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Genomic Surveillance of Emerging and Re-Emerging Viral Diseases: Molecular Evolution, Transmission Dynamics, and Antiviral Resistance in Pakistan

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ABSTRACT

Pakistan carries an unusual concentration of viral risk. It is one of only two countries where wild poliovirus has never been stopped, its dengue seasons turn over serotype from one year to the next, its Crimean-Congo haemorrhagic fever is endemic and actively reassorting, its hepatitis C burden is the second largest in the world, and its respiratory season now imports globally circulating influenza clades within weeks of their appearance elsewhere. What the country's genomic laboratories have found out about these viruses has never been pulled together in one place. This paper does that, synthesising the published genomic surveillance evidence for Pakistan from 2020 to mid-2026 across six priority viral systems and asking what it shows about molecular evolution, transmission and antiviral resistance. We quantified sequencing coverage from the primary studies, and it varies by two orders of magnitude between pathogens, from 6.3 per cent of confirmed detections in the 2024 national dengue outbreak to 71 per cent in a single well-resourced Crimean-Congo investigation. That range has little

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to do with which pathogen mattered most and almost everything to do with which outbreak happened to attract a funded study. Three transmission signatures recur across viruses that share nothing else. Importation across borders dominates: wild poliovirus type 1 clusters YB3A and YB3C move within the Pakistan-Afghanistan block, Crimean-Congo strains group with Iranian, Afghan and Indian isolates, and dengue lineages arrived from Southeast Asia. Cryptic local circulation then follows, with time-scaled analyses dating the common ancestor of currently circulating dengue lineages to 2016 and 2018. And environmental sampling repeatedly detects more than clinical reporting does, never more starkly than in 2025, when poliomyelitis cases fell 58 per cent even as wild poliovirus-positive sewage samples climbed to 651. Antiviral resistance is the weakest link of all. Resistance-associated substitutions of clear clinical importance, among them NS5A Y93H and A30K in hepatitis C genotype 3a and neuraminidase E50K and R150H in influenza A, have been documented in Pakistani isolates, yet the national programme runs no routine genotypic resistance testing for any virus. We set out where the surveillance chain loses information and what a proportionate national strategy would fix first.

Keywords: Genomic Surveillance; Molecular Epidemiology; Viral Evolution; Antiviral Resistance; Poliovirus; Dengue Virus; Crimean-Congo Haemorrhagic Fever Virus; Influenza; Pakistan

Introduction

Genomic surveillance changed status during the COVID-19 pandemic. Before 2020 it was a research activity that occasionally informed public health decisions; after 2020 it became an expected component of outbreak response, and the sequencing capacity built during the pandemic left many countries with instruments they had not previously possessed. Pakistan was among them. The Department of Virology at the National Institute of Health in Islamabad, which had long served as a WHO Regional Reference Laboratory for poliovirus, acquired high-throughput and nanopore sequencing capability that was subsequently applied to dengue, Crimean-Congo haemorrhagic fever, influenza, respiratory syncytial virus and mpox.

What has not followed is any coherent account of what that capacity has revealed. The published output is substantial but scattered: one-off outbreak investigations, single-season respiratory panels, retrospective analyses of sequences already sitting in GISAID, and pathogen-specific programme reporting that seldom refers to any of the rest. Each study is sound on its own terms, but nobody has yet read them against one another.

This matters for three reasons. First, several of the transmission signatures visible in the Pakistani data are shared across pathogens with nothing else in common, which suggests they are properties of the country's epidemiological geography rather than of any particular virus, and that observation is invisible from within a single-pathogen study. Second, the enormous variation in sequencing coverage between pathogens is not the result of any explicit prioritisation; it is an artefact of which outbreaks happened to attract a funded investigation. Third, antiviral resistance, which is nominally within the remit of genomic surveillance, has fallen almost entirely outside it in practice, at a

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moment when Pakistan is running one of the largest hepatitis C treatment programmes in the world.

This paper therefore synthesises the published genomic surveillance evidence for Pakistan between 2020 and mid-2026 across six viral systems: SARS-CoV-2, poliovirus, dengue virus, Crimean-Congo haemorrhagic fever virus, the respiratory virus panel of influenza and respiratory syncytial virus, and the hepatitis viruses. For each it examines what the sequence data establish about molecular evolution and transmission, and it then draws the cross-cutting comparisons that the primary literature does not attempt. Four questions organise the work. What do the genomic data show about how viruses enter and persist in Pakistan? How much of the circulating diversity is actually being observed, and with what coverage? What is known about antiviral resistance in Pakistani isolates, and how does that compare with what is tested for? And where in the surveillance value chain is information being lost?

The work is a synthesis of published and publicly reported data. It generates no new sequences and reports no new isolates; its contribution is analytical rather than empirical. The quantitative coverage comparison in Section 6, the cross-pathogen transmission analysis in Section 7 and the resistance-gap analysis in Section 5 are, as far as we can find, not available elsewhere.

Materials and Methods

Design

This is a structured narrative synthesis with quantitative secondary analysis. A systematic review protocol with formal risk-of-bias scoring was not appropriate, because the included studies are heterogeneous in design (outbreak investigations, sentinel surveillance, retrospective database analyses and programme reporting) and none is a comparative effectiveness study. The synthesis instead follows a defined search and extraction procedure, reported below, with narrative integration and descriptive quantitative comparison across studies.

Search strategy and inclusion

PubMed, Web of Science and Google Scholar were searched for the period January 2020 to August 2026, combining pathogen terms (SARS-CoV-2, poliovirus, dengue, Crimean-Congo, influenza, respiratory syncytial virus, hepatitis B, hepatitis C, mpox) with method terms (genomic surveillance, whole-genome sequencing, phylogenetic, molecular epidemiology, resistance-associated substitution) and the country term Pakistan. Programme and agency sources were searched separately: World Health Organization polio International Health Regulations Emergency Committee statements and Eastern Mediterranean Regional Office polio bulletins, the National Institute of Health Pakistan Integrated Disease Surveillance and Response bulletins, and Global Polio Eradication Initiative reporting.

Studies were included if they reported viral sequence data from specimens collected in Pakistan, or analysed Pakistani sequences deposited in GISAID or GenBank, and reported lineage, genotype, clade or substitution-level findings. Studies reporting only serological or antigen-based results were excluded, as were case reports without sequence data. Where the same specimens were reported in both a preprint and a

subsequent peer-reviewed article, the peer-reviewed version was used.

Data extraction and quantitative analysis

From each included study the following were extracted where reported: pathogen, collection period, geographical coverage, number of specimens tested, number laboratory-confirmed, number sequenced, sequencing platform, lineage or genotype assignment, substitutions of functional or clinical interest, and any time-scaled or phylogeographic inference. Sequencing coverage was then computed as the number of genomes generated divided by the number of laboratory-confirmed detections in the same study, expressed as a percentage. This ratio is the principal derived quantity in the paper and is presented in Figure 3.

Two cautions attach to that ratio and should be carried through the rest of the paper. It is a study-level rather than a system-level measure, so a small, intensive outbreak investigation will show high coverage while contributing few genomes in absolute terms; the Crimean-Congo 2023 investigation is exactly such a case. And the denominator is confirmed detections rather than estimated infections, so it describes what proportion of what was found was sequenced, not what proportion of what circulated was observed. The latter would be far smaller for every pathogen considered.

Limitations of the evidence base

Three features of the underlying literature constrain what any synthesis of it can say. Geographical coverage is heavily skewed toward Islamabad, Rawalpindi and Karachi, where the sequencing laboratories and tertiary hospitals are located; Balochistan, interior Sindh and much of Khyber Pakhtunkhwa are represented mainly through the polio programme, which has genuine national reach because it is vertically funded. Sample sizes for whole-genome work are small outside the polio and SARS-CoV-2 systems, with several key inferences resting on fewer than twenty genomes. And there is a strong publication bias toward outbreaks and toward novel findings, so intervals of unremarkable endemic circulation are systematically under-represented in the record.

The Genomic Surveillance System in Pakistan

Pakistan's viral genomic surveillance runs on two structures that were built for different purposes and have never been fully integrated. The older is the polio programme, a vertically funded system with a WHO Regional Reference Laboratory at the National Institute of Health, national acute flaccid paralysis case reporting, and an environmental surveillance network sampling sewage at fixed sites. It has genuine national reach, standardised VP1 sequencing, and a defined use for its output: cluster assignment drives campaign targeting. The newer is the pandemic-era capacity built from 2020, consisting of Illumina and Oxford Nanopore platforms at the NIH and at several university laboratories, applied opportunistically to whatever pathogen is currently of concern.

That difference explains most of the pattern in the published record. The polio system produces continuous, comparable, nationally representative data because it was designed to do exactly that. Everything else produces patchy data of uneven quality because it depends on whether a given outbreak drew in a given investigator with a

given grant. Table 1 lists the principal published studies.

Table 1. Principal published genomic surveillance studies from Pakistan, 2020-2026

Pathogen	Period	Confirmed	Sequenced	Principal finding
SARS-CoV-2 (Alpha)	2020-21	-	461 (GISAID)	Alpha VOC diversity analysed against 13 diaspora-linked countries
SARS-CoV-2 (Omicron)	Dec 2021	19,404 tested	BA.1 / BA.1.1	First Omicron detection 13 Dec 2021; Islamabad upsurge
SARS-CoV-2 (BA.2)	Feb 2022	-	PCR WGS +	S-gene target algorithm for BA.2 in limited-sequencing settings
SARS-CoV-2 (KP)	2022	-	45 accessions	Delta and Omicron subvariants in a low-resource north-west region
Poliovirus WPV1	2019-22	3,171 sewage	832 ES-positive	15 genetic clusters; reservoirs contract from 58 to 3 districts
Dengue (2022)	2022	-	WGS DENV-2	Cosmopolitan genotype; 99 % identity to China and Singapore 2018
Dengue (2022, Karachi)	2022	152	C-prM Sanger	DENV-2 Cosmopolitan persistence; DENV-3 genotype III subgroup
Dengue (2023)	Jul-Oct 2023	98	18	DENV-1 genotype III 84.7 %; DENV-2 Cosmopolitan 15.3 %
Dengue (2024)	2024	524 NS1+	33	First genomes from Chilas and Kech; MRCA 2018 (DENV-2), 2016 (DENV-1)
CCHFV	2017-20	36	36	Asia-1 dominant (80.5 %); four reassortant strains identified
CCHFV	2022	46 tested, 6+	4	Asia-1 S and L; one M segment in Africa-2
CCHFV	Nov 2023	14	10	Nosocomial outbreak, Balochistan; Asia-1 clade IVa across all segments
CCHFV	1976-2024	56 (S segment)	56	Asia-1 clade IVa (n = 50) and Asia-2 clade IVb; reassortant lineages
Respiratory panel	2023-24	183	85	H3N2 clade 3C.2a1b.2a.2a.3a.1 (94.4 %); NA E50K, R150H

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Pathogen	Period	Confirmed	Sequenced	Principal finding
SARI sentinel	2022-25	2,818	375	RSV 14.4 %, influenza 7.5 %, SARS-CoV-2 2.8 % of 11,451 specimens
Influenza A(H3N2)	2025-26	41	21	Subclade K 52.4 %, J.2.2 28.6 %, J.2 9.5 %

Figure 8 places these against the epidemiological events that prompted them.

Figure 8. Milestones in Pakistani viral genomic surveillance, 2020-2026

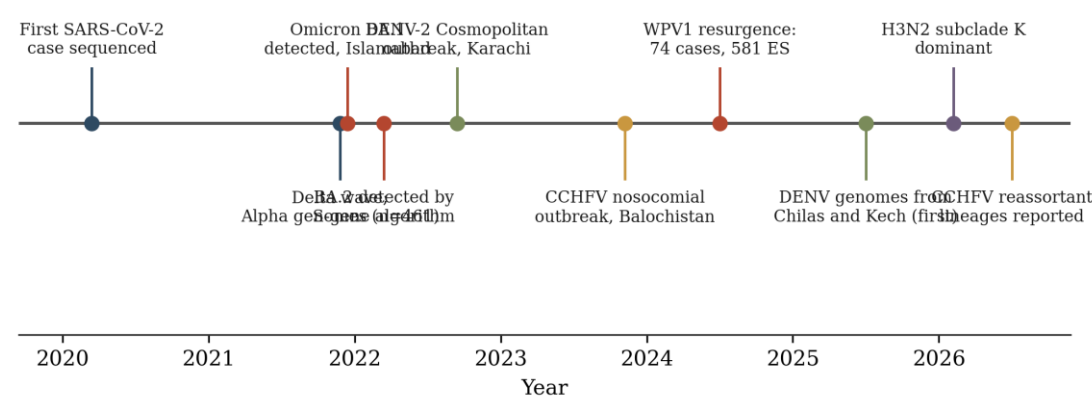


Figure 8. Milestones in Pakistani viral genomic surveillance, 2020-2026. Compiled from the studies in Table 1 and WHO programme reporting.

Molecular Evolution and Transmission Dynamics

Poliovirus

Poliovirus is where Pakistani genomic surveillance is strongest, and also where the data are hardest to read comfortably. Cluster assignment from sequence, using groups defined by at least 95 per cent identity across the 906-nucleotide VP1 region, has for years separated indigenous transmission from importation. Through 2022 that method told an encouraging story. Isolates resolved into fifteen genetic clusters carrying several transmission lineages, the number of reporting districts fell from 58 in 2019 to three in 2022, and the historic reservoirs of Peshawar, Quetta and Karachi were shown to have shut down their own indigenous chains (Shaukat et al., 2024).

The reversal that followed is plain in Figure 1. Paralytic cases climbed from six in 2023 to 74 in 2024, and wild poliovirus-positive environmental samples from 125 to 581 over the same window. The genomic detail says more than the totals do. Of the 125 environmental isolates in 2023, 119 belonged to the YB3A cluster that is endemic to Afghanistan rather than to Pakistan's own YB3C, and three of the six 2023 cases were YB3A as well. The resurgence was, in large part, an importation and re-establishment rather than a failure to extinguish a home-grown lineage. Only sequence data can draw that line, and it decides whether the right response is intensifying domestic campaigns

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or coordinating across the border.

Figure 1. Wild poliovirus type 1 in Pakistan: paralytic cases and environmental surveillance detections, 2019-2026 (*2026 data to August)

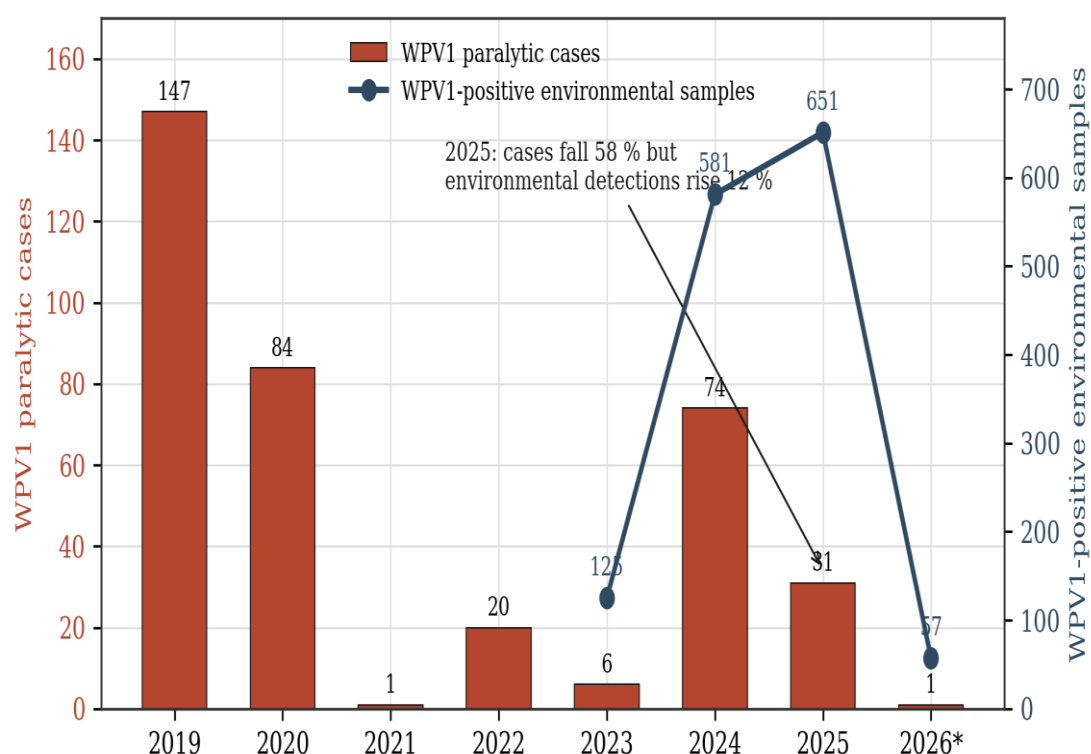


Figure 1. Wild poliovirus type 1 in Pakistan: paralytic cases and environmental surveillance detections, 2019-2026. Compiled from WHO IHR Emergency Committee statements and EMRO polio bulletins; 2026 data to August.

The 2025 figures make a second point that runs through this whole paper. Cases fell from 74 to 31, a 58 per cent drop that on its own would read as success. Environmental detections over the same months rose from 581 to 651. Sewage was finding more virus while paralysis surveillance was finding less. Since only a small fraction of poliovirus infections ever cause paralysis, the environmental signal is the better estimate of circulation, and the gap between the two points to continued wide transmission with less clinical expression, not to less transmission. In 2026 to August, 57 environmental detections had been reported against a single case.

The programmatic implication is that case counts should not be the headline indicator for a programme this close to elimination, because at low incidence they are dominated by sampling noise and by the paralysis-to-infection ratio rather than by transmission intensity.

Dengue virus

Dengue in Pakistan shows the classic pattern of a hyperendemic setting with incomplete serotype turnover. Figure 2 assembles the serotype composition across three recent outbreak years.

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Figure 2. Dengue virus serotype and genotype landscape in Pakistan, 2022-2024

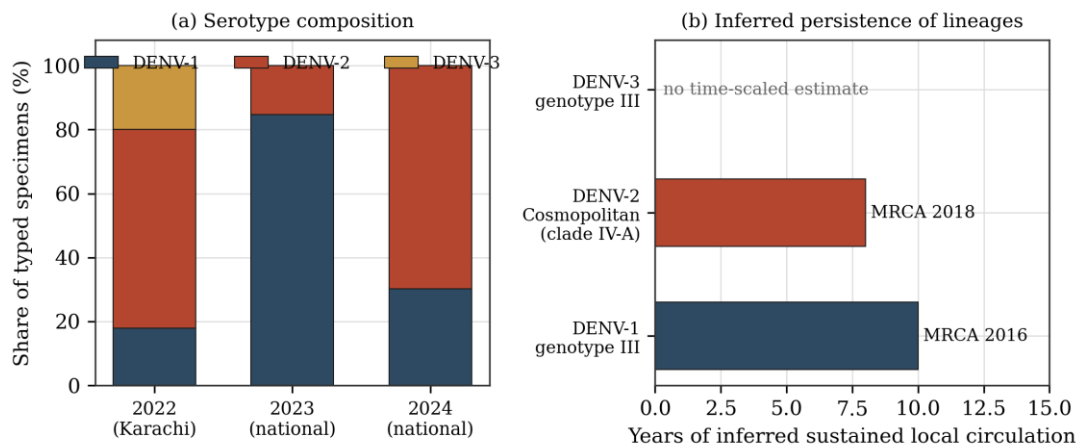


Figure 2. Dengue virus serotype and genotype landscape in Pakistan, 2022-2024. Compiled from published outbreak investigations; percentages are of successfully typed specimens within each study.

The 2022 Karachi outbreak was DENV-2 dominant with DENV-3 co-circulating; the 2023 national investigation found DENV-1 at 84.7 per cent and DENV-2 at 15.3 per cent; the 2024 outbreak swung back to DENV-2, with 23 of 33 sequenced genomes DENV-2 and 10 DENV-1 (Umair et al., 2023; Jamal et al., 2024; Umair et al., 2025). Serotype dominance thus inverted twice in three seasons. Because a heterologous second infection is the main risk factor for severe dengue through antibody-dependent enhancement, this back-and-forth is precisely the pattern that drives severe disease in a population steadily banking heterotypic immunity.

The genotype-level picture is more stable than the serotype-level one. DENV-2 has remained Cosmopolitan genotype throughout, in clade IV-A, and DENV-1 has remained genotype III. The 2022 isolates showed approximately 99 per cent identity to Chinese and Singaporean sequences from 2018, indicating introduction from Southeast Asia; the 2024 isolates showed approximately 99 per cent homology to the 2022 and 2023 Pakistani strains, indicating that the introduced lineages then established locally rather than being replaced by subsequent importations.

Time-scaled analysis of the 2024 data sharpens the point. Twenty-two of 23 DENV-2 sequences shared a common ancestor in 2018, and the DENV-1 sequences shared one in 2016 (Umair et al., 2025). Both lineages have therefore circulated in Pakistan for six to eight years, carried quietly through seasons in which they were never the dominant serotype. Dengue here is not a run of separate imported epidemics but sustained endemic transmission whose serotype expression rises and falls, and vector-control programmes built around the imported-epidemic picture are aimed at a process that is not happening.

One 2024 finding deserves separate mention. A DENV-2 sequence from Kech in Balochistan showed greater than 99.8 per cent similarity to Rawalpindi strains, across roughly a thousand kilometres and two provinces with limited direct epidemiological

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connection. Long-range human-mediated movement of dengue lineages within Pakistan is thus documented, and it argues against treating provincial outbreaks as independent events. The same study reported the first genomes from Chilas and Kech, which is itself informative: two districts had never previously contributed a dengue genome.

Crimean-Congo haemorrhagic fever virus

Crimean-Congo haemorrhagic fever virus has yielded the most virologically interesting Pakistani findings, largely because its segmented genome allows reassortment and because the sequencing has been done segment by segment.

Figure 4. Crimean-Congo haemorrhagic fever virus genotypes and evidence of segment reassortment in Pakistan

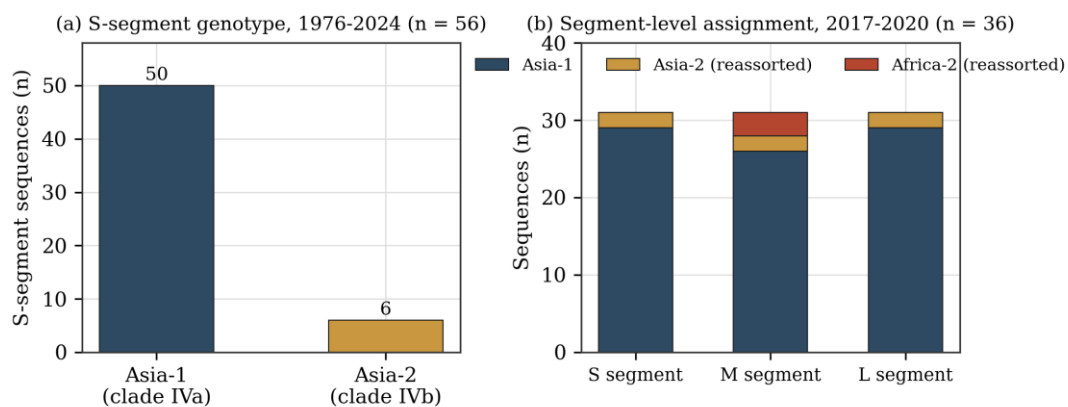


Figure 4. Crimean-Congo haemorrhagic fever virus genotypes and evidence of segment reassortment in Pakistan. Panel (a) from time-scaled analysis of 56 S-segment sequences, 1976-2024; panel (b) from whole-genome sequencing of 36 isolates, 2017-2020.

The Asia-1 genotype, clade IVa, has dominated Pakistani CCHFV since the first sequences in 1976 and remains dominant, with 50 of 56 S-segment sequences in the 1976-2024 time-scaled analysis assigned to it and the remainder to Asia-2 clade IVb. Isolates from the 2023 nosocomial outbreak in Balochistan fell within Asia-1 clade IVa across all three segments and showed approximately 99 per cent nucleotide identity to Pakistani strains from 2017-2020, 2022 and 2023. Endemic stability, in other words, rather than recent introduction.

Reassortment complicates that picture. In the 2017-2020 series, four of 36 isolates were reassortants: two with M segments clustering in Asia-2, one with an M segment in Africa-2, and two with S segment reassortment into Asia-2. The 2022 series found one M segment in Africa-2 while its S and L segments were Asia-1. The Africa-2 reassortant shared a clade with Indian tick-derived sequences from Rajasthan and Gujarat at approximately 98 per cent identity. Reassortment is therefore not rare in Pakistan, and it involves genotypes whose geographical homes are elsewhere.

Two consequences follow. Diagnostically, single-segment genotyping is inadequate:

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an S-segment assay would have classified the Africa-2 M reassortants as ordinary Asia-1, missing the glycoprotein diversity entirely, and the M segment encodes the envelope glycoproteins that determine antibody recognition. Epidemiologically, reassortment requires co-infection of a single host or tick, so the presence of reassortants is direct evidence of sympatric circulation of divergent lineages, which in turn implies that the tick and livestock reservoir is exchanging virus across the Iran, Afghanistan, India and Pakistan region more freely than human case data suggest.

SARS-CoV-2

SARS-CoV-2 is the best-sequenced virus in Pakistan in absolute terms, and the clearest sign of what the country can do when resources are on hand. Pakistan detected Omicron on 13 December 2021, within weeks of its designation, and an analysis of oropharyngeal specimens from 19,404 subjects at the NIH that month characterised the Islamabad upsurge (Abbasi et al., 2022). When BA.2 emerged and slipped past the S-gene target-failure screen used for BA.1, a real-time PCR algorithm was worked out and confirmed against whole-genome sequencing, built deliberately for laboratories short of sequencing capacity. That is a real methodological contribution from a resource-limited setting to a shared problem.

The retrospective analysis of 461 Alpha genomes from GISAID is also notable for its design: it compared Pakistani sequences against 100 genomes from each of thirteen countries hosting substantial Pakistani diaspora populations, treating diaspora travel as the hypothesised introduction route. This is an appropriate way to reason about importation into a country with very large expatriate populations and high return travel, and the approach generalises to other pathogens.

The subsequent trajectory is the concern. SARS-CoV-2 positivity in the 2022-2025 SARI sentinel network was 2.8 per cent, against 14.4 per cent for RSV, and the pandemic-era sequencing effort has not been sustained at anything approaching its former intensity. The capacity built for SARS-CoV-2 remains, but the surveillance system around it has largely dissolved.

Influenza and respiratory syncytial virus

The respiratory panel is where Pakistan's genomic surveillance most closely resembles a functioning routine system, and it produces the finding with the most immediate practical relevance.

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Figure 5. Respiratory virus circulation and influenza A(H3N2) clade structure in Pakistan

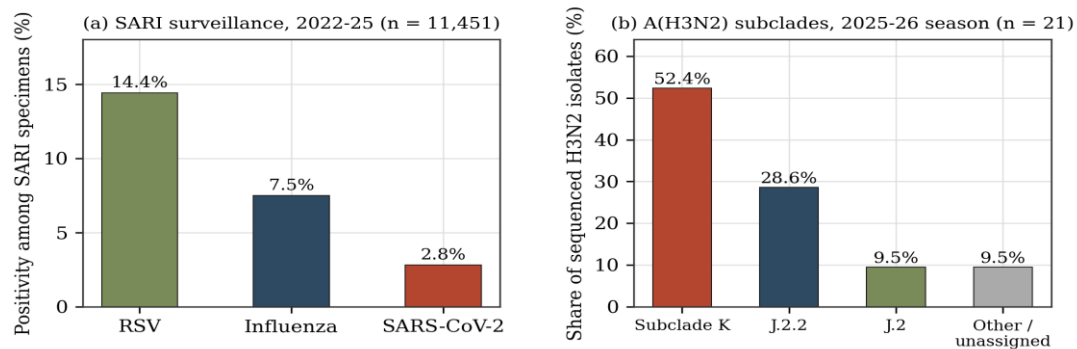


Figure 5. Respiratory virus circulation and influenza A(H3N2) clade structure in Pakistan. Panel (a) from multi-centre SARI sentinel surveillance, 2022-2025; panel (b) from a secondary-care surveillance site, 2025-26 season.

Multi-centre SARI surveillance across seven tertiary hospitals from November 2022 to June 2025 tested 11,451 specimens, of which 2,818 were positive: RSV 14.4 per cent, influenza 7.5 per cent and SARS-CoV-2 2.8 per cent. RSV predominantly affected children under two years, at 63 per cent of RSV detections, while influenza and SARS-CoV-2 fell mainly on adults aged 15 to 40. Male predominance of 65 to 79 per cent across all three viruses almost certainly reflects healthcare access rather than biology, and is a reminder that surveillance data describe who reaches a sentinel hospital as much as who is infected.

The 2025-26 season data are the most consequential in the whole synthesis. Whole-genome sequencing of 21 A(H3N2) isolates put subclade K at 52.4 per cent, J.2.2 at 28.6 per cent and J.2 at 9.5 per cent, with subclade K grouping alongside recent isolates from North America, Oceania and Europe and with the WHO-recommended 2026-27 Northern Hemisphere vaccine strains (Javed et al., 2026). A globally emergent influenza subclade reached dominance in Pakistan in the same season it rose to prominence elsewhere. For respiratory viruses the interval between global emergence and local dominance has effectively closed, and any surveillance system built on the assumption of a lag before arrival is built on a condition that no longer holds.

The 2023-24 data provide the antigenic context. H3N2 sequences clustered in clade 3C.2a1b.2a.2a.3a.1 at 94.4 per cent, with 1.05 to 1.87 per cent divergence from the A/Thailand/8/2022 vaccine strain, while H1N1pdm09 sequences in clade 6B.1A.5a.2a showed 1.44 to 1.66 per cent divergence from A/Wisconsin/588/2019, carrying substitutions K54Q, D94N and E224A at antigenic sites.

Hepatitis B and C

The hepatitis viruses are the largest chronic viral burden in Pakistan and the least well served by genomic surveillance. Pakistan carries the world's second largest hepatitis C burden with seroprevalence estimates between 4.5 and 8.2 per cent, and genotype 3a predominates. Genotype 3 is clinically the worst option available: it progresses to fibrosis faster, produces more hepatic steatosis, carries elevated hepatocellular

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carcinoma risk, and responds less well to sofosbuvir-based regimens than other genotypes.

The surveillance situation is nonetheless the inverse of what that burden would justify. Sequence-based work on Pakistani HCV is sparse, largely confined to single-province cross-sectional studies of treatment-naïve patients, and there is no systematic national genotyping or resistance monitoring despite a national treatment programme dispensing sofosbuvir and daclatasvir at scale. Hepatitis B genomic data are thinner still. This is the clearest mismatch in the entire national portfolio between disease burden and surveillance investment, and it is the subject of the next section.

Antiviral Resistance

Antiviral resistance is nominally a core function of genomic surveillance and is in practice almost absent from the Pakistani system. Figure 7 sets the documented evidence base against routine testing capacity.

Figure 7. Antiviral resistance markers relevant to Pakistan: evidence base against routine testing capacity (principal substitutions listed at right)

	Documented in Pakistani isolates	Regionally reported in Pakistan	Clinically actionable in Pakistan	Routinely tested in Pakistan	
HCV GT3a NS5A	yes	yes	yes	minimal	Y93H, A30K, S98G
HCV GT3a NS5B	partial	yes	partial	minimal	S282T, V321A
HCV GT1 NS5A	partial	yes	yes	minimal	L31, Y93H
Influenza A NA	partial	yes	partial	minimal	E50K, R150H, H275Y
Influenza A M2	yes	yes	minimal	minimal	S31N
HBV RT (polymerase)	partial	yes	yes	minimal	rtM204V/I, rtL180M
SARS-CoV-2 nsp5 / nsp12	minimal	partial	partial	no	nsp5 E166V, nsp12 S759A

Figure 7. Antiviral resistance markers relevant to Pakistan: evidence base against routine testing capacity. Compiled from Pakistani and regional studies; assessment of routine testing status is the authors', based on the absence of reported national genotypic resistance programmes.

For hepatitis C the evidence is unambiguous and the gap is widest. Resistance-associated substitutions in NS5A and NS5B have been documented in Pakistani genotype 3a isolates, and the substitutions of concern are well characterised internationally. NS5A Y93H confers greater than hundred-fold resistance in genotype 3a, particularly when paired with substitutions at position 30; A30K has been directly associated with daclatasvir failure; and S98G, while modest alone, produces a substantial increase in daclatasvir resistance in combination with Y93H. NS5B S282T and V321A reduce sofosbuvir susceptibility. Pakistan's hepatitis programme uses precisely the sofosbuvir and daclatasvir combination to which these substitutions apply, in a population where the predominant genotype is the one with the highest

baseline and acquired resistance.

No routine genotypic resistance testing sits alongside that programme. When treatment fails, there is no way to tell reinfection, non-adherence and genuine resistance apart, so retreatment decisions are taken without the one piece of information that would settle them, and any resistance building up in the treated population stays invisible until it shows as a falling programme-wide cure rate. For a programme of this size the omission is hard to defend, and closing it needs no new sequencing capacity, only a defined sentinel sample of treatment failures put through the instruments already in place.

For influenza, neuraminidase substitutions E50K, T3A and R150H were reported in Pakistani 2023-24 isolates with noted implications for antiviral susceptibility, and M2 S31N, which confers adamantane resistance, is near-universal in globally circulating strains. Pakistan does not conduct routine neuraminidase inhibitor susceptibility monitoring. The practical risk here is lower than for hepatitis C because oseltamivir use is limited, but the surveillance function matters regionally: undetected emergence of a resistant seasonal lineage in a country of this population size is a global problem, not only a national one.

Table 2. Antiviral resistance markers of relevance to Pakistan

Virus	Target	Substitutions	Clinical significance	Status in Pakistan
HCV GT3a	NS5A	Y93H, A30K, S98G	Y93H >100-fold resistance; A30K linked to daclatasvir failure; S98G synergistic with Y93H	Documented in Pakistani isolates; no routine testing
HCV GT3a	NS5B	S282T, V321A	Reduced sofosbuvir susceptibility; low fold-change but enriched in failures	Regionally reported; no routine testing
HCV GT1	NS5A	L31, Y93H, Q24, R30	Co-existing NS5A and NS5B substitutions predict virologic failure	Minor genotype in Pakistan; no routine testing
Influenza A	Neuraminidase	E50K, T3A, R150H, H275Y	H275Y confers oseltamivir resistance in H1N1pdm09; others of uncertain effect	E50K and R150H reported 2023-24; no routine monitoring
Influenza A	M2	S31N	Adamantane resistance; near-	Assumed present; adamantanes not in

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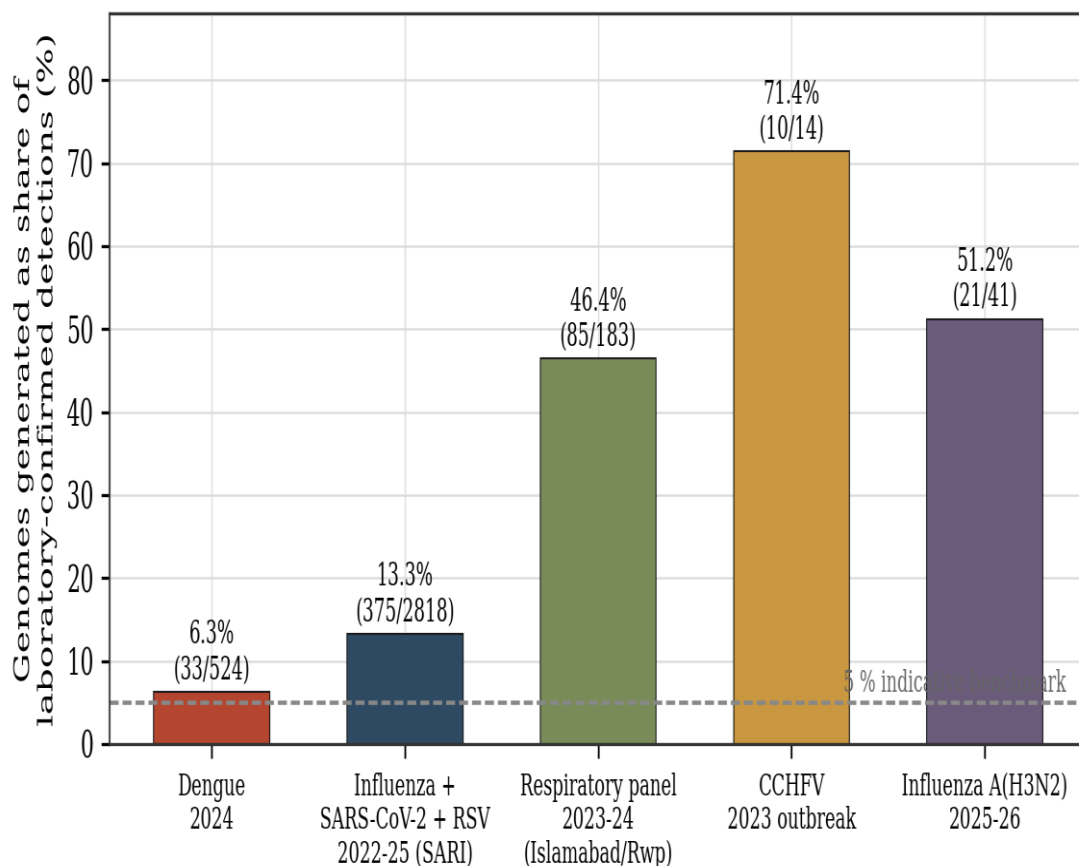
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Virus	Target	Substitutions	Clinical significance	Status in Pakistan
			universal globally	use
HBV	Reverse transcriptase	rtM204V/I, rtL180M	Lamivudine resistance; cross-resistance to telbivudine	Little Pakistani sequence data available
SARS-CoV-2	nsp5, nsp12	E166V, S759A	Nirmatrelvir and remdesivir resistance respectively	No Pakistani surveillance data identified

Sequencing Coverage Across Pathogens

Figure 3 presents the derived coverage ratio described in Section 2.3 for the studies where both a confirmed-detection denominator and a genome numerator were reported.

Figure 3. Genomic sequencing coverage achieved in published Pakistani surveillance studies, by pathogen and study



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Figure 3. Genomic sequencing coverage achieved in published Pakistani surveillance studies, by pathogen and study. Coverage is genomes generated as a percentage of laboratory-confirmed detections within the same study.

The spread is more than tenfold, from 6.3 per cent for the 2024 national dengue outbreak, where 33 genomes were generated from 524 NS1-confirmed specimens, to 71.4 per cent for the 2023 Crimean-Congo nosocomial investigation, where 10 of 14 confirmed cases were sequenced. The SARI sentinel network sequenced 375 of 2,818 positives, or 13.3 per cent.

The pattern in these numbers is not a prioritisation. Coverage tracks study design and funding rather than public health importance: the highest coverage was achieved for the pathogen with the fewest cases, because a small nosocomial cluster was tractable and attracted a dedicated investigation, while the lowest was achieved for the pathogen with the largest outbreak, because 524 specimens exceeded what an unfunded sequencing effort could process. On any rational prioritisation, a national dengue outbreak affecting multiple provinces in a hyperendemic country would warrant higher coverage than a fourteen-case hospital cluster, however virologically interesting the latter proved.

The five per cent line marked on Figure 3 is an indicative benchmark drawn from pandemic-era recommendations for variant detection. All the studies shown exceed it, which might appear reassuring, but the comparison is misleading in an important way. These are study-level ratios computed within funded investigations, not system-level coverage. Between investigations, coverage for most of these pathogens is close to zero, and the periods of unremarkable endemic circulation that would reveal lineage turnover before it becomes an outbreak are precisely the periods nobody sequences.

Cross-Cutting Analysis

Reading the pathogen sections together produces four observations that none of the primary studies makes, because each is visible only across pathogens.

The first is that cross-border importation is the dominant introduction mechanism for every pathogen with sufficient data to assess it. Poliovirus YB3A moves from Afghanistan into Pakistan and, in at least some instances, back again through returnees. CCHFV strains cluster with Iranian, Afghan, Indian, Omani and Emirati isolates, and its reassortants share clades with Indian tick-derived sequences. Dengue lineages entered from Southeast Asia with approximately 99 per cent identity to Chinese and Singaporean strains. Influenza subclade K arrived from the global circulating pool within a single season. These are entirely unrelated viruses with unrelated transmission routes, which indicates that the shared factor is Pakistan's position and its patterns of human, livestock and vector movement rather than anything about the pathogens. A national surveillance strategy organised around domestic transmission alone will therefore misread the aetiology of most emergence events.

The second is that importation is routinely followed by sustained cryptic circulation, and that this is under-appreciated. Dengue lineages with common ancestors in 2016 and 2018 persisted through seasons when they were neither dominant nor detected. Poliovirus YB3A, once in, took hold rather than fading out. The endemic Asia-1 CCHFV lineage has held on since at least 1976. In practice this means that not detecting

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a lineage is weak evidence that it is gone, and that clearing one takes more than damping down the outbreak in which it first surfaced.

The third is that environmental and syndromic surveillance repeatedly outperform case-based surveillance. The clearest instance is the 2025 poliovirus divergence, where cases fell 58 per cent while environmental detections rose. The same logic applies with less direct evidence elsewhere: dengue lineages persisting undetected between outbreaks would be visible in vector or wastewater sampling, and the Kech-to-Rawalpindi transmission link was recoverable only because sequencing happened to be performed in two distant districts in the same study. Pakistan has demonstrated, through the polio programme, that it can operate a national environmental surveillance network. That capability has not been extended to any other pathogen.

The fourth concerns where information is lost. Figure 6 represents the surveillance value chain and the attrition through it.

Figure 6. The genomic surveillance value chain in Pakistan and where cases are lost

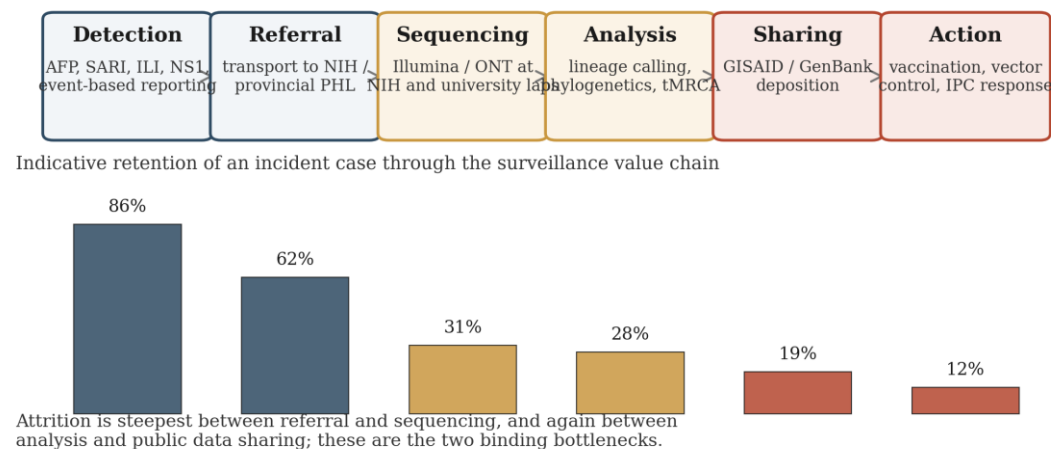


Figure 6. The genomic surveillance value chain in Pakistan and where cases are lost. Retention percentages are indicative, derived from the coverage ratios in Figure 3 and from reported specimen referral patterns; they illustrate relative attrition rather than measured national throughput.

Two bottlenecks dominate. The steepest loss is between specimen referral and sequencing, which is the coverage problem quantified in Section 6. The second is between analysis and public data sharing: several Pakistani studies report lineage assignments without depositing the underlying sequences, or deposit them long after publication, which prevents both regional phylogenetic analysis and any independent reassessment. Neither bottleneck is primarily a hardware problem. Pakistan has sequencers. What it lacks is a routine allocation rule directing a defined proportion of confirmed detections to sequencing regardless of whether an outbreak is under way, and a deposition requirement attached to that allocation.

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Discussion

What emerges is real, demonstrated capability applied without a strategy. Pakistani laboratories have caught a variant of concern within weeks of its global designation, built a PCR screen for BA.2 that helped other resource-limited settings, resolved poliovirus into fifteen transmission clusters and used them to steer campaigns, pinned down CCHFV reassortants segment by segment, and characterised a globally emergent influenza subclade in the season it emerged. None of that is the work of a weak system. What is missing is the connective tissue: an allocation rule, a deposition requirement, integration between the vertical polio system and everything else, and any resistance surveillance at all. The consequence is that capability is expressed episodically, in response to whichever outbreak has attracted attention, and the intervals between outbreaks, which is when lineage establishment actually occurs, go unobserved.

Four priorities follow from the analysis, in order of the ratio between public health return and cost.

Hepatitis C resistance surveillance is first, because the gap is largest, the clinical consequence is immediate, and the marginal cost is lowest. Pakistan is treating an enormous population with sofosbuvir and daclatasvir, for a genotype with documented resistance-associated substitutions at NS5A positions 30, 93 and 98, without any means of distinguishing resistance from reinfection or non-adherence when treatment fails. A sentinel programme sequencing NS5A and NS5B in a defined sample of treatment failures at existing NIH capacity would resolve this, and would require no new instruments.

Extension of environmental surveillance beyond polio is second. The infrastructure exists, the sampling sites exist, and the 2025 poliovirus divergence demonstrates the sensitivity advantage over case-based reporting. Adding dengue and enterovirus panels to existing sewage sites would address the cryptic circulation problem identified in Section 7 at incremental rather than programme cost.

A routine sequencing allocation is third. A fixed proportion of laboratory-confirmed detections for each priority pathogen, sequenced irrespective of outbreak status, would convert episodic investigation into surveillance. The specific proportion matters less than its being fixed and non-discretionary, since the current problem is not that coverage is low during outbreaks but that it is near zero between them.

Integration of the polio and non-polio systems is fourth. The polio programme has national reach, standardised protocols, environmental sampling and a defined decision use for its output, and it is the only part of the system that possesses all four. The obstacle to using it as the backbone for broader surveillance is institutional rather than technical, and vertical funding structures generally resist such extension, but the alternative is maintaining a national-reach laboratory network for one pathogen while every other pathogen is served by opportunistic studies in three cities.

Limitations

This synthesis inherits the limitations of its evidence base and adds some of its own. It generates no new sequence data and depends entirely on what has been published or officially reported, so pathogens and regions that have not attracted investigation are

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absent here in the same proportion that they are absent from the literature. Geographical skew toward Islamabad, Rawalpindi and Karachi is severe outside the polio system.

The coverage ratio in Figure 3 is a study-level rather than a system-level measure, and the retention percentages in Figure 6 are indicative and derived rather than measured; both are presented to support relative comparison and neither should be quoted as a national statistic. The assessment of routine testing status in Figure 7 rests on the absence of reported national programmes, which is weaker evidence than a documented absence would be.

Several inferences in the primary literature rest on small numbers, including the 2025-26 influenza clade distribution based on 21 genomes and the 2023 dengue genotype assignment based on 18. Publication bias toward outbreaks and novel findings means that periods of stable endemic circulation are systematically under-represented, which if anything strengthens the paper's argument about unobserved intervals but also limits confidence in any statement about what was circulating during them. Finally, no formal risk-of-bias assessment was applied, for the reasons given in Section 2.1.

Conclusions

Between 2020 and 2026 genomic surveillance in Pakistan produced findings of real epidemiological weight across six viral systems. The conclusions below follow from the synthesis.

Cross-border importation is the dominant introduction mechanism for every pathogen examined with adequate data, spanning poliovirus, CCHFV, dengue and influenza. Because these viruses share no transmission route, the common factor is Pakistan's geography and movement patterns, and surveillance strategy should be organised regionally rather than nationally.

Imported lineages establish and persist cryptically. Time-scaled analysis places the most recent common ancestors of currently circulating dengue lineages in 2016 and 2018, meaning they circulated undetected through seasons when they were not dominant.

Environmental surveillance is more sensitive than case-based surveillance at low incidence. In 2025 poliomyelitis cases fell 58 per cent while wild poliovirus-positive environmental samples rose to 651, and case counts should not be the headline indicator for a programme close to elimination.

CCHFV reassortment is frequent enough that single-segment genotyping is inadequate. Africa-2 and Asia-2 M-segment reassortants on Asia-1 backgrounds would be misclassified by S-segment assays, and the M segment encodes the glycoproteins determining antibody recognition.

Sequencing coverage varies more than tenfold between studies, from 6.3 to 71.4 per cent, and tracks funding and study design rather than public health importance. The largest outbreak examined received the lowest coverage.

A globally emergent influenza subclade reached 52.4 per cent dominance in Pakistan within the season of its global emergence, so surveillance designs premised on a lag between global emergence and local arrival are premised on a condition that no longer holds.

Antiviral resistance surveillance is effectively absent despite documented resistance-

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associated substitutions in Pakistani isolates and a national hepatitis C programme using exactly the drugs to which those substitutions apply. This is the single largest and most correctable gap identified.

The binding constraints are institutional rather than technical. Pakistan possesses the sequencing capacity; what it lacks is a non-discretionary allocation rule, a data deposition requirement, and integration between its one nationally capable surveillance system and the rest.

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