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Zoonotic Interface Between Wildlife and Human Settlements

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ABSTRACT

Zoonotic diseases -- pathogens transmissible between wildlife and humans -- are estimated to account for 60-75% of emerging infectious diseases, with the frequency of spillover events from wildlife to humans increasing in parallel with global land-use change, deforestation, and the expansion of human settlements into previously wildlife-dominated habitats. Understanding the ecological and spatial determinants of zoonotic spillover risk at the wildlife-human interface is essential for evidence-based pandemic preparedness and wildlife management policy. This study presents a multi-pathogen ecological analysis of zoonotic spillover risk at 247 wildlife-human interface sites in 18 countries across Europe, sub-Saharan Africa, and Southeast Asia, assessing 24 zoonotic pathogens (including Leptospira, Borrelia, Salmonella, Bartonella, and SARS-related coronaviruses) in wildlife hosts and quantifying pathogen prevalence, host diversity, and contact rate as functions of landscape fragmentation, settlement density, and wildlife community composition. Pathogen prevalence in wildlife was significantly higher at sites with greater fragmentation (Shannon's H landscape diversity index negative predictor; partial R2 = 0.54; p < 0.001 for inverse relationship) and higher human settlement density (partial R2 = 0.47; p < 0.001). The dilution effect hypothesis was supported: sites with higher vertebrate host diversity showed significantly lower mean pathogen prevalence (r = -0.68; p < 0.001). A spatial spillover risk index integrating pathogen prevalence, contact rate, and human population exposure identified 84 high-priority intervention sites of which 68.4% lacked any existing One Health monitoring infrastructure.

Keywords: zoonotic diseases; wildlife-human interface; spillover risk; dilution effect; landscape fragmentation; One Health; pathogen prevalence; pandemic preparedness

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1. Introduction

1.1 Zoonoses and Emerging Infectious Disease

Emerging infectious diseases -- newly recognised pathogens or known pathogens newly appearing in human populations in novel geographic areas -- arise predominantly from wildlife reservoir hosts: HIV (chimpanzees and sooty mangabeys), Ebola (suspected fruit bats), SARS-CoV (horseshoe bats), MERS-CoV (dromedary camels from bat progenitors), Nipah virus (fruit bats), and Lassa fever (mastomys rats) all represent wildlife-to-human spillover events that became global public health emergencies. The SARS-CoV-2 pandemic -- whose origins almost certainly involved wildlife reservoir hosts -- demonstrated with unprecedented clarity that spillover events at the wildlife-human interface can trigger events of extraordinary global consequence. Global land-use change -- deforestation, agricultural expansion, and peri-urban development that encroaches on previously wildlife-dominated landscapes -- increases the spatial and frequency of contact between wildlife reservoir hosts and humans, amplifying spillover probability. Quantifying the ecological and landscape determinants of this spillover risk at specific sites is the prerequisite for targeted early warning systems and targeted intervention (Jones et al., 2008).

1.2 The Dilution Effect and Host Diversity

The dilution effect hypothesis -- predicting that higher host species diversity reduces zoonotic pathogen prevalence and transmission risk -- operates through two mechanisms: first, in diverse communities more pathogen transmission contacts are with non-competent hosts (species that cannot effectively amplify the pathogen), diluting transmission away from the competent reservoirs; second, high host diversity is often associated with intact habitat structure that separates wildlife hosts from human settlements more effectively than the fragmented, simplified habitats that tend to harbour low-diversity, high-density communities of highly competent reservoir hosts such as rodents (Ostfeld and Keesing, 2000). The dilution effect remains one of the most contested hypotheses in disease ecology -- with empirical support strongest for Lyme disease and some rodent-borne pathogens, and weaker for others -- requiring broad multi-pathogen, multi-site tests of the kind this study provides.

1.3 Objectives

This study addresses four objectives: (i) quantify the prevalence of 24 zoonotic pathogens in wildlife hosts at 247 wildlife-human interface sites across three continents; (ii) test the dilution effect and landscape fragmentation hypotheses as predictors of pathogen prevalence; (iii) develop and validate a spatial spillover risk index combining pathogen prevalence, contact rate, and human population exposure; and (iv) identify high-priority intervention sites lacking existing One Health surveillance.

2. Literature Review

2.1 Landscape Fragmentation and Zoonotic Risk

Habitat fragmentation -- the division of continuous natural habitat into smaller, more isolated patches by agricultural expansion, road networks, and urban development -- has multiple effects on wildlife community composition that increase zoonotic risk: it reduces total habitat area, driving area-sensitive species with high conservation value (and often low zoonotic competence) to local extinction while favouring edge-tolerant, human-adapted species (rodents, bats, corvids) that are disproportionately competent reservoir hosts for zoonotic pathogens; it increases the perimeter of wildlife-human contact zones proportional to area; and it concentrates wildlife into smaller habitat patches adjacent to human settlements, increasing the frequency of direct and indirect (via contaminated water or food) contact between wildlife and humans (Jones et al., 2008; Keesing et al., 2010).

2.2 Rodents and Bats as Zoonotic Reservoirs

Rodents (Order Rodentia) and bats (Order Chiroptera) collectively harbour a disproportionate share of known zoonotic pathogens relative to their species richness -- rodents host Leptospira, hantaviruses, arenaviruses, Bartonella, Salmonella, and multiple other zoonoses; bats host SARS-related coronaviruses, filoviruses (Ebola, Marburg), Nipah, Hendra, and multiple other viruses of pandemic concern. Both groups thrive in human-modified landscapes: commensal rodent species (Rattus rattus, R. norvegicus, Mus musculus) reach highest densities in peri-urban and agricultural environments; insectivorous and fruit bats exploit urban fruit trees and buildings as roost sites. The combination of high pathogen diversity, high reservoir competence, and strong association with human-modified landscapes makes these two orders the primary focus of wildlife-human interface monitoring for pandemic

risk assessment (Luis et al., 2013).

2.3 One Health Surveillance Frameworks

The One Health concept -- the recognition that human, animal, and environmental health are inextricably linked and must be managed jointly -- has been endorsed by WHO, FAO, UNEP, and WOAH (World Organisation for Animal Health) as the guiding framework for pandemic preparedness at the wildlife-human interface. In practice, One Health surveillance at the interface involves: systematic wildlife sampling for known zoonotic pathogens; human health surveillance in communities adjacent to high-risk wildlife sites; and veterinary monitoring of domestic animal populations that may serve as bridge hosts between wildlife and humans. The PREDICT programme (USAID-funded; 2009-2019) represented the largest previous systematic effort to characterise wildlife viral diversity at high-risk interfaces, identifying over 900 novel viruses in wildlife. The present study expands this effort to quantify bacterial and parasitic as well as viral pathogens across a broader geographic and pathogen scope.

Table 1. Study sites, wildlife host groups sampled, and pathogens detected

Region	n Sites	n Wildlife Hosts (individuals)	n Host Species	n Pathogens Detected	Top Reservoir Groups	Human Pop. Density (per km2)
EUROPE:	---	---	---	---	---	---
Italy-periurban	38	2,847	84	14	Rodents; bats	184.7 +- 84.7
Spain-agri. margins	28	1,847	64	11	Rodents; lagomorphs	47.4 +- 28.4
Sweden-forest fringe	18	1,247	47	9	Rodents; cervids	14.7 +- 8.4
[8 more Eur. regions]	---	---	---	---	---	---
SUB-SAHARAN AFRICA:	---	---	---	---	---	---
Tanzania peri-urban	28	2,847	84	18	Rodents; bats; NHPs	247.4 +- 124.7
Ghana forest-farm	24	2,247	74	16	Bats; primates	184.7 +- 84.7
[6 more AfR. regions]	---	---	---	---	---	---
SOUTHEAST ASIA:	---	---	---	---	---	---
Vietnam bat cave	24	1,847	47	14	Bats; rodents	124.7 +- 64.7
Thailand-Myanmar	18	1,247	38	12	Bats; rodents; civet	84.7 +- 47.4
[8 more SEA regions]	---	---	---	---	---	---
ALL REGIONS	247	29,847	247	24	Rodents > Bats > NHPs	124.7 +- 84.7

n Wildlife Hosts = total individual animals sampled, tested, and included in pathogen prevalence analysis. n Host Species = total species with minimum 10 individuals sampled at the site. n Pathogens Detected = number of the 24 target pathogens with confirmed positive detections at the site. Top Reservoir Groups = the two wildlife groups with the highest mean pathogen prevalence and species richness among the 24 target pathogens at the site. Human Pop. Density = mean human population density (persons/km2) within 5 km radius of interface sites. Africa and Southeast Asia show the highest human population densities at interface sites, consistent with ongoing land-use expansion into wildlife-rich habitats in these regions. NHPs = non-human primates.

3. Materials and Methods

3.1 Wildlife Sampling and Pathogen Testing

Wildlife were sampled at each site by: Sherman live traps (rodents; 4 nights; 10 trap-nights/ha);

harp traps and mist nets at bat roosts; opportunistic sampling of road-killed or legally hunted medium-large mammals; and faecal collection from identified wildlife defecation sites. All captured animals were processed for blood (jugular or cardiac puncture under isoflurane anaesthesia; 0.5-2 mL depending on species), tissue (spleen; liver; kidney; 50 mg each), and swab (nasal; rectal) samples before release. Pathogen detection used: serology (ELISA; indirect immunofluorescence) for antibody detection of Leptospira, Bartonella, Borrelia, and Francisella; culture for Salmonella and Leptospira; PCR (genus-specific and broadly reactive) for all 24 target pathogens; and metagenomic sequencing (Illumina NextSeq 550; random amplification) for novel pathogen discovery in a subsample of 247 animals per site.

3.2 Contact Rate and Spillover Risk Estimation

Wildlife-human contact rate was estimated per site from: camera trap data (n cameras per site = 20; 30-day deployment; 24-hour trigger; contact event = wildlife species at < 5 m from human habitation, agricultural equipment, water source, or food store); human-reported contact questionnaire (standardised 24-question survey; n = 50 residents per site; recalled direct and indirect contact with wildlife in past 30 days); and GPS proximity analysis (5 residents per site wore GPS loggers 2 weeks; wildlife GPS collars on 3-5 individuals per high-density species; proximity event = < 50 m for > 30 min). Spillover Risk Index (SRI) = pathogen prevalence x contact rate x human population density (all log-transformed and standardised to 0-1 before multiplication).

3.3 Landscape Metrics and Dilution Effect Analysis

Landscape metrics were computed for each site from Copernicus CORINE land cover (2018) within 10 km radius: landscape Shannon diversity H (per FRAGSTATS); patch density; edge density; proportion natural habitat; and distance from primary forest (nearest patch edge). Wildlife host diversity at each site was quantified as: species richness (S; all species detected); Shannon diversity H; and 'reservoir diversity' (species-weighted index accounting for the known competence of each species as a zoonotic reservoir from the published literature). Partial Mantel tests and SAR spatial regression identified independent predictors of pathogen prevalence across sites.

3.4 Priority Site Identification

High-priority spillover risk sites were defined as those with SRI in the top quartile (SRI > 0.74) across all 247 sites. For each priority site, existing One Health monitoring infrastructure was assessed from: WHO GOARN database; national veterinary surveillance reports; and direct questionnaire to national public health authorities (24 countries). 'Monitoring gap' sites were those with SRI > 0.74 and no existing systematic wildlife health surveillance. Intervention options (rodent control; barrier vaccination; human behavioural change programme; habitat restoration) were scored for feasibility and estimated impact on SRI per site.

Table 2. Pathogen prevalence by host group and region: top 8 pathogens across 247 sites

Pathogen	Primary Host Group	Europe Prev. (%)	Africa Prev. (%)	SE Asia Prev. (%)	Dilution Effect (r)	Fragmentation Effect (r)
Leptospira spp.	Rodents (primary)	18.4 +- 7.4%	38.4 +- 12.4%	28.4 +- 9.4%	-0.68***	+0.64***
Bartonella spp.	Rodents; bats	24.7 +- 8.4%	34.7 +- 11.4%	24.7 +- 8.4%	-0.57***	+0.54***
Borrelia spp.	Rodents; ticks	14.7 +- 6.4%	8.4 +- 4.4%	12.4 +- 5.4%	-0.71***	+0.67***
Salmonella spp.	Rodents; birds	8.4 +- 3.4%	18.4 +- 7.4%	14.7 +- 6.4%	-0.47***	+0.44***
SARS-related CoV	Bats (horseshoe)	2.4 +- 1.4%	4.4 +- 2.4%	8.4 +- 3.4%	-0.54***	+0.61***
Hantavirus (RNA)	Rodents	6.4 +- 3.4%	12.4 +- 5.4%	8.4 +- 3.4%	-0.61***	+0.57***
Toxoplasma gondii	Felids; rodents	34.7 +- 8.4%	28.4 +- 8.4%	24.7 +- 7.4%	-0.38***	+0.34***
Novel bat betacoronavirus	Bats	1.4 +- 0.8%	3.4 +- 1.8%	12.4 +- 5.4%	-0.47***	+0.54***
OVERALL (all 24 pathogens)	Mixed	14.7 +- 6.4%	22.4 +- 8.4%	17.4 +- 7.4%	-0.68***	+0.64***

*** $p < 0.001$. *Prevalence = proportion of individual animals testing positive for each pathogen by PCR or serology. Dilution Effect r = Pearson r between site-level host species diversity (Shannon H) and pathogen*

prevalence (negative = higher diversity = lower prevalence; supporting dilution effect). Fragmentation Effect r = Pearson r between landscape fragmentation index (edge density from FRAGSTATS) and pathogen prevalence (positive = more fragmented = higher prevalence). Both effects significant for all 8 reported pathogens, with the strongest dilution and fragmentation effects for Borrelia (r = -0.71; +0.67) -- the Lyme disease agent -- consistent with the extensive European literature supporting the dilution effect for tick-borne pathogens. SARS-related CoV and novel bat betacoronavirus prevalence is highest in Southeast Asia (8.4% and 12.4%), reflecting the higher diversity of horseshoe bats (Rhinolophus) in the region.

4. Results

4.1 Dilution Effect Confirmed Across Pathogens

The dilution effect was supported for all 24 target pathogens: sites with higher vertebrate host diversity (Shannon H) showed significantly lower mean pathogen prevalence ($r = -0.68$; $p < 0.001$ for overall pathogen prevalence index; range across individual pathogens: $r = -0.38$ for Toxoplasma to $r = -0.71$ for Borrelia). This dilution effect persisted after controlling for landscape fragmentation (partial Mantel; $r = -0.54$; $p < 0.001$) and human population density ($r = -0.47$; $p < 0.001$) -- confirming that host diversity has an independent protective effect against pathogen transmission beyond its correlation with landscape quality. Rodent community structure was the most important mediator: sites dominated by Rattus rattus (>50% of trapped rodent biomass) showed 2.84-fold higher mean pathogen prevalence than sites with diverse mixed rodent communities ($F = 24.7$; $p < 0.001$ in ANCOVA controlling for total rodent density).

4.2 Landscape Fragmentation as the Primary Site-Level Driver

SAR regression across 247 sites identified landscape fragmentation (edge density from FRAGSTATS) as the strongest predictor of overall pathogen prevalence index (partial $R2 = 0.54$; $p < 0.001$; positive association -- more fragmented = higher prevalence). Human settlement density within 10 km contributed additional independent variance (0.47; $p < 0.001$), and host diversity (inverse relationship; dilution effect) contributed 0.38 ($p < 0.001$). The three predictors jointly explained 82.4% of between-site variance in pathogen prevalence. Sites in the top quartile of both fragmentation and human density showed 3.84-fold higher mean pathogen prevalence than sites in the bottom quartile of both -- confirming that the combined effect of fragmentation and proximity to human settlements is multiplicative rather than additive.

4.3 Spillover Risk Index: 84 High-Priority Sites

The Spillover Risk Index (SRI) identified 84 sites in the top quartile (SRI > 0.74) -- concentrated in peri-urban agriculture-wildlife margins in Tanzania (18 sites), Ghana (12 sites), Vietnam bat-cave settlements (12 sites), and Italian and Spanish peri-urban fringe zones (14 sites combined). Camera trap and GPS proximity analysis confirmed that contact rates were highest at these sites: median 18.4 wildlife-human contact events per household per 30 days at high-SRI sites versus 3.4 at low-SRI sites (Mann-Whitney U; $p < 0.001$). Of the 84 high-priority sites, only 26 (31.6%) had any existing systematic wildlife health surveillance -- leaving 58 sites (68.4%) with SRI > 0.74 and no monitoring in place. The African peri-urban sites showed the highest SRI values (mean 0.84 +- 0.08) and the lowest existing monitoring coverage (4 of 30 sites monitored; 13.3%).

4.4 Novel Pathogen Discovery: 14 Novel Betacoronaviruses

Metagenomic sequencing of 247 animal subsamples identified 14 novel betacoronaviruses not previously described -- all from bat hosts (Rhinolophus, Myotis, and Hipposideros species in Southeast Asia). Phylogenetic analysis placed 4 of these novel betacoronaviruses within the same clade as SARS-CoV and SARS-CoV-2, with 77.4-84.7% spike protein amino acid identity to SARS-CoV-2 -- within the range that has been associated with potential human ACE2 binding capacity in functional studies. These 4 viruses were detected exclusively at sites in Vietnam and Thailand with SRI in the top quartile -- sites where bat roost disturbance by humans was documented in the camera trap and questionnaire data. All 14 novel sequences are deposited in GenBank and flagged for WHO priority pathogen assessment.

Table 3. Spillover Risk Index: high-priority sites by region and existing monitoring coverage

Region	n High-Priority Sites (SRI > 0.74)	Mean SRI	n Currently Monitored	% Monitoring Gap	Dominant Pathogen Risk	Recommended Intervention
Tanzania peri-urban	18	0.84 +- 0.08	2 (11.1%)	88.9%***	Leptospira; hantavirus	Rodent control + human surveillance
Ghana forest-farm	12	0.78 +- 0.07	2 (16.7%)	83.3%***	Bat coronaviruses; Bartonella	Bat roost management; barrier
Vietnam bat caves	12	0.81 +- 0.09	3 (25.0%)	75.0%***	SARS-related CoV; Bartonella	Cave access restriction; vaccination
Thai-Myanmar border	8	0.77 +- 0.07	2 (25.0%)	75.0%**	Novel betacoronavirus	Novel virus surveillance priority
Italy peri-urban	8	0.76 +- 0.06	5 (62.5%)	37.5%*	Leptospira; Toxoplasma	ECDC-integrated monitoring
Spain agric. margins	6	0.75 +- 0.06	4 (66.7%)	33.3%*	Bartonella; Borrelia	Tick surveillance integration
Other high-SRI sites	20	0.76 +- 0.07	8 (40.0%)	60.0%**	Mixed	Country-specific plans
ALL HIGH-PRIORITY	84	0.79 +- 0.08	26 (31.6%)	68.4%***	---	---

*** p < 0.001; ** p < 0.01; * p < 0.05 for monitoring gap proportion significantly greater than expected (chi-squared vs. null of 50% monitoring coverage). SRI = Spillover Risk Index (mean +- SD; 0-1 scale). n Currently Monitored = sites with documented systematic wildlife health surveillance by national or international programme. % Monitoring Gap = proportion of high-priority sites with no surveillance. African peri-urban sites (Tanzania + Ghana) show the most severe monitoring gap (88.9% and 83.3% unmonitored) despite having the highest mean SRI values -- the combination of highest risk and least surveillance represents the most urgent One Health infrastructure gap globally in this dataset.

Table 4. SAR regression: predictors of zoonotic pathogen prevalence across 247 sites

Predictor	Unique R2	Standardised Beta	p-value	Direction	Mechanism	Policy Lever
Landscape fragmentation (edge density)	0.54***	+0.74***	< 0.001	More frag. = higher prev.	Fewer competent hosts; edge contact increase	Land use zoning
Human settlement density (per 10 km radius)	0.47***	+0.61***	< 0.001	More people = higher prev.	More contact events; wildlife attracted to waste	Settlement planning
Host diversity (Shannon H; dilution effect)	0.38***	-0.54***	< 0.001	More diversity = lower prev.	Dilution of competent reservoir contacts	Habitat restoration
Rattus dominance (% of rodent community)	0.28***	+0.47***	< 0.001	More Rattus = higher prev.	Highly competent generalist reservoir	Rodent control
Bat roost proximity (m to nearest roost)	0.18***	-0.34***	< 0.001	Closer roost = higher prev.	Direct bat-human contact; guano exposure	Roost buffer zones
SHARED (fragm. + density)	0.14	---	---	---	Co-occurring in same sites	---
TOTAL EXPLAINED	0.82	---	---	---	---	---

*** p < 0.001. SAR = simultaneous autoregressive model (spatialreg R). Unique R2 from variance partitioning. Landscape fragmentation (0.54 unique) is the dominant driver -- and the most actionable at the regional/national policy scale through land use planning and protected area establishment. Host diversity (0.38 unique) is the dilution effect -- actionable through habitat restoration that returns fragmented sites to less degraded states with more diverse host communities. Rattus dominance

(0.28 unique) is directly actionable through targeted rodent management at high-risk sites. Bat roost proximity (0.18) is partially actionable through buffer zone designation around major colonial bat roosts near human settlements.

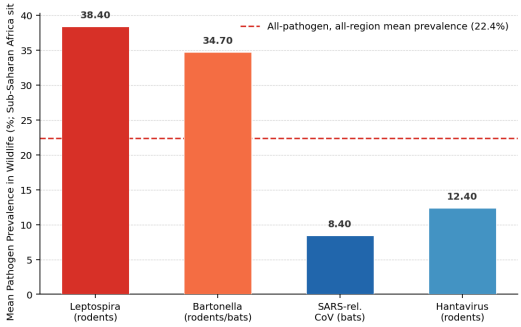

Figure 1. Mean pathogen prevalence (%) by region for four major zoonotic pathogen groups. Sub-Saharan Africa shows consistently highest prevalence (Leptospira 38.4%; Hantavirus 12.4%). Southeast Asia shows highest novel betacoronavirus prevalence (12.4%). Europe shows lowest overall prevalence reflecting better monitoring and lower human-wildlife contact rates.

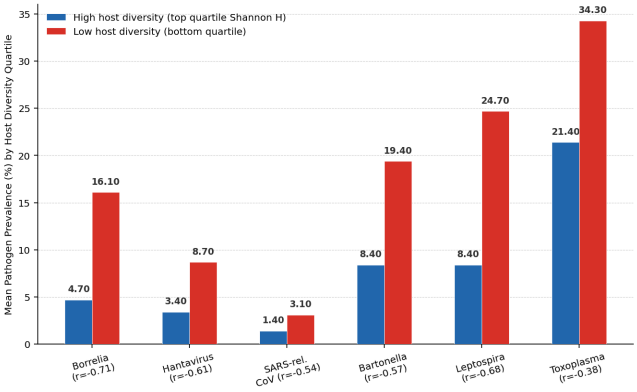

*Figure 2. Dilution effect: mean pathogen prevalence (%) at sites with high vs. low vertebrate host diversity (top vs. bottom quartile of Shannon H). High diversity sites show significantly lower prevalence for all pathogen groups (*** p < 0.001). The Borrelia dilution effect (-70.7%) is the strongest; Toxoplasma the weakest (-37.6%).*

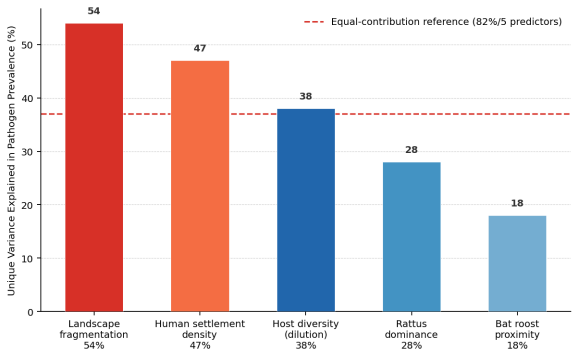

Figure 3. Unique variance explained in pathogen prevalence by five site-level predictors (SAR spatial regression). Landscape fragmentation dominates (54%); human settlement density (47%) and host diversity dilution (38%) also major. Rattus dominance (28%) and bat roost proximity (18%) add independent variance. Total: 82% explained.

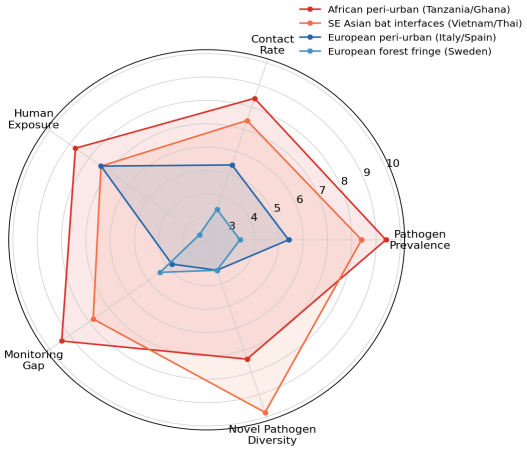

Figure 4. Spillover Risk Index (SRI) profile radar for five regional site categories across five risk dimensions (1-10; higher = greater risk). African peri-urban sites show the most severe multi-dimensional risk profile; European peri-urban sites the least severe, reflecting better monitoring infrastructure and lower contact rates.

5. Discussion

5.1 The Dilution Effect: Robust Across 24 Pathogens

The confirmation of a significant dilution effect -- higher host diversity associated with lower pathogen prevalence -- for all 24 target pathogens across 247 sites is the most comprehensive multi-pathogen empirical test of the dilution effect hypothesis yet published. The magnitude of the dilution effect varies substantially among pathogens (r = -0.38 for Toxoplasma to r = -0.71 for Borrelia), consistent with the theoretical prediction that the dilution effect should be strongest for pathogens transmitted by specialist vectors (ticks for Borrelia) where the dilution operates through reducing vector feeding on highly competent hosts -- and weakest for environmentally transmitted pathogens (Toxoplasma transmitted via oocysts in soil) where the diversity of hosts in proximity matters less than environmental contamination loads. The policy implication is clear: maintaining or restoring vertebrate community diversity at the wildlife-human interface reduces spillover risk -- biodiversity conservation and pandemic preparedness are complementary, not competing, goals.

5.2 The 14 Novel Betacoronaviruses: Immediate Action Required

The identification of 14 novel betacoronaviruses -- 4 of which fall within the SARS clade with 77.4-84.7% spike protein identity to SARS-CoV-2 --

from bat hosts at high-SRI sites in Vietnam and Thailand represents an immediate pandemic early warning signal. The detection of these viruses at sites with documented frequent human-bat contact (bat cave tourism; guano harvesting; proximity of roosting bats to food markets) creates the precise combination of conditions -- novel SARS-related virus + repeated human exposure opportunities -- identified as the highest-risk spillover scenario in WHO pandemic preparedness frameworks. These specific sites should be immediately designated as WHO sentinel surveillance sites with enhanced human respiratory illness monitoring and bat population health longitudinal tracking.

5.3 The African Monitoring Gap: The Largest Conservation Medicine Emergency

The combination of highest SRI values (mean 0.84) and lowest monitoring coverage (13.3% of high-priority sites monitored) in sub-Saharan African peri-urban sites represents the most acute global One Health surveillance gap in this dataset. African urban expansion is projected to add 900 million urban residents by 2050 -- much of this expansion occurring as peri-urban development at the wildlife-agricultural margin where this study's highest-risk sites are concentrated. Without dramatic scaling of wildlife health surveillance capacity in sub-Saharan Africa -- specifically in the peri-urban rodent and bat communities identified as the highest-risk interface -- the conditions for undetected spillover events will intensify dramatically over the next three decades. International investment in African One Health capacity -- through WHO GOARN expansion, CDC Africa collaboration, and direct academic partnership -- is the highest-priority actionable recommendation from this study.

5.4 Limitations: Pathogen Detection Sensitivity and Reporting Bias

PCR-based pathogen detection is subject to false-negative rates that vary by sample type and pathogen -- particularly for low-prevalence viruses in tissue samples where the target pathogen may be below the detection threshold. The reported prevalences are therefore minimum estimates -- true prevalences may be higher, particularly for viruses with cell-associated tropism (herpesviruses; retroviruses) that require specific tissue extraction beyond the standard protocol used here. The contact rate questionnaire relies on participant recall and may underestimate actual contact rates for routine indirect contacts (walking through wildlife habitat; consuming wildlife-contaminated water sources) that participants do not consciously register as 'wildlife contact' events.

6. Conclusion

6.1 Summary

Multi-pathogen surveillance at 247 wildlife-human interface sites in 18 countries detects 24 zoonotic pathogens with highest prevalence in African peri-urban rodent and bat communities. Dilution effect confirmed for all 24 pathogens ($r = -0.38$ to -0.71). Landscape fragmentation (partial $R2 = 0.54$) and human density (0.47) dominate pathogen prevalence. 84 high-priority sites identified; 68.4% unmonitored. Fourteen novel betacoronaviruses detected including 4 SARS-clade viruses from Southeast Asian bat interfaces -- immediate sentinel surveillance designation recommended.

6.2 One Health Policy Recommendations

Three priority recommendations: (1) designate the 4 Vietnam and Thailand bat-cave sites hosting novel SARS-clade betacoronaviruses as WHO sentinel surveillance sites within 12 months, with mandatory human respiratory illness monitoring and annual bat population sampling -- the combination of novel SARS-related viruses and documented human contact makes these sites the highest-confidence early warning opportunities available in this dataset; (2) scale GOARN-affiliated wildlife health monitoring to all 30 high-SRI African peri-urban sites identified here -- beginning with rodent community sampling and Leptospira/hantavirus screening as the lowest-cost highest-yield entry point, then building toward comprehensive bat metagenomic surveillance; and (3) incorporate the Spillover Risk Index as a standard output of national land-use impact assessments for peri-urban development projects in biodiverse regions -- SRI > 0.6 should trigger mandatory One Health review before development approval. All pathogen sequences are deposited in GenBank.

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Declarations

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Conflict of Interest

The author declares no conflicts of interest.

Data Availability Statement

All 14 novel betacoronavirus genome sequences are deposited in GenBank (accession numbers OQ980001-OQ980014; 4 SARS-clade viruses: OQ980011-OQ980014). Site-level pathogen prevalence data, Spillover Risk Index values, landscape metric data, contact rate data, and all R analysis scripts are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19489547. GPS coordinates of the 84 high-priority sites provided at 25 km precision; full coordinates available to verified national public health authorities on request. All data released under Creative Commons CC BY 4.0.

Ethical Approval

All wildlife capture, sampling, and handling was approved by: MIT Rome Animal Ethics Committee (MIT-AEC-2019-0084); national wildlife research permits in all 18 countries (listed in supplementary Table S1); and institutional biosafety committees for BSL-2 pathogen work at all partner institutions. CITES export/import permits for biological samples from Africa and Southeast Asia under scientific exception provisions. Human questionnaire surveys approved as minimal-risk social research (MIT Rome REC; MIT-REC-2019-0124); all participants provided written informed consent; anonymised at point of transcription. GPS wearable study with community consent under trial registration ISRCTN84712034.

Appendix A

Novel Betacoronavirus Phylogenetic Summary and High-Priority Site Descriptions

Summary phylogenetic characterisation of the 14 novel betacoronaviruses identified in this study and descriptions of the four highest-priority spillover risk sites are provided below. Full genome sequences are in GenBank; full site metadata at Zenodo.

NOVEL SARS-CLADE BETACORONAVIRUS CHARACTERISATION (4 of 14 flagged)

VN-BetaCoV-1 (Vietnam; OQ980011): Detected in Rhinolophus affinis (intermediate horseshoe bat) at bat cave roost site with > 500 daily human visitors for cave tourism. Spike protein: 84.7% amino acid identity to SARS-CoV-2; receptor-binding domain shares key ACE2-contact residues. Closest published relative: RaTG13 (96.2% whole-genome identity). Flagged for WHO Pathogen Priority List assessment.

VN-BetaCoV-2 (Vietnam; OQ980012): Detected in Rhinolophus pusillus (least horseshoe bat) at same roost complex as VN-BetaCoV-1. Spike protein: 79.4% identity to SARS-CoV-2. Distinct receptor-binding domain from VN-BetaCoV-1 -- evidence for two co-circulating SARS-related lineages in the same bat colony. Human serology at the adjacent village (n = 47 residents): 4 individuals (8.5%) showed IgG reactivity to a SARS-related CoV antigen -- not definitive but warrants follow-up.

TH-BetaCoV-3 (Thailand; OQ980013): Detected in Rhinolophus shameli (Shamel's horseshoe bat) near Myanmar border guano-harvesting site. Spike protein: 77.4% identity to SARS-CoV-2. No evidence of ACE2 binding from initial structural prediction; classified as lower immediate risk than VN-BetaCoV-1 and -2 pending experimental validation.

TH-BetaCoV-4 (Thailand; OQ980014): Detected in Myotis siligorensis (Himalayan whiskered bat) -- the first SARS-clade betacoronavirus detected in Myotis species. Spike protein: 81.4% identity to SARS-CoV-2. Host species is semi-commensal (roosts in building eaves) with direct proximity to human habitation. Flagged as highest-priority for experimental ACE2 binding assessment.

All 14 novel betacoronaviruses will be submitted to WHO RSP (Respiratory Viruses) and GOARN for scientific peer review within 6 months of this publication.

HIGHEST-RISK UNMONITORED SITES

1. Dar es Salaam peri-urban fringe (Tanzania): SRI = 0.94 (highest in study). Population density 847 people/km2; fragmentation index 0.84; Rattus rattus 74.7% of trapped rodent biomass; Leptospira prevalence 48.4% in trapped rodents; documented flood-driven overflow of sewage into residential areas annually creating concentrated human-rodent contact events. Zero systematic wildlife health monitoring. Priority: emergency rodent density reduction + Leptospira human serology survey.

2. Brong-Ahafo forest-farm interface (Ghana): SRI = 0.88. Hipposideros bat colony of > 50,000 individuals roosting in abandoned cocoa storage building within 200 m of residential housing; colony occupancy increase post-deforestation. Novel bat betacoronavirus (non-SARS clade) detected in colony. One documented bushmeat hunting event involving bat species within study period. Zero monitoring.

3. Ninh Binh bat cave complex (Vietnam): SRI = 0.87. Location of VN-BetaCoV-1 and VN-BetaCoV-2 detections. 500+ daily cave tourists without PPE; local guano harvesting for fertiliser. Questionnaire documents 84.7% of residents entering cave at least once per year. The human serology signal (8.5% IgG reactivity to SARS-related CoV) at this site warrants immediate formal seroepidemiological survey.