

The National Burden and Policy Implications of Human Immunodeficiency Virus Among Blood Donors in Pakistan: A Systematic Review and Meta-Analysis (1988-2026)

Usman Waheed^{1,2}, Sana Zulfikar³, Shahnam Shahid^{3,4}, Usama Qamar^{1,2}, Noore Saba⁵, Joana Bikulciene⁶, Abuzar Ghaffari⁴, Omar Khawar Khokhar⁷, Sara Sameer⁸, Akhlaaq Wazeer⁹

¹Department of Allied Health Sciences, Islamabad Medical & Dental College, Islamabad, Pakistan

²Hajra & Sharif Ibrahim Billoo Postgraduate Research Laboratory, Islamabad Medical & Dental College, Islamabad, Pakistan

³Department of Pathology and Blood Bank, Pakistan Institute of Medical Sciences, Islamabad, Pakistan

⁴Department of Medical Laboratory Technology, Faculty of Allied Health & Biological Sciences, Ibadat International University, Islamabad, Pakistan

⁵Safe Blood Transfusion Project, Peshawar Regional Blood Centre, Provincial Ministry of Health, Khyber Pakhtunkhwa, Pakistan

⁶Institute of Experimental and Clinical Medicine, Vilnius, Lithuania

⁷Department of Trauma and Orthopaedics, Macclesfield District General Hospital, East Cheshire NHS Trust, Cheshire, England, United Kingdom

⁸Department of Pathology and Blood Bank, Dr. Akbar Niazi Teaching Hospital, Islamabad, Pakistan

⁹Department of Medical Laboratory Technology, Mirpur Institute of Medical Sciences, Mirpur, Azad Jammu & Kashmir, Pakistan

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*Correspondence:

drusman.waheed1@gmail.com

ORCID: <https://orcid.org/0000-0002-5760-5671>

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ABSTRACT

Introduction: Human immunodeficiency virus (HIV) remains a major public health challenge and poses a significant threat to blood transfusion safety. Although numerous studies have investigated HIV prevalence among blood donors in Pakistan, a comprehensive national estimate has not previously been available.

Objective: This systematic review and meta-analysis aimed to determine the prevalence, temporal trends, and geographical distribution of HIV among healthy blood donors in Pakistan from 1988-2026.

Methodology: A systematic literature search was conducted to identify studies reporting HIV prevalence among blood donors in Pakistan from 1988 to 2026. Eligible studies were screened according to PRISMA guidelines. Data on study characteristics, sample size, HIV-positive cases, geographical location, and screening methodologies were extracted. Random-effects meta-analysis was performed to estimate pooled prevalence, while subgroup analyses were conducted by province, study period, and screening technique. Heterogeneity, publication bias, and sensitivity analyses were also assessed.

Results: A total of 125 studies comprising 12,663,749 blood donors were included. Overall, 13,892 donors tested positive for HIV, yielding an overall national prevalence of 0.109% (95% CI: 0.107-0.111). The random-effects pooled prevalence was 0.070% (95% CI: 0.049-0.099). Significant heterogeneity was observed across studies ($I^2 = 99.7\%$, $p < 0.001$). Regionally, the highest prevalence was observed in Sindh and Gilgit-Baltistan, followed by Balochistan, Islamabad Capital Territory, Khyber Pakhtunkhwa, Punjab, and Azad Jammu & Kashmir. Temporal analysis demonstrated a significant increase in HIV prevalence over time (OR per year = 1.144; 95% CI: 1.139-1.149; $p < 0.001$). Studies using NAT and CLIA-based assays reported higher detection rates than those using ELISA or rapid diagnostic tests. Leave-one-out sensitivity analysis confirmed the robustness of the pooled estimate, while funnel plot asymmetry suggested possible small-study effects or publication bias.

Conclusion: The study provides comprehensive national synthesis of HIV prevalence among blood donors in Pakistan. Although the overall prevalence is relatively low, the increasing temporal trend and marked regional variations highlight challenges to blood safety.

Keywords: HIV; AIDS; Blood donors; Blood safety; Transfusion-transmitted infections; Seroprevalence; Pakistan

Introduction

Human immunodeficiency virus (HIV) is a retrovirus that progressively impairs the human immune system, ultimately leading to acquired immunodeficiency syndrome (AIDS) and increased susceptibility to opportunistic infections and life-threatening conditions.¹⁻³

Despite decades of global efforts, HIV remains a major public health concern worldwide. According to the Joint United Nations Programme on

HIV/AIDS (UNAIDS), approximately 91.4 million individuals have been infected with HIV since the beginning of the epidemic, with 44.1 million deaths attributed to AIDS-related illnesses. In 2024 alone, an estimated 40.8 million people were living with HIV globally, with 1.3 million new infections reported. Women and girls accounted for 45% of new HIV infections and 53% of all people living with HIV. Furthermore, 87% of individuals living with HIV were aware of

their status, while 77% were receiving antiretroviral therapy (ART).⁴

Although significant progress has been made in diagnosis and treatment, evidenced by increased awareness of HIV status and expanded access to antiretroviral therapy,⁴ persistent challenges such as stigma, inequitable access to healthcare, and undiagnosed infections continue to hinder efforts to eliminate the epidemic.⁵⁻⁸

HIV has profound consequences not only for individuals but also for families and national economies, primarily due to loss of productivity, increased healthcare costs, and long-term social impacts.⁹⁻¹¹ In response, coordinated global strategies focusing on prevention, early diagnosis, and treatment have led to measurable reductions in disease burden.¹²⁻¹⁴ However, the sustainability of these gains remains threatened in resource-limited settings and conflict affected regions.^{15,16}

In Pakistan, HIV epidemiology presents unique challenges. The National AIDS Control Programme (NACP) estimates that approximately 330,000 people were living with HIV by the end of 2025. Of these, only about 25.6% are registered with the NACP, and approximately 18.4% are receiving ART through designated treatment centers. This significant gap between estimated and registered cases highlights underdiagnosis, limited access to care, and weaknesses in surveillance systems.¹⁷ Additionally, periodic outbreaks reported in different regions of the country underscore systemic vulnerabilities in infection control practices and public health surveillance mechanisms.¹⁸⁻²³ In response to growing outbreaks, the Prime Minister of Pakistan constituted a national task force in May 2026 to investigate the alleged mishandling of HIV-related issues in the country, representing a potentially significant step toward strengthening HIV governance and public health oversight.²⁴

Blood transfusion services play a critical role in healthcare delivery, particularly for patients undergoing surgery, trauma management, obstetric care, and chronic transfusion-dependent conditions such as thalassaemia. However, transfusion-transmitted infections (TTIs), including hepatitis B virus (HBV), hepatitis C virus (HCV), HIV/AIDS, syphilis, and malaria, remain a persistent threat to blood safety. In

Pakistan, HIV screening in blood banks began in 1988²⁵ but was not mandatory until 1995, when federal government directives required screening of all donated blood for three major TTIs (HBV, HCV, HIV). Subsequent legislative developments, including blood safety laws introduced between 1997 and 2004 (with later revisions), established regulatory frameworks to ensure safer transfusion practices, promote voluntary blood donations, eliminate paid donations, and screening for five TTIs.²⁶⁻³¹

Despite these advancements, blood safety continues to be compromised by weak governance, infrastructural limitations, variability screening practices, disparities in quality assurance and implementation of standards across blood centres and the evolving dynamics of the HIV epidemic, particularly among high-risk populations.³²⁻³⁶ The phenomenon of 'bridging populations', where infections spread from high-risk groups to the general population, poses an additional risk to blood donors and recipients.³⁷ Furthermore, although serological screening has improved safety, it does not entirely eliminate the risk of transfusion-transmitted HIV, especially during the window period of infection.³⁸⁻⁴⁰

Blood recipients, especially those requiring frequent transfusions such as patients with thalassaemia, remain particularly vulnerable to TTIs.⁴¹⁻⁴³ Therefore, robust epidemiological data are essential for informed decision-making, resource allocation, and the development of targeted interventions to enhance blood safety.

A number of individual studies have investigated the prevalence of HIV among blood donors in Pakistan; however, these studies are often limited by small sample sizes, regional focus, and methodological heterogeneity, making it difficult to derive nationally representative estimates. To date, there is a lack of comprehensive synthesis of available evidence at the national level.

Systematic review and meta-analysis provide a powerful methodological approach to aggregate data from multiple studies, enhance statistical power, and generate more precise estimates of disease burden. By combining evidence across diverse settings and populations, this approach enables a more comprehensive understanding of HIV prevalence and its distribution among blood donors. Therefore, the present analysis aims to determine the pooled prevalence of HIV infection

among blood donors in Pakistan, assess temporal trends, and evaluate geographical variations. The findings of this study are expected to contribute to evidence-based policymaking, strengthen blood safety strategies, and guide future research and public health interventions aimed at reducing the burden of HIV in Pakistan.

Methodology

Protocol registration

A preliminary literature search and concept validation were conducted prior to protocol development to ensure the scientific rationale of the proposed study, avoid duplication of previously addressed research questions, and confirm the availability of sufficient literature for review. A preliminary search was performed in the PROSPERO database (International Prospective Register of Systematic Reviews) and the Database of Abstracts of Reviews of Effects (DARE) to identify similar or ongoing studies. The protocol for this study was subsequently registered in the PROSPERO database under the registration number CRD420261405362. As the study was based exclusively on previously published data, ethical approval was not required. The search strategy, study selection, data extraction, and reporting of results were conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 Statement.⁴⁴

Inclusion criteria

Articles were included in the study if they met the following criteria: published between January 1988 and June 2026; availability of full text; reported prevalence of HIV among blood donors in Pakistan; published in English language; observational studies, including cross-sectional and cohort studies, as well as national or provincial surveillance reports; mentioned clearly the screening test used for HIV detection; and reported both the number of donors screened and the number of reactive cases. The review question was formulated using the CoCoPop framework for prevalence studies as follows (1) Condition: HIV prevalence, (2) Context: Pakistan, and (3) Population: Blood donors.

Exclusion criteria

The following types of publications were excluded: review articles, conference

proceedings, case reports, commentaries, case series, editorials, qualitative studies, opinion papers, posters, duplicate publications, studies with inconsistent data, studies reporting self-reported HIV prevalence, studies conducted on Pakistani nationals residing abroad, and studies that did not meet the inclusion criteria. To minimize the risk of duplicate inclusion of the same blood donor population across multiple studies, the retrieved literature was rigorously screened during the study selection and data extraction phases. Potential overlap was assessed by comparing the study location, study period, institution, and other relevant characteristics. When similarities were identified, full-text articles were reviewed in detail to determine whether overlapping participant populations existed, and duplicate data were excluded accordingly.

Search strategy

A comprehensive literature search was conducted across multiple electronic databases, including PubMed, Scopus, ScienceDirect, Web of Science, Embase, PakMediNet, Google Scholar, and the Cochrane Library (including both the Cochrane Database of Systematic Reviews and the Cochrane Central Register of Controlled Trials). Additional searches were performed in World Health Organization (WHO) databases and national health repositories to identify relevant reports and grey literature. The search covered the period from January 1988 to June 2026. The search strategy included the keywords: "Human Immunodeficiency Virus" OR "HIV" OR "HIV/AIDS" AND "Blood Donors" AND "Prevalence" OR "anti-HIV" OR "Epidemiology" AND "Pakistan". Boolean operators ("AND" and "OR") were applied to refine the search strategy. An advanced search was also performed in PubMed using relevant Medical Subject Headings (MeSH) terms related to HIV. In addition, the reference lists of retrieved articles and relevant review papers were manually screened to identify additional eligible studies. The database search was independently conducted by two reviewers (UW and SZ) between April 28 and June 10, 2026.

Outcome of interest

The primary outcome of interest was the seroprevalence of HIV among blood donors in

Pakistan. HIV prevalence estimates included in the analysis were based on laboratory detection of HIV infection through biological assessment of blood samples using the diagnostic methods reported in the included studies.

Study screening and selection

The study selection process was conducted in two stages. In the first stage, all records retrieved from the databases were imported into EndNote™ X8.2 for duplicate removal. Titles and abstracts were independently screened by two reviewers (UW and SZ) to assess study relevance. In the second stage, the full texts of potentially eligible articles were independently reviewed by two reviewers (SS and NS) against the predefined inclusion criteria. Any disagreements regarding study eligibility were resolved through discussion with a third reviewer (UQ). The overall study selection process was illustrated using a PRISMA flow diagram, showing the number of records identified, screened, excluded, and ultimately included in the review. This study formed part of a broader research project assessing the seroprevalence of transfusion-transmissible infections, including HBV, HCV, HIV, and syphilis among blood donors in Pakistan. Owing to the substantial volume of data, the project was divided into four separate analyses, each focusing on an individual transfusion-transmissible infection.

Data extraction

A standardized data extraction form was developed to ensure uniformity in data collection from the studies included for narrative synthesis and meta-analysis. Data extraction was performed independently by the authors UW, AW, AG, SZ, SS, and UQ, while any discrepancies or conflicts were resolved through discussion with JB and OKK. The extracted variables included (a) study characteristics: author(s), publication year, study location, and study design; (b) population characteristics: number of donors, age, gender, and study setting (urban or rural); (c) outcome measures: HIV prevalence among blood donors (reported as percentage or proportion); (d) screening methods: type of diagnostic assay used, including rapid diagnostic tests (RDTs), enzyme-linked immunosorbent assay (ELISA), chemiluminescent immunoassay (CLIA), and polymerase chain reaction/nucleic acid testing (PCR/NAT). Any discrepancies in extracted data

were resolved through consensus discussions to ensure accuracy and minimize bias. The extracted data were stored in Microsoft Excel 2021 (Microsoft Corporation, Redmond, Washington, USA). To further ensure data accuracy and consistency, all extracted data underwent a secondary verification process, during which the same reviewers cross-checked studies different from those they had initially extracted.

Quality assessment

Two review authors (UW and JB) independently assessed the methodological quality of the included studies to minimize assessment errors and reduce the influence of individual bias or preconceptions. To improve the reliability and consistency of the evaluations, the reviewers first discussed the application of the assessment tool and pilot-tested the risk-of-bias checklist on a sample of articles before commencing the formal assessment process. Any discrepancies in quality assessment were resolved through discussion between the two reviewers. In cases where consensus could not be reached, a third reviewer (OKK) was consulted, and final decisions were made with appropriate justification. The Joanna Briggs Institute (JBI) Critical Appraisal Checklists for cross-sectional and cohort studies were used for quality assessment.⁴⁵ The JBI checklist comprises eight items for cross-sectional studies and eleven items for cohort studies.⁴⁶ These tools assessed potential sources of bias related to participant recruitment, measurement of outcomes and independent variables, and statistical analysis. Each checklist item was rated as “yes,” “no,” or “unclear.” Quality scores were calculated based on the total number of “yes” responses. For cross-sectional studies, articles scoring 7-8, 4-6, and 0-3 points were categorized as having low, moderate, and high risk of bias, respectively. For cohort studies, scores of 9-11, 5-8, and 0-4 points indicated low, moderate, and high risk of bias, respectively.

Statistical analysis

The extracted data were exported to STATA version 17 (StataCorp 2021, College Station, TX, USA) for statistical analysis. A narrative synthesis of all included studies was initially conducted, whereby eligible articles were summarized according to publication year, geographic region, study design, and sample size. For the meta-

analysis, the number of HIV-reactive blood donors and the total number of successfully screened donors were used to calculate the pooled prevalence of HIV. The pooled prevalence estimates were generated using the “metaprop” command in STATA. The findings were presented through descriptive text and forest plots displaying individual study estimates, confidence intervals, and the overall pooled effect size. Statistical heterogeneity among studies was assessed using the Cochran Q test and quantified with the I^2 statistic, with $p < 0.05$ considered indicative of significant heterogeneity.

A meta-regression analysis was performed using study year as a continuous moderator variable. For each study, HIV prevalence was calculated as the proportion of HIV-positive donors relative to the total number screened. A random-effects meta-regression model was then fitted to assess the association between study year and reported HIV prevalence, while accounting for between-study heterogeneity. Because prevalence estimates were small and highly variable across studies, the proportions were modeled on the logit scale. The regression coefficient (β) represented the average annual change in log-odds of HIV prevalence with increasing study year. The coefficient was exponentiated to obtain the odds ratio (OR) per year with its corresponding 95% confidence interval (CI). Statistical significance was assessed using the p-value of the regression coefficient. A bubble plot was generated to visually present the meta-regression findings. In this plot, each bubble represents an individual study; the position of the bubble reflects the study year and the reported HIV prevalence, while the size of the bubble is proportional to the study weight or sample size. The fitted regression line and its 95% confidence band were superimposed to illustrate the overall temporal trend.

A subgroup analysis was performed to assess variation in HIV prevalence according to the screening technique used in the included studies. Screening methods were categorized as rapid diagnostic test (RDT), enzyme-linked immunosorbent assay (ELISA), chemiluminescence-based immunoassays (CLIA/CMIA/ECLIA), nucleic acid testing (NAT), and mixed methods, where more than one screening platform was used. For each study, HIV prevalence was calculated as the number of HIV-

positive blood donors divided by the total number of donors screened and expressed as a percentage. Pooled prevalence estimates for each screening-method subgroup were calculated, and 95% confidence intervals were used to indicate the precision of the estimates. Differences in HIV prevalence across screening techniques were assessed using the chi-square test for subgroup differences, with statistical significance set at $p < 0.05$.

Publication bias was evaluated using a funnel plot of transformed proportions against sample size, while asymmetry was further assessed using Egger’s regression test, where $p < 0.05$ suggested potential publication bias. Descriptive statistics, including mean, median, and range, were calculated to illustrate the distribution of HIV prevalence across studies. A time-series analysis (1988-2026) was also performed to explore temporal trends and identify fluctuations or changes in HIV prevalence over time. Additionally, a leave-one-out sensitivity analysis was conducted using the “metaninf” command to identify potential sources of heterogeneity and assess the robustness of the pooled estimates. The final results were presented in the form of text, tables, and figures.

Ethical considerations

As this study was based exclusively on previously published literature, the units of analysis were the published articles rather than individual human participants. Therefore, informed consent from study participants was not required. Likewise, no additional ethical approval was necessary. It was assumed that all included studies had obtained ethical clearance from their respective institutional review boards or ethics committees. Furthermore, the ethical and methodological principles outlined in the PRISMA statement were strictly followed to ensure transparency, accuracy, reproducibility, and integrity of the systematic review findings.

Results

Figure 1 depicts the PRISMA flow diagram for study selection. A total of 2,765 records were identified through database searches, with an additional 23 records obtained through handcomb searching. After removing 519 duplicates, 2,269 records remained for title and abstract screening. Following full-text assessment and resolution of

disagreements by consensus, 125 unique studies were included in the systematic review and meta-analysis.⁴⁷⁻¹⁷¹

Detailed characteristics of the included studies are presented in Table 1. A total of 125 studies conducted between 1988 and 2026 were included in the systematic review, comprising 12,663,749 blood donors, of whom 13,892 tested positive for HIV. The overall crude prevalence of HIV among blood donors in Pakistan was 0.109% (95% CI: 0.107-0.111). Using a random-effects meta-analysis, the pooled prevalence was estimated at 0.070% (95% CI: 0.049-0.099). Considerable between-study heterogeneity was observed ($I^2 = 99.7\%$, Cochran's $Q = 35,086.4$, $df = 124$, $p < 0.001$), supporting the use of a random-effects model (Fig. 2).

Jammu & Kashmir (0.03%; 95% CI: 0.021-0.057). Differences in HIV prevalence among provinces were statistically significant (χ^2 for subgroup differences, $p < 0.001$).

The earliest data were reported from Karachi in 1988 (Mujeeb *et al.*),⁴⁷ while the most recent studies were reported in 2026 from Mardan and Karachi.¹⁷⁰⁻¹⁷¹ The included studies were predominantly cross-sectional and employed a range of screening assays including RDTs, ELISA, CLIA/CMIA/ECLIA, NAT, and one study utilizing a combination of methods. ELISA was the most frequently used assay (39.2%), followed by CLIA-based methods (31.2%), RDTs (20%), NAT (8.8%), and 1 mixed-method study (0.8%). A gradual transition from conventional serological screening to chemiluminescence and nucleic acid

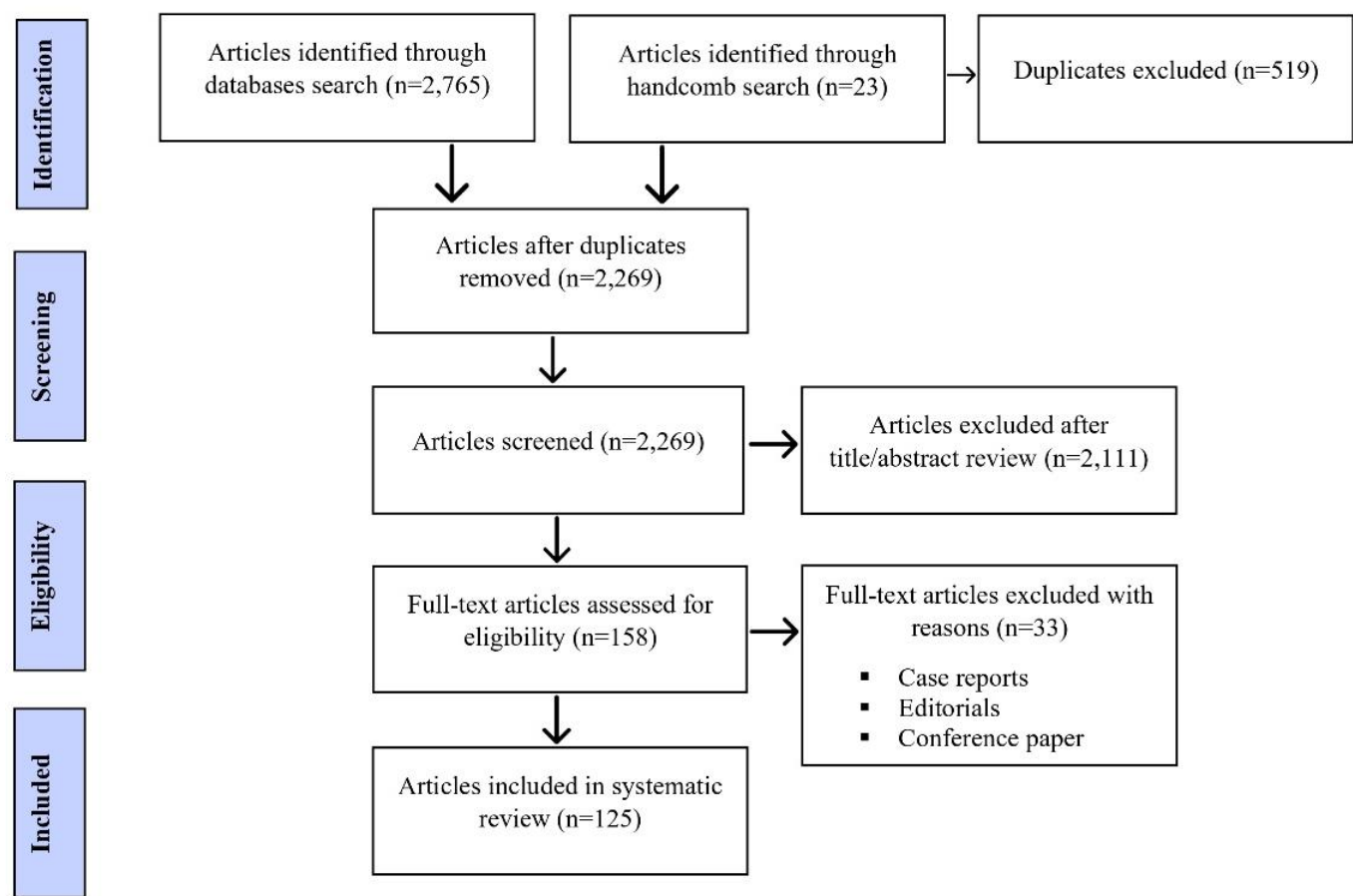


Fig. 1: Flowchart of the process through which articles were selected based on PRISMA guidelines

The highest HIV prevalence (Fig. 3) was observed in Sindh (0.143%; 95% CI: 0.141-0.146), followed by Gilgit-Baltistan (0.142%; 95% CI: 0.039-0.519), Balochistan (0.140%; 95% CI: 0.104-0.188), Islamabad Capital Territory (0.12%; 95% CI: 0.117-0.132), Khyber Pakhtunkhwa (0.11%; 95% CI: 0.103-0.116), Punjab (0.04%; 95% CI: 0.039-0.043), and Azad

testing (NAT) was evident after 2010, reflecting improvements in diagnostic sensitivity. Subgroup analysis according to screening methodology demonstrated significant differences in HIV prevalence ($p < 0.001$) indicating that screening methodology contributed to variability in reported prevalence estimates. Studies employing mixed screening approaches yielded the highest pooled

Table 1: Studies (n=125) included in the systematic review that assessed the prevalence of HBV among blood donors across Pakistan

S. No.	Author and Year	Province/Region	Sample Size	HIV Reactive (%)	Technique
1.	Mujeeb <i>et al.</i> , 1988 ⁴⁷	Karachi, Sindh	523	3 (0.6%)	ELISA
2.	Khanani <i>et al.</i> , 1988 ⁴⁸	Karachi, Sindh	121	1 (0.82%)	ELISA
3.	Mujeeb <i>et al.</i> , 1991 ⁴⁹	Karachi, Sindh	1,655	3 (0.18%)	ELISA
4.	Raziq <i>et al.</i> , 1993 ⁵⁰	Peshawar, KP	33,402	0	RDT
5.	Mujeeb <i>et al.</i> , 1993 ⁵¹	Karachi, Sindh	666	0	ELISA
6.	Kayani <i>et al.</i> , 1994 ⁵²	Karachi, Sindh	32,127	1 (0.003%)	ELISA
7.	Kakepto <i>et al.</i> , 1996 ⁵³	Karachi & Hyderabad, Sindh	51,127	10 (0.02%)	ELISA
8.	Iqbal and Rehan, 1996 ⁵⁴	Lahore, Punjab	12,482	0	ELISA
9.	Mujeeb <i>et al.</i> , 2000 ⁵⁵	Karachi, Sindh	612	0	RDT
10.	Ahmed <i>et al.</i> , 2000 ⁵⁶	Abbottabad, KP	960	0	RDT
11.	Rahman <i>et al.</i> , 2002 ⁵⁷	Punjab	1,176,284	12 (0.001%)	ELISA
12.	Khattak <i>et al.</i> , 2002 ⁵⁸	Rawalpindi, Punjab	103,858	7 (0.007%)	ELISA
13.	Mumtaz <i>et al.</i> , 2002 ⁵⁹	Rawalpindi, Punjab	553	0	RDT
14.	Khan <i>et al.</i> , 2002 ⁶⁰	Peshawar, KP	23,278	2 (0.008%)	ELISA
15.	Shiekh <i>et al.</i> , 2004 ⁶¹	Quetta, Balochistan	5,000	11 (0.22%)	ELISA
16.	Asif <i>et al.</i> , 2004 ⁶²	ICT	3,430	8 (0.25%)	CLIA
17.	Zaidi <i>et al.</i> , 2004 ⁶³	Peshawar, KP	41,047	0	ELISA
18.	Mahmood <i>et al.</i> , 2004 ⁶⁴	Multan, Punjab	6,000	0	ELISA
19.	Sirhindi <i>et al.</i> , 2005 ⁶⁵	Lahore, Punjab	18,216	0	RDT
20.	Jehangir <i>et al.</i> , 2006 ⁶⁶	Multan, Punjab	25,631	0	ELISA
21.	Khan <i>et al.</i> , 2006 ⁶⁷	Liaquatpur, Punjab	1,426	0	RDT
22.	Fayyaz <i>et al.</i> , 2006 ⁶⁸	Bahawalpur, Punjab	27,938	0	ELISA
23.	Sultan <i>et al.</i> , 2007 ⁶⁹	Lahore, Punjab	41,498	11 (0.03%)	ELISA
24.	Azam <i>et al.</i> , 2007 ⁷⁰	Karachi, Sindh	688	1 (0.14%)	RDT
25.	Khan <i>et al.</i> , 2007 ⁷¹	Rawalpindi, Punjab	139,163	0	RDT
26.	Bhatti <i>et al.</i> , 2007 ⁷²	Rawalpindi, Punjab	94,177	4 (0.004%)	ELISA
27.	Siddiqui <i>et al.</i> , 2008 ⁷³	Multan, Punjab	1,104	0	RDT
28.	Niazi <i>et al.</i> , 2008 ⁷⁴	Rawalpindi, Punjab	4,427	0	CLIA
29.	Manzoor <i>et al.</i> , 2009 ⁷⁵	Lahore, Punjab	6,659	3 (0.05%)	ELISA
30.	Shah <i>et al.</i> , 2009 ⁷⁶	Lahore, Punjab	60,719	3 (0.006%)	ELISA
31.	Qazi <i>et al.</i> , 2010 ⁷⁷	Peshawar, KP	100	0	RDT
32.	Shaikh <i>et al.</i> , 2010 ⁷⁸	Karachi, Sindh	2,000	0	ELISA
33.	Khan <i>et al.</i> , 2011 ⁷⁹	Peshawar, KP	3,915	0	ELISA
34.	Borhany <i>et al.</i> , 2011 ⁸⁰	Karachi, Sindh	5,717	0	ELISA
35.	Safi <i>et al.</i> , 2011 ⁸¹	Peshawar, KP	62,251	28 (0.045%)	ELISA
36.	Attaullah <i>et al.</i> , 2012 ⁸²	Peshawar, KP	127,828	77 (0.06%)	ELISA
37.	Waheed <i>et al.</i> , 2012 ⁸³	ICT	10,145	0	ELISA
38.	Umair <i>et al.</i> , 2012 ⁸⁴	Mirpur, AJK	8,927	0	ELISA
39.	Zahra <i>et al.</i> , 2013 ⁸⁵	Karachi, Sindh	13,170	7 (0.05%)	CLIA
40.	Butt <i>et al.</i> , 2013 ⁸⁶	Kasur, Punjab	833	0	RDT
41.	Irfan <i>et al.</i> , 2013 ⁸⁷	Karachi, Sindh	108,598	111 (0.10%)	CLIA
42.	Khattak <i>et al.</i> , 2014 ⁸⁸	Peshawar, KP	6,000	8 (0.13%)	ELISA
43.	Kamran <i>et al.</i> , 2014 ⁸⁹	Rawalpindi, Punjab	300	0	RDT
44.	Moiz <i>et al.</i> , 2014 ⁹⁰	Karachi, Sindh	42,830	29 (0.06%)	NAT
45.	Zaheer <i>et al.</i> , 2014 ⁹¹	ICT	160,376	27 (0.017%)	ELISA
46.	Moiz <i>et al.</i> , 2015 ⁹²	Karachi, Sindh	2,023,379	142 (0.001%)	CLIA
47.	Sheikh <i>et al.</i> , 2015 ⁹³	Gwadar, Balochistan	300	3 (1.0%)	ELISA
48.	Dossal <i>et al.</i> , 2015 ⁹⁴	Karachi, Sindh	23,559	12 (0.051%)	ELISA
49.	Niazi <i>et al.</i> , 2015 ⁹⁵	Rawalpindi, Punjab	56,772	12 (0.02%)	NAT
50.	Qasim <i>et al.</i> , 2015 ⁹⁶	Mirpur, AJK	8,927	0	ELISA
51.	Hussain <i>et al.</i> , 2015 ⁹⁷	Multan, Punjab	48,020	5 (0.01%)	RDT
52.	Wazeer <i>et al.</i> , 2015 ⁹⁸	Mirpur, AJK	4,622	0	ELISA
53.	Waheed <i>et al.</i> , 2016 ⁹⁹	ICT	65,376	45 (0.6%)	CLIA
54.	Raza <i>et al.</i> , 2016 ¹⁰⁰	Karachi, Sindh	33,595	34 (0.10%)	NAT
55.	Niazi <i>et al.</i> , 2016 ¹⁰¹	Rawalpindi, Punjab	160,552	26 (0.02%)	ELISA
56.	Ghani <i>et al.</i> , 2016 ¹⁰²	Rawalpindi, Punjab	626,413	66 (0.01%)	CLIA
57.	Sial <i>et al.</i> , 2016 ¹⁰³	Lahore, Punjab	29,522	20 (0.07%)	CLIA
58.	Sultan <i>et al.</i> , 2016 ¹⁰⁴	Karachi, Sindh	148,268	172 (0.11%)	CLIA
59.	Ghafoor <i>et al.</i> , 2016 ¹⁰⁵	Rahim Yar Khan, Punjab	1,500	2 (0.13%)	RDT
60.	Ahmed <i>et al.</i> , 2016 ¹⁰⁶	Peshawar, KP	8,349	1 (0.01%)	ELISA
61.	Arshad <i>et al.</i> , 2016 ¹⁰⁷	Karachi, Sindh	16,602	6 (0.04%)	CLIA
62.	Saeed <i>et al.</i> , 2017 ¹⁰⁸	Lahore, Punjab	18,274	4 (0.02%)	RDT

63.	Zameer <i>et al.</i> , 2017 ¹⁰⁹	Lahore, Punjab	10,048	11 (0.11%)	RDT
64.	Memon <i>et al.</i> , 2017 ¹¹⁰	Hyderabad, Sindh	4,683	3 (0.06%)	CLIA
65.	Batool <i>et al.</i> , 2017 ¹¹¹	Peshawar, KP	41,033	30 (0.07%)	CLIA
66.	Ahmad <i>et al.</i> , 2017 ¹¹²	Peshawar, KP	3,679	4 (0.10%)	CLIA
67.	Sultan <i>et al.</i> , 2017 ¹¹³	Karachi, Sindh	16,957	14 (0.08%)	NAT
68.	Raza <i>et al.</i> , 2018 ¹¹⁴	Karachi, Sindh	16,660	12 (0.7%)	NAT
69.	Awan <i>et al.</i> , 2018 ¹¹⁵	ICT	30,470	49 (0.16%)	NAT
70.	Azeem <i>et al.</i> , 2018 ¹¹⁶	KP	204,942	144 (0.07%)	ELISA
71.	Zeeshan <i>et al.</i> , 2018 ¹¹⁷	Mardan, KP	4,552	2 (0.04%)	CLIA
72.	Saba & Afridi 2018 ¹¹⁸	Peshawar, KP	4,237	15 (0.35%)	CLIA
73.	Akhtar <i>et al.</i> , 2018 ¹¹⁹	Multan, Punjab	7,270	38 (0.52%)	RDT
74.	Ali <i>et al.</i> , 2018 ¹²⁰	Karachi, Sindh	49,687	11 (0.02%)	CLIA
75.	Siddiqui <i>et al.</i> , 2011 ¹²¹	ICT	847	2 (0.24%)	CLIA
76.	Aslam <i>et al.</i> , 2019 ¹²²	Multan, Punjab	1,014	0	RDT
77.	Mengal <i>et al.</i> , 2019 ¹²³	Quetta, Balochistan	23,814	24 (0.10%)	CLIA
78.	Ahmed <i>et al.</i> , 2019 ¹²⁴	Muzaffarabad, AJK	10,632	14 (0.13%)	CLIA
79.	Rauf & Cheema 2019 ¹²⁵	Faisalabad, Punjab	6,594	12 (0.18%)	CLIA
80.	Zahoor <i>et al.</i> , 2019 ¹²⁶	Peshawar, KP	11,760	19 (0.16%)	CLIA
81.	Rana <i>et al.</i> , 2019 ¹²⁷	Karachi, Sindh	3,610	2 (0.06%)	CLIA
82.	Zaheer <i>et al.</i> , 2019 ¹²⁸	ICT	75,348	70 (0.09%)	CLIA
83.	Jahan <i>et al.</i> , 2019 ¹²⁹	Faisalabad, Punjab	1,942	10 (0.5%)	ELISA
84.	Sadiq <i>et al.</i> , 2019 ¹³⁰	Nawabshah, Sindh	37,845	2,389 (6.33%)	CLIA
85.	Haq <i>et al.</i> , 2020 ¹³¹	Peshawar, KP	8,634	63 (0.73%)	NAT
86.	Saeed <i>et al.</i> , 2020 ¹³²	ICT	18,500	476 (5.1%)	CLIA
87.	Ghazanfer & Osman 2021 ¹³³	Sialkot, Punjab	84,305	10 (0.01%)	RDT
88.	Sijjeel <i>et al.</i> , 2021 ¹³⁴	ICT	143,053	237 (0.16%)	CLIA
89.	Saeed and Ullah 2021 ¹³⁵	ICT	312,320	124 (0.04%)	ELISA
90.	Arshad <i>et al.</i> , 2021 ¹³⁶	Lahore, Punjab	200	1 (0.5%)	ELISA
91.	Qadir <i>et al.</i> , 2021 ¹³⁷	Karachi, Sindh	29,732	168 (0.5%)	CLIA
92.	Saba <i>et al.</i> , 2021 ¹³⁸	Peshawar, KP	41,817	96 (0.23%)	CLIA
93.	Bhatti <i>et al.</i> , 2021 ¹³⁹	Sialkot, Punjab	400	1 (3.84%)	RDT
94.	Samo <i>et al.</i> , 2021 ¹⁴⁰	Hyderabad, Sindh	353	3 (0.8%)	RDT
95.	Khan <i>et al.</i> , 2021 ¹⁴¹	Peshawar, KP	119,263	21 (0.01%)	ELISA
96.	Ghazanfar <i>et al.</i> , 2022 ¹⁴²	Rawalpindi, Punjab	223,242	0	RDT
97.	Bhatti <i>et al.</i> , 2022 ¹⁴³	ICT	120,968	125 (0.1%)	NAT
98.	Ullah <i>et al.</i> , 2022 ¹⁴⁴	Multan, Punjab	7,858	1 (0.01%)	ELISA
99.	Ahmed <i>et al.</i> , 2022 ¹⁴⁵	Karachi, Sindh	23,656	15 (0.06%)	ELISA
100.	Shah <i>et al.</i> , 2023 ¹⁴⁶	Peshawar, KP	6,311	0	ELISA
101.	Sabir <i>et al.</i> , 2023 ¹⁴⁷	Rawalpindi, Punjab	308,767	806 (0.26%)	NAT
102.	Wazeer <i>et al.</i> , 2023 ¹⁴⁸	Mirpur, AJK	7,356	0	CLIA
103.	Zorob <i>et al.</i> , 2023 ¹⁴⁹	Rawalpindi, Punjab	15,405	29 (0.19%)	CLIA
104.	Amjed <i>et al.</i> , 2023 ¹⁵⁰	Lahore, Punjab	2,236	9 (0.40%)	CLIA
105.	Raza <i>et al.</i> , 2023 ¹⁵¹	Faisalabad, Punjab	1,500	1 (0.06%)	RDT
106.	Aslam <i>et al.</i> , 2023 ¹⁵²	Lahore, Punjab	6,380	2 (0.03%)	ELISA
107.	Zeeshan <i>et al.</i> , 2023 ¹⁵³	Peshawar, KP	6,378	6 (0.1%)	ELISA
108.	Qadir <i>et al.</i> , 2023 ¹⁵⁴	DI Khan, KP	3,329	9 (0.27%)	CLIA
109.	Jadoon <i>et al.</i> , 2023 ¹⁵⁵	Laki Marwat, KP	100	0	RDT
110.	Hassni <i>et al.</i> , 2024 ¹⁵⁶	ICT	523	5 (1.0%)	CLIA
111.	Jamal <i>et al.</i> , 2024 ¹⁵⁷	Sindh	4,199,195	6,711 (0.015%)	RDT, ELISA & CLIA
112.	Babar <i>et al.</i> , 2024 ¹⁵⁸	Swabi, KP	10,939	44 (0.40%)	ELISA
113.	Saeed <i>et al.</i> , 2024 ¹⁵⁹	Lahore, Punjab	6,616	6 (2.3%)	CLIA
114.	Waheed <i>et al.</i> , 2024 ¹⁶⁰	Karachi, Sindh	56,732	151 (0.30%)	CLIA
115.	Saba <i>et al.</i> , 2024 ¹⁶¹	Peshawar, KP	62,185	151 (0.24%)	CLIA
116.	Sarwar <i>et al.</i> , 2024 ¹⁶²	Faisalabad, Punjab	491	0	ELISA
117.	Naz <i>et al.</i> , 2024 ¹⁶³	DI Khan, KP	25,000	152 (0.61%)	ELISA
118.	Hussain <i>et al.</i> , 2025 ¹⁶⁴	Skardu, GB	1,400	2 (0.14%)	RDT
119.	Akbar <i>et al.</i> , 2025 ¹⁶⁵	Quetta, Balochistan	1,680	5 (0.3%)	ELISA
120.	Sabir <i>et al.</i> , 2025 ¹⁶⁶	Rawalpindi, Punjab	275,014	367 (0.13%)	NAT
121.	Ullah <i>et al.</i> , 2025 ¹⁶⁷	ICT	9,213	13 (0.14%)	CLIA
122.	Kousar <i>et al.</i> , 2025 ¹⁶⁸	Karachi, Sindh	46,455	0	NAT
123.	Shah <i>et al.</i> , 2025 ¹⁶⁹	Malakand, KP	94,716	220 (0.23%)	CLIA
124.	Iqbal <i>et al.</i> , 2026 ¹⁷⁰	Mardan, KP	63,847	45 (0.07%)	CLIA
125.	Sajjad <i>et al.</i> , 2026 ¹⁷¹	Karachi, Sindh	8,265	25 (0.30%)	ELISA
Total			12,663,749	13,892 (0.109%)	

RDT: Rapid Diagnostic Test; ELISA: Enzyme Linked Immunosorbent Assay; CLIA: Chemiluminescence Immunoassay; NAT: Nucleic Acid Testing; AJK: Azad Jammu & Kashmir; ICT: Islamabad Capital Territory

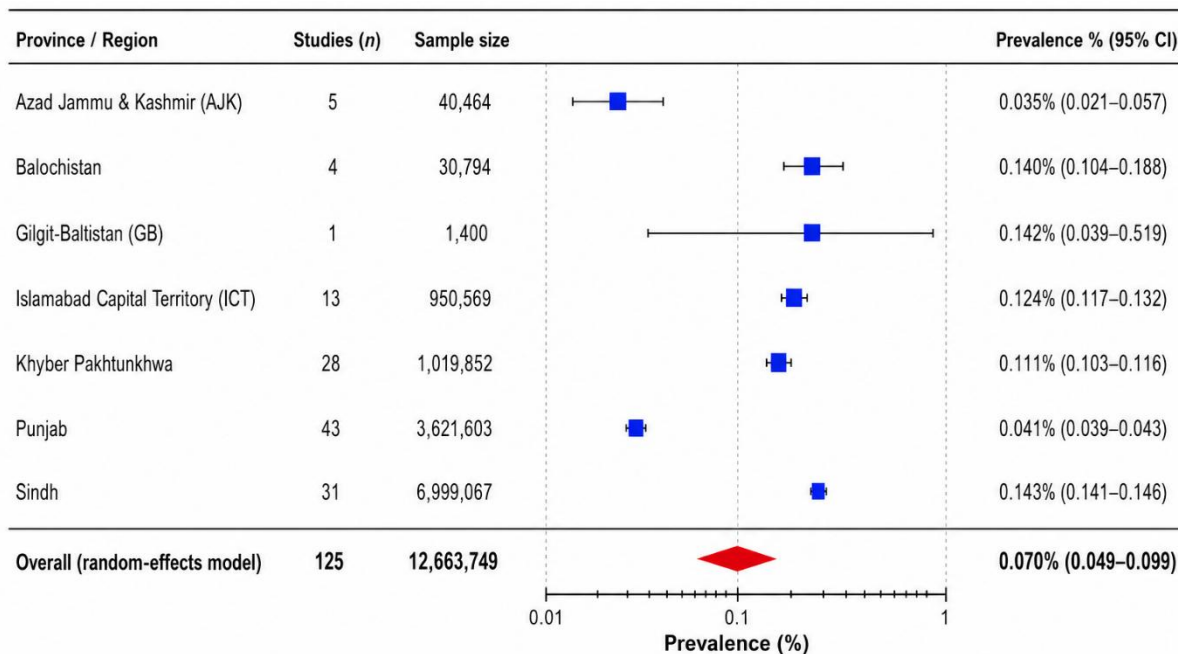


Fig. 2: Forest plot of pooled HIV prevalence by province/region among blood donors in Pakistan

prevalence (0.160%), followed by NAT (0.158%), CLIA/CMIA/ECLIA (0.121%), ELISA (0.027%), and RDTs (0.013%). These findings (Fig. 4) likely reflect differences in analytical sensitivity and the ability of advanced screening assays to detect infections during the serological window period of HIV.

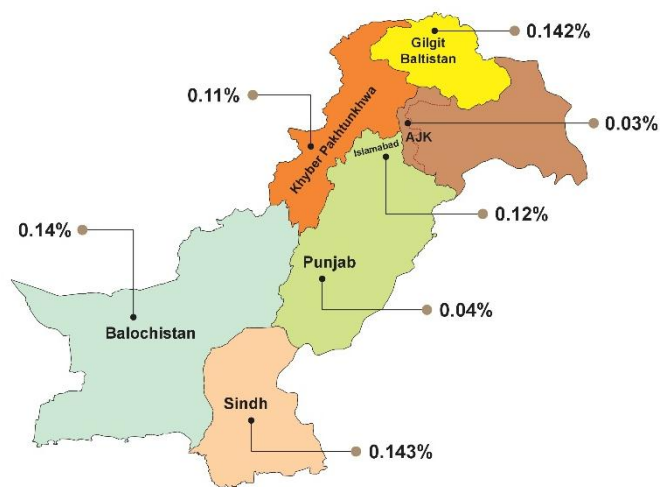


Fig. 3: Geographical distribution of HBV prevalence across Pakistan

Temporal analysis demonstrated (Fig. 5) a statistically significant increasing trend in HIV prevalence among blood donors over the study period ($p < 0.001$). Meta-regression using study year as a continuous covariate showed that the odds of HIV positivity increased by approximately 14.4% per calendar year (OR = 1.144; 95% CI: 1.139–1.149; $p < 0.001$).

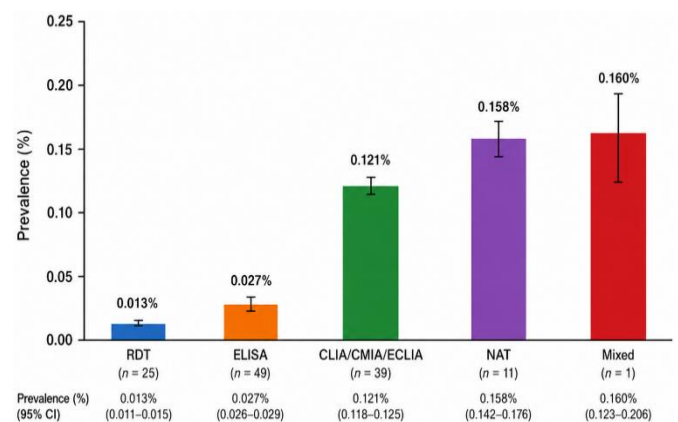


Fig. 4: HIV prevalence analysis according to screening methodology

Period-wise analysis showed that prevalence increased from 0.004% during 1988–2009 to 0.017% during 2010–2015, peaked at 0.223% during 2016–2020, and remained elevated at 0.150% during 2021–2026, indicating a sustained upward trend in HIV prevalence among blood donors.

Meta-regression analysis demonstrated a statistically significant increasing trend in HIV prevalence among blood donors in Pakistan over time. Study year was positively associated with HIV prevalence, with a regression coefficient of $\beta = 0.1346$ (95% CI: 0.1300–0.1392; $p < 0.001$). The meta-regression bubble plot (Fig. 6) visually confirmed this upward trend, showing that studies conducted in more recent years generally

reported higher HIV prevalence compared with earlier studies. Each bubble in figure 6 represents one study, with bubble size proportional to study size or statistical weight. The upward regression line indicates a significant increase in HIV prevalence over time). Although some dispersion of values was observed, the overall slope of the regression line remained consistently positive, indicating a clear temporal increase in HIV prevalence among blood donors. These findings are in agreement with the period-wise temporal

analysis, which showed that prevalence rose from very low levels in the earlier years to substantially higher levels in recent periods. Visual inspection of the funnel plot suggested asymmetry, which was supported by Egger's regression test (intercept = -4.42, $p = 0.011$), indicating the presence of potential publication bias or small-study effects. However, the substantial clinical and methodological heterogeneity across studies may also have contributed to the observed asymmetry (Fig. 7).

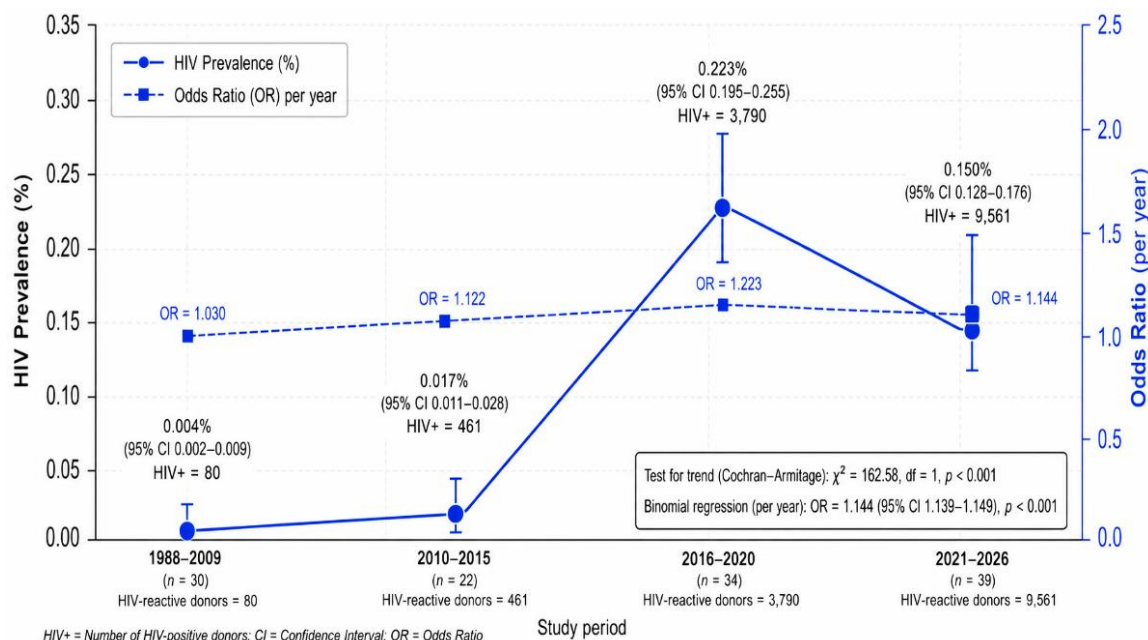


Fig. 5: Temporal trend analysis of HIV prevalence in blood donors

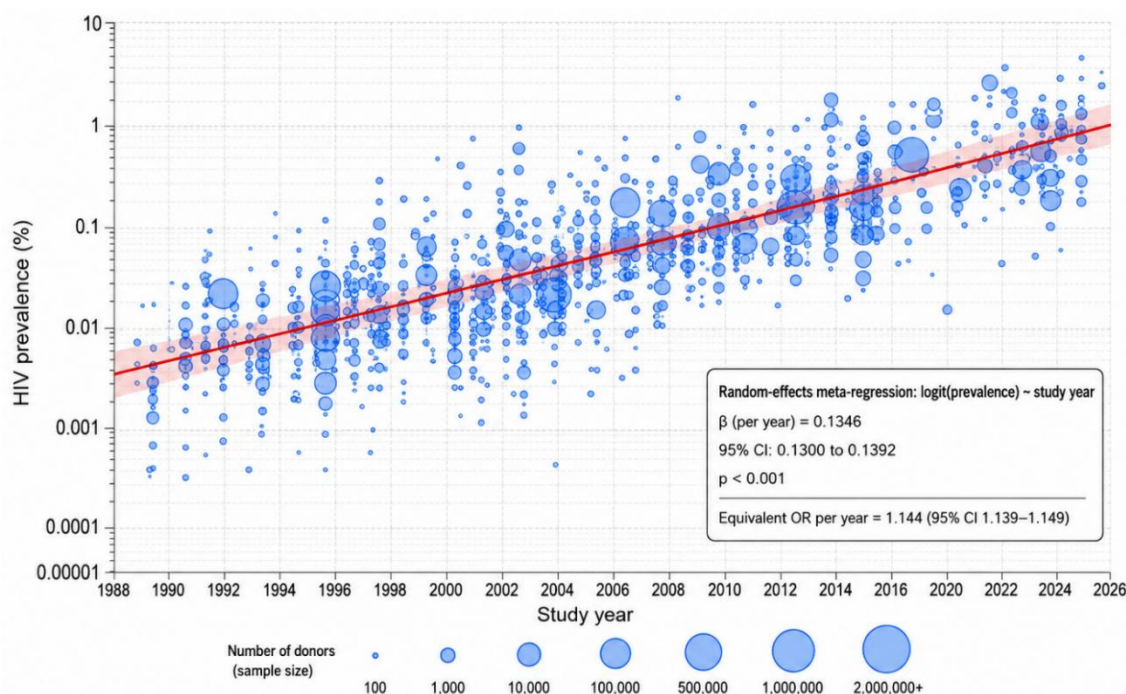


Fig. 6: Meta-regression bubble plot showing the relationship between study year and HIV prevalence among blood donors in Pakistan

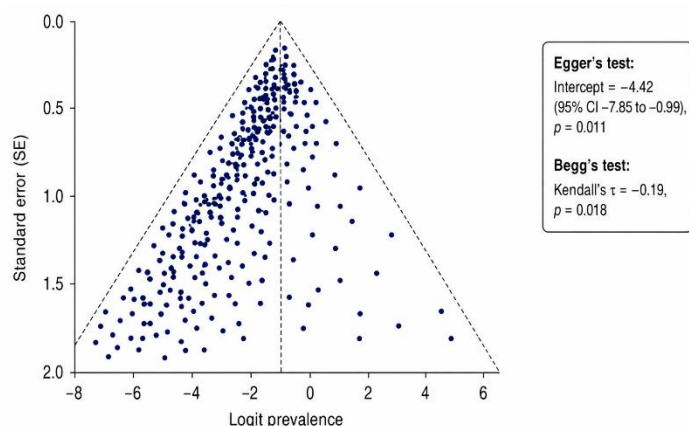


Fig. 7: Funnel plot showing visual asymmetry suggesting potential publication bias, small-study effects, or heterogeneity

Leave-one-out sensitivity analysis using the metaninf approach demonstrated (Fig. 8) that the pooled prevalence remained stable following sequential exclusion of each individual study, ranging from 0.067% to 0.075%, compared with the overall pooled estimate of 0.070% (95% CI: 0.049-0.099). Exclusion of the study by Sadiq *et al.*, (2019)¹³⁰ produced the largest change in the pooled estimate (0.075%) and reduced heterogeneity ($I^2 = 98.6\%$), suggesting that this study contributed substantially to between-study variability. Nevertheless, no single study materially altered the overall findings, confirming the robustness of the meta-analysis.

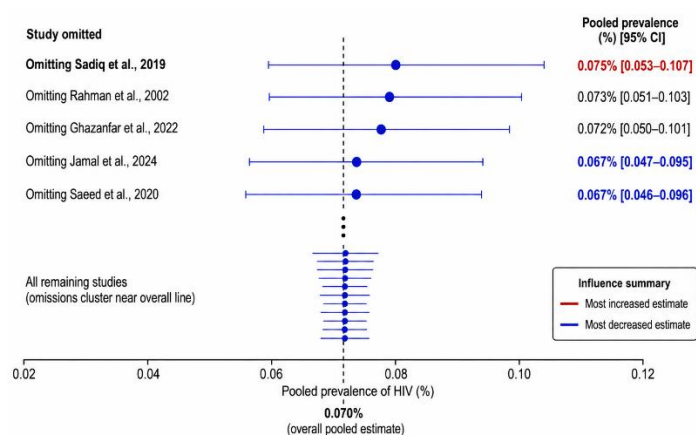


Fig. 8: Leave-one-out sensitivity analysis using the metaninf approach

Discussion

HIV infection remains a major threat to blood transfusion safety, particularly in developing countries. In Pakistan, the majority of blood donations (82%)¹⁷² are collected from family or

replacement donors rather than voluntary non-remunerated blood donors (VNRBDs), which represent the gold standard recommended by WHO, ISBT and national blood safety legislation for ensuring a safe and sustainable blood supply.¹⁷³⁻¹⁷⁵

Replacement donation occurs when relatives or friends donate blood on behalf of a patient requiring transfusion. This practice may increase the risk of transfusion-transmissible infections (TTIs), including HIV, as donors often feel compelled to donate despite having limited knowledge of their health status or potential risk factors. Consequently, strengthening voluntary blood donation programme remains essential for improving blood safety and reducing the transmission of infectious diseases through transfusion.¹⁷⁶

Although blood donor screening and HIV testing have improved at many blood centres, Pakistan still lacks a standardized system for notifying blood donors of their HIV test results and ensuring timely linkage to HIV testing, care, and treatment services. This gap not only limits access to early diagnosis and treatment but also represents a missed opportunity for preventing onward transmission and strengthening public health surveillance.

This systematic review and meta-analysis represent the first comprehensive synthesis of HIV prevalence among blood donors in Pakistan, encompassing 125 studies conducted over a period of nearly four decades (1988-2026) and including more than 12.6 million blood donations. The overall prevalence of HIV among blood donors was found to be 0.109%, indicating that although HIV prevalence in the donor population remains relatively low compared with the general epidemic in some high-risk groups, it continues to pose a significant challenge to blood safety in the country.

The prevalence of HIV among blood donors in Pakistan (0.109%) is lower than that reported from several countries in sub-Saharan Africa but remains higher than that observed in many Asian and high-income settings. A 2023 study by Thakur *et al.*, from India reported a pooled HIV prevalence of 0.20% among blood donors,¹⁷⁷ which is almost twice the estimate observed in the present study, whereas substantially lower

prevalences have been reported from China (0.00992%),¹⁷⁸ Bangladesh (0.01%),¹⁷⁹ Iran (0.0047%),¹⁸⁰ and Afghanistan (0.04%).¹⁸¹ The relatively lower prevalence in these countries may reflect stronger donor selection systems, greater reliance on voluntary non-remunerated blood donation, and more comprehensive public health interventions aimed at HIV prevention and control.

In contrast, considerably higher HIV prevalences have been documented in several African countries, including South Africa (0.381%),¹⁸² Ethiopia (0.5%),¹⁸³ Nigeria (1.1%),¹⁸⁴ Sudan (2.61%),¹⁸⁵ Uganda (1.9%),¹⁸⁶ and Kenya (3.5%).¹⁸⁷ These differences are likely attributable to variations in the underlying burden of HIV in the general population, the stage of the epidemic, socioeconomic determinants, healthcare infrastructure, and the effectiveness of blood safety programmes. Although Pakistan continues to be classified as a country with a concentrated epidemic, the observed increase in HIV prevalence among blood donors over time suggests that the infection is progressively extending beyond traditional high-risk groups and poses an increasing challenge to transfusion safety.¹⁸⁸

A notable finding of this study is the substantial geographical variation in HIV prevalence across provinces and regions. Sindh exhibited the highest burden, followed by Balochistan and Islamabad Capital Territory, whereas Punjab and Azad Jammu & Kashmir showed comparatively lower prevalence rates. The higher prevalence observed in Sindh may partly reflect the well-documented HIV outbreaks reported in the province over the past decade, particularly among vulnerable populations, as well as its large urban centres and population mobility.^{189,190} Similarly, the findings from Balochistan may be influenced by cross-border migration, limited healthcare access, and relatively weaker surveillance systems. Such regional disparities emphasize the need for province-specific interventions and targeted blood safety strategies. The temporal analysis demonstrated a significant increase in HIV prevalence among blood donors over time, particularly after 2015. The increasing slope observed in the meta-regression suggests that the burden of HIV in the donor population is not static and may be rising in parallel with the evolving HIV epidemic in the

country. This has important implications for blood safety, as blood donors are often considered a relatively low-risk population. A rising trend in donor seroprevalence may indicate broader transmission in the community, possible bridging from high-risk groups to the general population, and the need for strengthened donor selection and surveillance systems. These findings are consistent with reports indicating an expanding HIV epidemic in Pakistan, driven by increased transmission among key populations and subsequent bridging into the general population.¹⁹¹⁻¹⁹² The emergence of multiple localized outbreaks, together with growing concerns regarding infection control practices and unsafe medical procedures, has heightened national attention toward HIV prevention and control. The constitution of a national task force by the Government of Pakistan in May 2026 further reflects the seriousness of the evolving epidemic and the urgent need for coordinated public health responses.

Differences in HIV prevalence according to screening methodology were also evident. Studies employing NAT and CLIA-based assays reported higher prevalence rates compared with those using conventional ELISA and RDTs. This likely reflects the superior analytical sensitivity of advanced screening technologies, particularly their ability to detect infections during the serological window period. Therefore, differences in screening methodology may partly explain the heterogeneity observed across studies. These findings support the need for quality-assured, highly sensitive screening platforms and standardized testing strategies to improve blood safety and obtain more accurate estimates of HIV burden among blood donors in Pakistan. The gradual introduction of NAT in selected regional blood centres and tertiary care institutions therefore represents an important advancement in enhancing blood safety. Nevertheless, widespread implementation remains constrained by financial and infrastructural limitations, particularly in resource-limited settings.

The present meta-analysis demonstrated considerable between-study heterogeneity, which may be attributed to differences in geographical location, study period, donor demographics, blood donation practices, screening techniques, and sample sizes. The leave-one-out sensitivity analysis confirmed the robustness of the pooled

estimate, as no individual study substantially altered the overall prevalence. Although certain studies contributed more prominently to heterogeneity, the overall conclusions remained unchanged, indicating the stability and reliability of the findings. The presence of funnel plot asymmetry and significant Egger's and Begg's tests suggest potential publication bias or small-study effects; however, the marked methodological and regional diversity among studies may also account for this observation.

The findings of this review have important policy implications for Pakistan's blood transfusion system. Expansion of voluntary non-remunerated blood donation, universal implementation of high-sensitivity screening technologies such as NAT, improved donor education, and stronger integration between NACP and blood transfusion services will be critical to preventing further increases in transfusion-transmitted HIV and timely public health interventions. Strengthening haemovigilance and regulatory enforcement through the blood transfusion authorities across all public and private blood centres should therefore remain a national priority.

This study has several strengths, including its large sample size, comprehensive geographical coverage, and inclusion of nearly four decades of data. Nevertheless, certain limitations should be acknowledged. The included studies exhibited substantial heterogeneity with respect to methodology and screening techniques. Several regions, particularly Gilgit-Baltistan and Balochistan, were underrepresented, limiting the precision of regional estimates. In addition, many studies relied solely on serological assays without confirmatory testing or nucleic acid testing, potentially resulting in underestimation or overestimation of the true prevalence. Despite these limitations, the present work provides the most comprehensive evidence to date regarding

HIV prevalence among blood donors in Pakistan and establishes an important baseline for future surveillance, policy formulation, and research.

Conclusion

The current analysis included 125 studies and more than 12.6 million blood donors over a 39-year period, providing the first comprehensive national estimate of HIV prevalence among blood donors in Pakistan. Although the overall prevalence remains relatively low (0.109%), significant geographical disparities and an increasing temporal trend underscore the evolving nature of the HIV epidemic and its implications for blood safety. Strengthening voluntary non-remunerated blood donation, expanding access to high-sensitivity screening technologies (e.g. NAT), enhancing haemovigilance, and integrating blood donor surveillance with NACP are essential to further reduce the risk of transfusion-transmitted HIV and ensure a safe and sustainable blood supply in Pakistan.

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Data sharing statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declaration of interests

The authors declare that they have no competing interests.

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