-size calculation was reported because the activity was designed as routine reservoir surveillance with a fixed field effort. For the molecular analysis, all captured rats for which kidney specimens were collected were included. *Suncus murinus* captures were recorded as small-mammal by-catch but were not included in the rodent success-trap denominator or the rat qPCR analysis.

2.3 Variables and measurements

Captured animals were identified morphometrically using body weight, body and tail length, head/skull, ear and hind-foot measurements, sex-specific characteristics, and dorsal/ventral coloration. The principal rodent species were *Rattus tanezumi* and *Rattus norvegicus*. Ectoparasites were collected by combing the entire body; recovered specimens were transferred with forceps into screw-cap vials containing 70% alcohol [15].

Rodent density was summarized using the programmatic success-trap index specified in the surveillance report and Indonesian operational guidance: number of rats captured divided by number of deployed traps, multiplied by 100 [11,15]. This operational denominator is the number of traps rather than trap-nights and should therefore not be interpreted as a conventional trap-night capture rate. The general flea index was calculated as the number of fleas recovered divided by the number of rats examined. For comparison with the 2024 surveillance benchmark, a success-trap index above 1% was interpreted as exceeding the national environmental-health threshold applied at the time; the benchmark for *Leptospira*-positive rats was zero [12,15].

For molecular testing, captured rats were dissected and kidney tissue was collected. Real-time polymerase chain reaction (qPCR) was performed according to the Balai Labkesmas Makassar working instruction for biomolecular detection of *Leptospira* in rat kidneys using an EvaGreen PCR kit [14,15]. The source report did not provide the amplified gene target, cycle-threshold decision rule, analytical limit of detection, or run-level quality-control results; accordingly, this manuscript reports the laboratory findings as qPCR positivity for *Leptospira* sp. and does not infer organism viability or serovar identity.

2.4 Statistical analysis

Counts and percentages were recalculated from the tabulated site and sample-level records in the source report. When narrative summaries conflicted with tabulated/sample-level counts, the tabulated and qPCR roster data were treated as authoritative. This verification identified a narrative inconsistency in the Bowong Cindea species count and an arithmetic discrepancy in the site-specific flea index; the manuscript therefore reports 3 *R. tanezumi* and 1 *R. norvegicus* in Bowong Cindea and recalculates the flea index from its stated numerator and denominator. The Biringkassi flea index is 1/5 = 0.20, while the overall flea index is 1/21 = 0.048.

Descriptive analyses were performed in Python 3.13.5 with statsmodels 0.14.6. For qPCR positivity proportions, 95% confidence intervals were calculated using the Wilson score method. Because of the small number of rats and the non-probability, operational surveillance design, no formal between-site or between-species hypothesis tests were performed. Results are interpreted descriptively and not as causal or population-prevalence estimates.

2.5 Ethical considerations

The activity was conducted as routine governmental public-health surveillance by Balai Laboratorium Kesehatan Masyarakat Makassar in collaboration with district and primary-health-care partners. The source report did not state an institutional animal ethics committee approval number, animal-use permit, or formal exemption determination. These administrative and animal-welfare details must be verified and completed by the responsible institution before journal submission, consistent with HIGIENE publication ethics for studies involving animals.

3 Results

Across the three areas, 150 traps were deployed among 75 households. Twenty-seven small mammals were captured: 21 rats and six *Suncus murinus*. The report recorded 25 trap placements with captures; 15 were indoors and 10 outdoors, and two trap placements captured two animals. Among rats, *Rattus tanezumi* was more frequent (12/21; 57.1%) than *Rattus norvegicus* (9/21; 42.9%). Jollo contributed 12 of the 21 rats, Biringkassi five, and Bowong Cindea four.

Table 1. Small mammals captured and operational rodent success-trap index by surveillance area, Pangkep, September 2024

Area	Traps	R. tanezumi	R. norvegicus	S. murinus	Rats (n)	Success-trap (%)
Bowong Cindea	50	*3*	*1*	*0*	4	8.0
Biringkassi	50	*0*	*5*	*5*	5	10.0
Jollo	50	*9*	*3*	*1*	12	24.0
Total	150	*12*	*9*	*6*	21	14.0

Note: The success-trap index uses rats only and follows the operational formula in the 2024 surveillance report: rats captured / deployed traps × 100. S. murinus was recorded as non-rodent by-catch.

The success-trap index was highest in Jollo (24%), followed by Biringkassi (10%) and Bowong Cindea (8%). Thus, each site exceeded the 1% benchmark applied in the 2024 surveillance program. One flea was recovered from a rat in Biringkassi and two lice were recovered in Jollo. Recalculated from the stated formula, the site-specific general flea index was 0.20 in Biringkassi and 0 in the other two areas; the overall index was 0.048. These values remained below the programmatic flea-index benchmark of 2 used in the source report [12,15].

Figure 2. Spatial distribution of small-mammal capture outcomes in Jollo (A), Biringkassi (B), and Bowong Cindea (C). Symbols in the original maps indicate the number captured at each mapped trap point ("Jumlah Tikus tertangkap"). Source: 2024 surveillance report [15].

Georeferenced field mapping showed capture-positive trap points in all three surveillance areas (Figure 2). Jollo had the greatest number of captured rats and the highest operational success-trap index; the source mapping also recorded two trap placements with two captured small mammals, whereas capture-positive points in Biringkassi and Bowong Cindea were fewer. These maps provide spatial context for the aggregate trapping indices and help identify locations for targeted follow-up surveillance and rodent-control activities.

All 21 captured rats had kidney specimens tested by qPCR. *Leptospira* sp. was detected by qPCR in seven rats (33.3%; 95% CI 17.2-54.6). Positive rats were identified in all three surveillance areas: 2/4 in Bowong Cindea, 1/5 in Biringkassi, and 4/12 in Jollo. By species, positivity was 3/12 (25.0%) among *R. tanezumi* and 4/9 (44.4%) among *R. norvegicus*. The confidence intervals were wide, reflecting the small denominators, and the species difference was not formally tested.

Table 2. Renal *Leptospira* sp. qPCR positivity by surveillance area and rat species

Stratum	Tested (n)	Positive (n)	Positive (%)	95% CI (%)
Overall	21	7	33.3	17.2-54.6
Bowong Cindea	4	2	50.0	15.0-85.0
Biringkassi	5	1	20.0	3.6-62.4
Jollo	12	4	33.3	13.8-60.9
R. tanezumi	12	3	25.0	8.9-53.2
R. norvegicus	9	4	44.4	18.9-73.3

Note: CI = confidence interval. Wilson score intervals are shown. qPCR positivity indicates detection of Leptospira sp. in kidney tissue; the assay target and viability of organisms were not established in the source report.

Site-level rodent density and molecular positivity did not show the same ordering. Jollo had the highest success-trap index (24%) but an intermediate qPCR positivity proportion (33.3%), whereas Bowong Cindea had the lowest success-trap index (8%) but the highest observed molecular proportion (50.0%). Given the very small site-specific denominators, these values should not be interpreted as evidence of a statistically meaningful inverse pattern; they instead illustrate that density and infection indicators provide different surveillance information.

4 Discussion

This surveillance identified two concurrent signals of environmental-health concern: rodent density above the operational benchmark in all three areas and renal *Leptospira* sp. qPCR positivity in one-third of tested rats. Molecular-positive rats occurred in Bowong Cindea, Biringkassi, and Jollo and included both dominant commensal species, *R. tanezumi* and *R. norvegicus*. Together, these findings indicate that the reservoir signal was not confined to a single neighborhood or rat species. The practical implication is that control strategies focused only on the site with the largest number of rats would risk overlooking areas where fewer captured rats nevertheless include infected animals.

The overall molecular positivity of 33.3% is biologically plausible in the context of heterogeneous rodent infection reported elsewhere in Southeast Asia. A multicountry study found an average rodent infection prevalence of approximately 7%, with strong variation across localities and habitats [3]. In Malaysian Borneo, Blasdell et al. reported 31.6% overall rodent positivity and substantially higher detection in urban settings [4]. In Bogor, Indonesia, Koizumi et al. isolated *Leptospira* from 25 of 80 *R. norvegicus* (31.3%) [5]. Conversely, Sunaryo and Priyanto reported PCR positivity of 4.8% in *R. tanezumi* and 12.5% in *R. norvegicus* from Bantul, while other species showed different levels [6]. A 2025 Surabaya study reported considerably higher PCR positivity in urban rats [7]. Direct numerical comparison is inappropriate because trapping frame, habitat,

season, laboratory target, and diagnostic method differ among studies; nevertheless, the collective evidence shows that rodent carriage can vary substantially even within Indonesia.

R. norvegicus showed a numerically higher qPCR positivity proportion than *R. tanezumi* in this surveillance (44.4% vs 25.0%), but the small sample makes this difference highly uncertain. Both species are epidemiologically relevant because they occupy niches that bring them into contact with human settlements. *R. norvegicus* is strongly associated with sewers, drains, ground-level food resources, and moist environments, whereas *R. tanezumi* is highly adapted to buildings and elevated structures. The presence of both species, combined with more capture-positive trap placements indoors than outdoors, indicates a close household interface. Similar Indonesian work has detected *Leptospira* in both species [9], while genomic work in Bogor highlights the potential importance of *R. norvegicus* in maintaining diverse *Leptospira* lineages [5].

The site pattern deserves particular attention. Jollo had the highest operational success-trap index, but Bowong Cindea had the largest observed molecular proportion. Density and qPCR positivity are therefore not interchangeable indicators. The georeferenced capture maps add a spatial layer to this interpretation by showing where trap points yielded animals within each surveillance area (Figure 2), information that is lost when results are summarized only as site-level percentages. High density may increase the frequency of human-rodent encounters and environmental contamination opportunities, whereas molecular positivity reflects the proportion of captured animals with a positive qPCR result for *Leptospira* sp. at a particular point in time. A robust surveillance system should track both and retain georeferenced field data for repeat mapping. Repeated measurements could clarify whether spatial capture patterns and qPCR positivity shift across seasons, rainfall patterns, food availability, sanitation changes, or control activities.

The ectoparasite findings were comparatively limited. Only one flea and two lice were recovered, and the recalculated general flea index remained below the benchmark used in the surveillance report. These findings do not reduce the leptospirosis concern because leptospirosis is not primarily flea-borne; the key pathway is exposure to urine from infected mammals or contaminated environments. Ectoparasite monitoring remains useful for broader rodent-borne disease surveillance, particularly for plague risk, but should be interpreted as a distinct surveillance domain rather than a proxy for *Leptospira* transmission.

From an environmental-health perspective, the findings support integrated rather than single-method rodent control. Physical trapping can reduce local rodent abundance, while sanitation measures should reduce food, water, shelter, and access to buildings. The source surveillance report recommended removal of waste accumulation and food residues, elimination of standing water, improved food storage, maintenance of building structures, and sustained rodent control [15]. Chemical rodenticides may have a role within an integrated program but require careful selection, placement, safety management, and evaluation to limit risks to humans, domestic animals, and non-target wildlife. Control effectiveness should be assessed with pre-specified indicators rather than assumed from the amount of bait or number of traps deployed.

The molecular signal also argues for stronger linkage with human surveillance. The 2024 field activity was initiated in a district with recent leptospirosis activity, yet this study did not include human specimens, exposure histories, or environmental water/soil sampling. Consequently, the data cannot establish transmission from the captured rats to human cases. A One Health design that samples humans, animals, and environmental media in a coordinated temporal and spatial framework would provide stronger evidence about transmission pathways. A recent systematic review found that only a small fraction of leptospirosis field studies simultaneously investigated all three One Health compartments and emphasized recurring weaknesses in study design and analytic integration [8]. Pangkep offers a relevant setting for such repeated multicompartmental surveillance.

Several limitations should frame interpretation. First, only three operational surveillance areas and 21 rats were included, producing imprecise site- and species-specific estimates and limiting external validity. Second, participating households were not selected through a documented probability-sampling procedure, and the fixed trapping effort was designed for surveillance rather than prevalence estimation. Third, the success-trap indicator used the programmatic denominator of deployed traps rather than trap-nights; it is appropriate for comparison with the national operational benchmark used in 2024 but should not be compared directly with studies using trap-night capture rates. Fourth, the source report did not document the qPCR gene target, Ct threshold, analytical sensitivity, or run-level quality-control findings, limiting interpretation of diagnostic performance and preventing inference about species/serovar or viability. Fifth, the survey represented one short time window, so seasonal dynamics could not be evaluated. Finally, no concurrent human or environmental samples were available, precluding direct linkage between rodent positivity and human disease.

Despite these constraints, the study has practical strengths. It combined standardized trapping, morphometric species identification, ectoparasite examination, georeferenced field activity, and kidney qPCR within a local public-health response. The manuscript also recalculated ratios from tabulated counts rather than reproducing inconsistent narrative summaries. For programmatic decision-making, the most defensible interpretation is therefore that all three areas had substantial rodent presence and at least one molecular-positive rat, warranting continued surveillance and control rather than a claim about population-level infection prevalence.

5 Conclusion

Rodent surveillance in Bowong Cindea, Biringkassi, and Jollo identified operational success-trap indices of 8-24%, all above the 1% benchmark applied in the 2024 program, and *Leptospira* sp. qPCR positivity in 7 of 21 rat kidneys (33.3%). Both *Rattus tanezumi* and *Rattus norvegicus* were molecular-positive, and positive rats occurred in every surveillance area. The findings support sustained integrated rodent management, environmental sanitation, strengthened clinical vigilance and laboratory diagnosis for human leptospirosis, and repeated One Health surveillance that incorporates rodent, human, and environmental samples. Future work should use probability-based or clearly defined sentinel sampling, standardized trap-night metrics alongside national program indicators, complete qPCR quality-assurance reporting, and molecular typing where feasible.

6 Declarations

Ethics approval and consent to participate

This work was derived from routine governmental rodent surveillance. The source report did not provide an animal ethics approval number, permit identifier, or formal exemption determination. The responsible institution must verify and supply the applicable animal-welfare and administrative approval information before submission.

Consent for publication

Not applicable; no identifiable human participant information is reported.

Funding

The surveillance activity was implemented by Balai Laboratorium Kesehatan Masyarakat Makassar in collaboration with local health authorities. The source report did not identify a specific external research grant.

Competing interests

Individual competing-interest declarations were not included in the source surveillance report and must be completed by all listed authors before submission.

Author contributions (CRediT)

Proposed authorship is based on the technical team named in the source report. Erlina Hamzah: conceptualization, methodology, investigation, data curation, writing - original draft, project administration. Devi Gusni Yanti: methodology, investigation, data curation

Data availability

Aggregated data supporting the findings are included in this article. Access to underlying field and laboratory surveillance records is subject to approval by the responsible public-health institution.

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Generative AI statement

Generative AI was used to assist with manuscript restructuring and language editing. Numerical results were checked against the source surveillance report and recalculated from its tabulated counts. The authors remain responsible for verifying data, citations, originality, interpretation, and the final submitted text.

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