

The National Burden of Hepatitis B Among Blood Donors in Pakistan: A Systematic Review and Meta-Analysis (1991-2025)

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ABSTRACT

Introduction: Hepatitis B virus (HBV) infection remains one of the most significant global health challenges, capable of causing both acute and chronic liver disease. Ensuring safe transfusion practices is critical to minimize HBV transmission. Regular estimation of HBV burden among blood donors provides valuable insights into infection trends, helps identify high-risk regions, and guides evidence-based policies for prevention and control.

Objective: This systematic review and meta-analysis aim to assess the burden of HBV among blood donors in Pakistan from 1991 to 2025, offering insight into the epidemiology and regional disparities in the prevalence of the virus.

Methodology: The protocol for this systematic review was registered with PROSPERO under the registration CRD420251183056. A comprehensive literature search was carried out across multiple electronic databases, including PubMed, Scopus, ScienceDirect, Web of Science, Embase, PakMediNet, Google Scholar, and the Cochrane Library. The search covered the period from January 1991 to November 2025. A standardized data extraction form was developed to ensure uniformity. The methodological quality of included studies was assessed using a 12-point checklist adapted from the Downs and Black tool.

Results: A total of 137 records representing 139 unique studies were included in the review. The overall pooled prevalence of HBV among blood donors in different provinces and regions of Pakistan, based on the 35 years of data from 1991 to 2025, was 1.10% (110,126 positives out of 10,015,962 samples). Among the 139 studies, 132 (94.96%) screened for HBsAg, while 7 (5.04%) screened for HBV DNA. There was a consistent decline in HBV seroprevalence from above 4% in the 1990s to around 1% in recent years (2021-2025). Marked regional disparities persist across Pakistan, with Gilgit-Baltistan (3.63%) and Khyber Pakhtunkhwa (2.07%) exhibiting higher HBV prevalence.

Conclusion: The collective evidence from 139 studies indicates a steady epidemiological transition from intermediate toward low endemicity of HBV among Pakistan's donor population. The results not only highlight the substantial progress made in blood safety in Pakistan but also provide an essential baseline for post-2025 HBV elimination targets, aligning with the WHO's goal of reducing new hepatitis infections by 90% by 2030.

Keywords: Epidemiology, Seroprevalence, Hepatitis B, HBV, Hepatitis B surface antigen, Pakistan, Meta analysis

Introduction

Hepatitis B virus (HBV) infection remains one of the most significant global health challenges, capable of causing both acute and chronic liver disease. Chronic HBV infection can lead to cirrhosis, hepatocellular carcinoma, and death.

The World Health Organization (WHO) estimates that approximately 1.1 million deaths occurred from HBV-related complications in 2023, underscoring its continued public health importance.¹ Despite the availability of a highly effective vaccine, an estimated 254 million

individuals worldwide were living with chronic hepatitis B in 2024, with notable regional variations.² Each year, HBV is responsible for around 1.2 million new infections globally.³

The global burden of HBV infection is unevenly distributed. The Western Pacific and African regions bear the greatest burden, harboring 116 million and 81 million chronically infected individuals, respectively. The Eastern Mediterranean region (including Pakistan) accounts for about 60 million cases, followed by

Southeast Asia (18 million), Europe (14 million), and the Americas (5 million).⁴ Nearly 80% of countries have declared viral hepatitis a serious public health concern,⁵ and the majority of chronic carriers reside in Asia and Africa.⁶

Recognizing the magnitude of this threat, the WHO Global Health Sector Strategy (2016-2030) called for the elimination of viral hepatitis as a public health problem by 2030.⁷ The elimination goals were defined as a 90% reduction in new infections and a 65% reduction in mortality compared with 2015 levels. The World Health Assembly (in 2016) further set a target of achieving an HBsAg prevalence below 0.1% among children under five years by 2030.⁸

Although there is no specific curative treatment for acute HBV infection, supportive care focuses on alleviating symptoms such as dehydration, vomiting, and diarrhea. In severe cases, acute liver failure may occur, which can be fatal.⁹ Accurately estimating HBV-related mortality, however, remains challenging due to underreporting and misclassification on death certificates.^{10,11}

Universal hepatitis B vaccination, recommended by the WHO since 1992, has proven to be one of the most effective public health measures for controlling HBV transmission.¹² Global vaccination coverage has significantly reduced HBV prevalence among children under five years, declining from 4.4% in 1990 to 0.9% in 2020.¹³ In Pakistan, the estimated vaccination coverage is approximately 74%, though regional disparities persist.¹⁴

HBV transmission occurs primarily through exposure to infected blood or body fluids.¹⁵ In recognition of this risk, the WHO has long recommended that all donated blood be screened for major transfusion-transmissible infections (TTIs), including hepatitis viruses. Blood safety has therefore become a cornerstone of public health policy since the early 2000s.¹⁶ The recruitment of voluntary non-remunerated blood donors (VNRBDs) and the promotion of regular repeat donations have been shown to improve the overall safety of blood supplies.^{17,18}

In Pakistan, hepatitis B remains a major public health burden, affecting an estimated 5-6 million

individuals and causing approximately 12,000 deaths annually.¹⁴ Transmission occurs through multiple routes, including perinatal exposure, unsafe injections, unsterile medical procedures, and transfusions of unscreened blood. Blood donors, though generally considered a low-risk group, may serve as an important sentinel population for estimating the prevalence of HBV and other TTIs in the community. Variations in infection rates among blood donors are influenced by socioeconomic status, geographic region, and the background prevalence of HBV in the general population.^{19,20}

Pakistan has made notable progress in improving blood safety. Screening for hepatitis B surface antigen (HBsAg) began in Pakistan during the mid-1980s and became mandatory following the first provincial blood safety legislation enacted in Sindh in 1997, later adopted by other provinces.²¹⁻²³ The introduction of the national immunization programme, which includes neonatal HBV vaccination, has contributed to changing the epidemiological landscape of hepatitis B in the country. The national survey by the Ministry of Health estimated the HBsAg prevalence in the general population at 2.5%.²⁴ To address the hepatitis epidemic, the National Hepatitis Strategic Framework (2017-2021) was developed by the Ministry of National Health Services, Regulation, and Coordination (M/o NHSR&C), emphasizing prevention, surveillance, and safe blood transfusion practices. Additionally, provincial initiatives such as the Punjab Hepatitis Action Plan and the Sindh Hepatitis Action Plan further strengthen local responses to HBV control.

Ensuring safe transfusion practices is critical to minimizing HBV transmission. Although achieving absolute zero risk of transfusion-transmitted infection is not feasible, continuous advancements in screening technologies, improved blood banking infrastructure, donor education, and strict regulatory oversight are essential.

Regular estimation of HBV burden among blood donors provides valuable insights into infection trends, helps identify high-risk regions, and guides evidence-based policies for prevention and control.

Therefore, the objective of this systematic review and meta-analysis is to consolidate available data on the prevalence of HBV infection among blood donors in Pakistan between 1991 and 2025, analyze temporal trends, evaluate screening practices, and provide recommendations to strengthen national blood safety strategies.

Methodology

Protocol Registration

A preliminary search was conducted 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 projects. The protocol for this systematic review was registered with PROSPERO under the registration CRD420251183056. As the study is based exclusively on previously published data, no ethical approval was required. This review adhered to the Centre for Evidence-Based Practice standards and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines²⁵ to ensure transparency, reproducibility, and methodological rigor.

Eligibility Criteria

Eligibility criteria were defined using the PICOS (Population, Intervention/Exposure, Comparison, Outcome, Study design) framework, i.e. population (blood donors), intervention/exposure (serological or molecular evidence of hepatitis B virus infection), comparison (no formal comparison groups were required, but studies reporting prevalence differences by region (urban vs. rural) or donor type were included), outcome (prevalence of HBV infection among blood donors, reported as a percentage or proportion), study design (cross-sectional, observational, and cohort studies, as well as national or provincial surveillance reports, were included). Case reports, editorials, reviews, commentaries, and studies with incomplete or duplicated data were excluded.

Search Strategy

A comprehensive literature search was carried out 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 Controlled Register of Controlled Trials). Additional searches were conducted in World Health Organization databases and national health repositories for relevant reports.

The search covered the period from January 1991 to November 2025. Keywords and Medical Subject Headings (MeSH) terms used included "Hepatitis B" OR "HBV" AND "Blood donors" AND "Prevalence" OR "HBsAg" OR "Epidemiology" AND "Pakistan." Boolean operators ("AND," "OR") were applied to refine the search. No language restrictions were initially imposed; however, only studies published in English were included in the final analysis. Reference lists of retrieved articles and relevant reviews were also hand-searched to identify additional eligible studies.

Exclusion criteria included duplicate records, case reports, reviews, editorials, self-reported HBV prevalence, or studies on Pakistani nationals residing abroad. Only studies reporting primary data on HBV incidence or prevalence among blood donors were included.

Study Selection

The study selection process involved two stages. In the first stage, all records retrieved from databases were imported into EndNote™ X8.2²⁶ for duplicate removal. Titles and abstracts were screened independently by two reviewers (UW and SS) to assess relevance. In the second stage, full-text articles of potentially eligible studies were reviewed independently by two reviewers (UW and NS) against the inclusion criteria. Any disagreements were resolved through discussion or by consulting a third reviewer. The overall selection process was illustrated using a PRISMA flow diagram, depicting the number of records identified, screened, excluded, and finally included in the review.

Data Extraction

A standardized data extraction form was developed to ensure uniformity. Two reviewers (UW and OKK) independently extracted data, which were then cross verified for accuracy.

Extracted variables included study characteristics (author(s), publication year, study location, and design; population characteristics (number of donors, age, gender, and setting (urban or rural)); outcome measures (HBV prevalence among blood donors (as a percentage or proportion); and screening methods (type of assay used (e.g., RDT, ELISA, CLIA, PCR). Discrepancies were resolved through discussion to maintain accuracy and reduce bias.

Quality Assessment

The methodological quality of included studies was assessed using a 12-point checklist adapted from the Downs and Black tool, as applied in previous reviews. Three reviewers (NH, AG, and UA) independently scored each study, with results cross-checked by two additional reviewers (UQ and AB). The checklist evaluated: clarity of objectives, study design, representativeness of participants, recruitment period, sample size adequacy, handling of missing data, reporting of demographics and confounders, description of detection methods, and identification of biases and outcomes. Studies were categorized into three quality grades, i.e. Grade A (Excellent): 9-12 points, Grade B (Moderate): 5-8 points, and Grade C (Low): 1-4 points.

Statistical Analysis

Quantitative synthesis was conducted using STATA version 14.2 (StataCorp, College Station, TX, USA). A random-effects model was applied to account for between-study variability. Heterogeneity was evaluated using the Cochran Q test and quantified with the I^2 statistic; heterogeneity was considered significant when $p < 0.05$. Publication bias was assessed through a Funnel plot of transformed proportions versus sample size, with asymmetry evaluated using Egger's test ($p < 0.1$ indicating potential bias).

Pooled prevalence estimates were visualized using a forest plot, displaying individual study estimates, confidence intervals, and the overall summary effect. Descriptive statistics (mean, median, and range) were also computed to illustrate the distribution of HBV prevalence across studies. A time-series analysis (1991-2025) was performed to explore temporal trends

and identify fluctuations or declines in HBV prevalence.

Ethical Considerations

Since the review utilized data extracted from previously published studies, no additional ethical approval was necessary. It is assumed that all included studies had obtained ethical clearance from their respective institutions. The ethical principles outlined in the PRISMA statement were strictly followed to ensure transparency, accuracy, and reproducibility of the findings.

Results

Figure 1 represents the process (flow diagram) by which articles were selected based on PRISMA guidelines. The initial search identified 2,056 records, with an additional 13 records found through handcomb searching. After removing duplicates ($n=344$), 1,725 records remained for title and abstract screening. Following full-text screening and consensus on disagreements, 137 records representing 139 unique studies were included in the review.²⁷⁻¹⁶³

Detailed information on the studies included and their characteristics is shown in Table 1. The overall pooled prevalence of HBV among blood donors in different provinces and regions of Pakistan, based on the 35 years data from 1991 to 2025, was 1.10% (110,126 positives out of 10,015,962 samples). Although substantial heterogeneity exists among studies, the long-term trend demonstrates a gradual decline from $>4\%$ during the early 1990s to $<1\%$ in the most recent surveillance years (2020-2025).

The earliest data was reported from Karachi in 1991 (Zuberi et al.),²⁷ while the most recent studies were reported from 2025 in Skardu, Karachi, Quetta, and Faisalabad.¹⁶⁰⁻¹⁶³ Study designs were predominantly cross-sectional, with testing performed through Rapid Diagnostic Tests (RDTs), Enzyme-Linked Immunosorbent Assay (ELISA), Chemiluminescence Immunoassay (CLIA), and Nucleic Acid Testing (NAT). The diagnostic transition from serology-based screening to molecular testing was evident after 2010, improving detection sensitivity and lowering apparent prevalence estimates in more recent years.

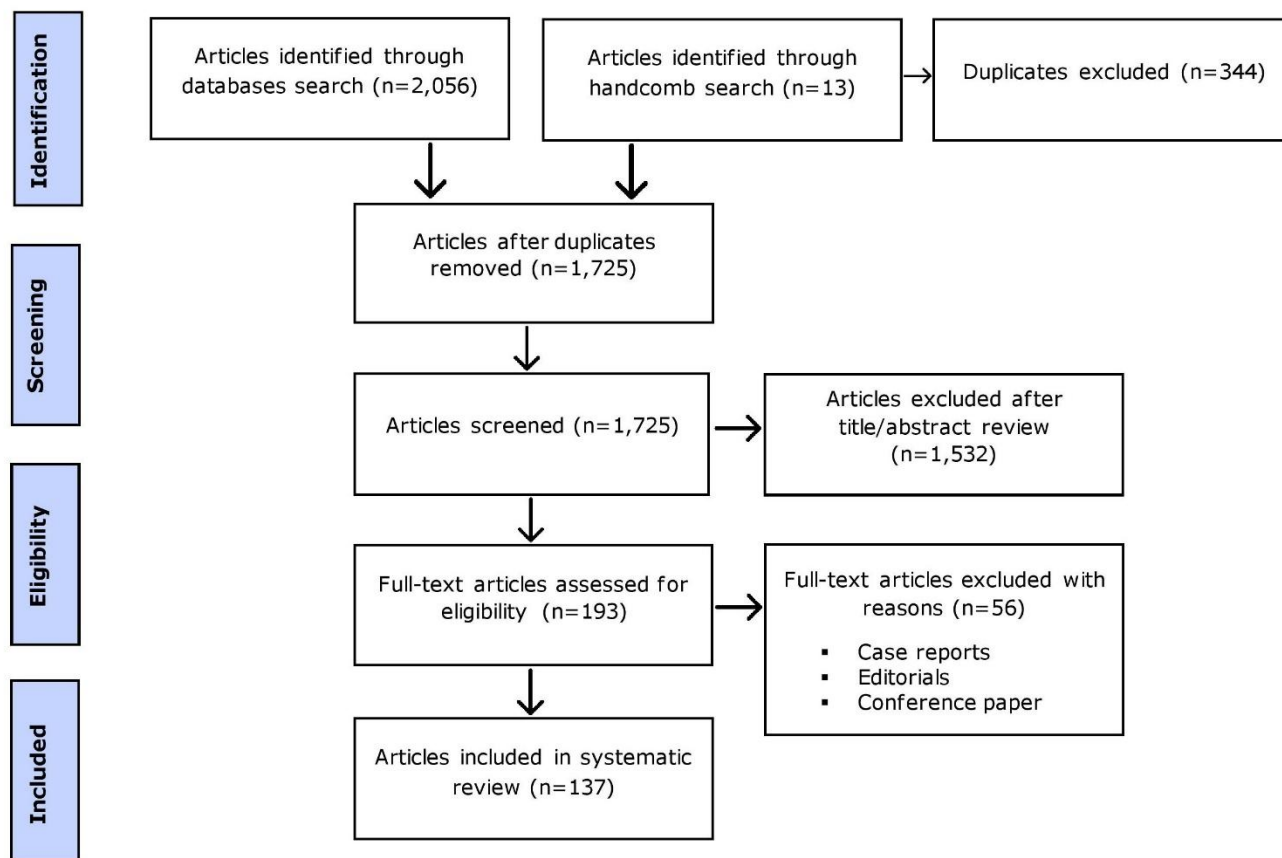


Fig. 1: Flowchart of the process through which articles were selected based on PRISMA guidelines

Among the 139 studies, 132 (94.96%) screened for HBsAg while 7 (5.04%) screened for HBV DNA. Regarding the screening assays used, 23.74% (n=33) studies utilized rapid diagnostic test (RDT), 44.60% (n=62) enzyme-linked immunosorbent assay (ELISA), 25.90% (n=36) chemiluminescence immunoassay (CLIA), 5.04% (n=7) nucleic acid testing (NAT)/polymerase chain reaction (PCR), and 0.72% (n=1) radioimmunoassay (RIA).

Among the included studies, ELISA remained the most frequently applied technique until 2015. From 2015 onward, CLIA and NAT became standard at major regional blood centres (e.g., Islamabad, Rawalpindi, Karachi, Lahore). Regional distribution of studies revealed that majority of the studies were conducted in the province of Punjab 39.56% (n=55), followed by Sindh 23.74% (n=33), Khyber Pakhtunkhwa 17.99% (n=25), Islamabad Capital Territory 10.07% (n=14), Balochistan 2.88% (n=4), Azad Jammu & Kashmir 3.60% (n=5), and Gilgit-Baltistan 2.15% (n=3).

Table 2 shows the pooled subgroup prevalence of HBV among blood donors, stratified by province. High prevalence pockets persisted in northern highland regions (Gilgit-Baltistan 3.63%, parts of Khyber Pakhtunkhwa > 2%) and isolated rural districts of southern Punjab and interior Sindh, likely reflecting lower vaccination coverage and limited donor education. In contrast, urban blood centres of Karachi and Islamabad demonstrated sustained reduction below 1% after 2020.

From the 139 records studied in this systematic review, the overall sample size was 10,015,962 that tested the prevalence of HBV in blood donors. The sample size across the studies ranged from 91 (Punjab)³² to 4.19 million (Sindh).¹⁵⁹ There was a wide variation in HBV prevalence among individual studies, e.g. it ranged from 0.0027%¹⁵⁹ and 0.04%¹⁶¹ (in Sindh), 0.14% (in AJK)¹⁵⁶, and 0.01% (in ICT)¹³⁶ to 44% in Sindh Province.²⁷ Fig. 2 shows a map of Pakistan to visually represent the geographical distribution of HBV prevalence across Pakistan, highlighting regional variations.

Table 1: Studies included in the systematic review that assessed the prevalence of HBV among blood donors across Pakistan

Sr. No.	Author and Year	Province/Region	Sample size	HBV Positive (%)	Technique
1.	Zuberi <i>et al.</i> , 1991 ²⁷	Karachi, Sindh	163	71 (44%)	RIA
2.	Tanwani <i>et al.</i> , 1996 ²⁸	ICT	20,787	555 (2.67%)	RDT
3.	Mujeeb <i>et al.</i> , 1996 ²⁹	Karachi, Sindh	839	41 (4.9%)	ELISA
4.	Bhatti <i>et al.</i> , 1996 ³⁰	Rawalpindi, Punjab	760	49 (6.5%)	ELISA
5.	Kakepto <i>et al.</i> , 1996 ³¹	Karachi-Hyderabad, Sindh	51,257	1,173 (2.28%)	ELISA
6.	Rehman <i>et al.</i> , 1996 ³²	Lahore, Punjab	91	4 (5%)	ELISA
7.	Rehman <i>et al.</i> , 1996 ³³	Karachi, Sindh	7,209	245 (3.4%)	RDT
8.	Chaudary <i>et al.</i> , 1996 ³⁴	Lahore, Punjab	200	4 (2.0%)	RDT
9.	Bukhari <i>et al.</i> , 1999 ³⁵	Lahore, Punjab	27,057	920 (3.4%)	ELISA
10.	Hashmi <i>et al.</i> , 1999 ³⁶	Faisalabad, Punjab	435	9 (2.06%)	ELISA
11.	Mujeeb <i>et al.</i> , 2000 ³⁷	Karachi, Sindh	612	13 (2.21%)	ELISA
12.	Khattak <i>et al.</i> , 2002 ³⁸	Rawalpindi, Punjab	103,858	3,427 (3.3%)	ELISA
13.	Mumtaz <i>et al.</i> , 2002 ³⁹	Rawalpindi, Punjab	580	34 (5.86%)	RDT
14.	Mahmood <i>et al.</i> , 2004 ⁴⁰	Multan, Punjab	6,000	202 (3.37%)	RDT
15.	Asif <i>et al.</i> , 2004 ⁴¹	ICT	3,430	82 (2.39%)	ELISA
16.	Khokhar <i>et al.</i> , 2004 ⁴²	ICT	47,538	1,221 (2.56%)	ELISA
17.	Ahmed <i>et al.</i> , 2004 ⁴³	Peshawar, KP	4,000	76 (1.9%)	ELISA
18.	Sirhindi <i>et al.</i> , 2005 ⁴⁴	Lahore, Punjab	18,216	612 (3.36%)	RDT
19.	Akhtar <i>et al.</i> , 2005 ⁴⁵	Karachi, Sindh	351,309	7,048 (2%)	ELISA
20.	Waseem <i>et al.</i> , 2005 ⁴⁶	Lahore, Punjab	1,680	50 (2.97%)	ELISA
21.	Baqi <i>et al.</i> , 2006 ⁴⁷	Karachi, Sindh	300	10 (3.4%)	ELISA
22.	Fayyaz <i>et al.</i> , 2006 ⁴⁸	Bahawalpur, Punjab	27,938	752 (2.69%)	RDT
23.	Mujeeb <i>et al.</i> , 2006 ⁴⁹	Karachi, Sindh	7,325	344 (4.7%)	ELISA
24.	Alam <i>et al.</i> , 2006 ⁵⁰	Central Punjab	2,038	98 (4.83%)	RDT
25.	Khan <i>et al.</i> , 2006 ⁵¹	Liaquatpur, Punjab	1,426	85 (5.96%)	RDT
26.	Aziz <i>et al.</i> , 2006 ⁵²	Skardu, GB	850	71 (8.4%)	ELISA
27.	Mirza <i>et al.</i> , 2006 ⁵³	Bahawalpur, Punjab	15,550	504 (3.24%)	ELISA
28.	Sharif <i>et al.</i> , 2006 ⁵⁴	Rawalpindi, Punjab	2,558	72 (2.8%)	ELISA
29.	Masood <i>et al.</i> , 2007 ⁵⁵	Rawalpindi, Punjab	1,428	35 (2.45%)	ELISA
30.	Sultan <i>et al.</i> , 2007 ⁵⁶	Lahore, Punjab	41,498	921 (2.22%)	ELISA
31.	Bhatti <i>et al.</i> , 2007 ⁵⁷	Karachi, Sindh	94,177	2,033 (2.16%)	CLIA
32.	Khan <i>et al.</i> , 2007 ⁵⁸	Quetta, Balochistan	1,474	71 (4.8%)	RDT
33.	Ishaq <i>et al.</i> , 2007 ⁵⁹	Thatta, Sindh	310	18 (5.8%)	RDT
34.	Ijaz <i>et al.</i> , 2007 ⁶⁰	Lahore, Punjab	7,431	113 (1.52%)	RDT
35.	Azam <i>et al.</i> , 2007 ⁶¹	Karachi, Sindh	688	31 (4.5%)	RDT
36.	Alam <i>et al.</i> , 2007 ⁶²	Gilgit-Baltistan	8,949	328 (3.66%)	RDT
37.	Chaudhary <i>et al.</i> , 2007 ⁶³	Rawalpindi, Punjab	1,428	34 (2.45%)	ELISA
38.	Mujeeb <i>et al.</i> , 2007 ⁶⁴	Karachi, Sindh	21,125	697 (3.3%)	ELISA
39.	Farooqi <i>et al.</i> , 2007 ⁶⁵	Peshawar, KP	17,539	396 (2.28%)	RDT
40.	Nazar <i>et al.</i> , 2008 ⁶⁶	Karachi, Sindh	11,459	196 (1.71%)	RDT
41.	Mujeeb <i>et al.</i> , 2008 ⁶⁷	Rural Sindh	5,345	305 (5.7%)	ELISA
42.	Waheed <i>et al.</i> , 2009 ⁶⁸	ICT	18,202	351 (1.92%)	ELISA
43.	Manzoor <i>et al.</i> , 2009 ⁶⁹	Lahore, Punjab	6,659	113 (1.7%)	ELISA
44.	Shah <i>et al.</i> , 2010 ⁷⁰	Peshawar, KP	32,042	632 (1.97%)	ELISA
45.	Shaikh <i>et al.</i> , 2010 ⁷¹	Karachi, Sindh	2,000	78 (3.9%)	ELISA
46.	Borhany <i>et al.</i> , 2011 ⁷²	Karachi, Sindh	5,717	103 (1.81%)	ELISA
47.	Anwar <i>et al.</i> , 2011 ⁷³	Lahore, Punjab	16,695	467 (2.79%)	ELISA
48.	Khan <i>et al.</i> , 2011 ⁷⁴	Peshawar, KP	3,915	81 (2.07%)	ELISA
49.	Hussain <i>et al.</i> , 2011 ⁷⁵	Lahore, Punjab	855	60 (7%)	ELISA
50.	Waheed <i>et al.</i> , 2012 ⁷⁶	ICT	10,145	396 (3.91%)	ELISA
51.	Attaullah <i>et al.</i> , 2012 ⁷⁷	Peshawar, KP	127,828	3432 (2.68%)	ELISA
52.	Umair <i>et al.</i> , 2012 ⁷⁸	Mirpur, AJK	8,927	150 (1.68%)	ELISA
53.	Tunio <i>et al.</i> , 2013 ⁷⁹	Hyderabad, Sindh	2,696	49 (1.82)	CLIA
54.	Irfan <i>et al.</i> , 2013 ⁸⁰	Karachi, Sindh	108,598	2,068 (1.90%)	CLIA
55.	Khan <i>et al.</i> , 2013 ⁸¹	Khyber Pakhtunkhwa	7,148	132 (1.85%)	NAT

56.	Chaudhry <i>et al.</i> , 2013 ⁸²	Lahore, Punjab	2,155	21 (1.3%)	NAT
57.	Zehra <i>et al.</i> , 2013 ⁸³	Karachi, Sindh	13,170	242 (1.84%)	CLIA
58.	Moiz <i>et al.</i> , 2014 ⁸⁴	Karachi, Sindh	42,830	555 (1.30%)	CLIA
59.	Iqbal <i>et al.</i> , 2014 ⁸⁵	Rahim Yar Khan, Punjab	2,17,847	6,407 (2.9%)	RDT
60.	Zaheer <i>et al.</i> , 2014 ⁸⁶	ICT	160,376	3,769 (2.35%)	ELISA
61.	Pervaiz <i>et al.</i> , 2015 ⁸⁷	Punjab	5,894	222 (3.8%)	RDT
62.	Niazi <i>et al.</i> , 2015 ⁸⁸	Rawalpindi, Punjab	56,772	719 (1.27%)	NAT
63.	Wazir <i>et al.</i> , 2015 ⁸⁹	Mirpur, AJK	8,927	150 (1.68%)	RDT
64.	Hussain <i>et al.</i> , 2015 ⁹⁰	Multan, Punjab	48,020	1,113 (2.32%)	RDT
65.	Niazi <i>et al.</i> , 2016 ⁹¹	Rawalpindi, Punjab	160,552	2,385 (1.48%)	ELISA
66.	Sial <i>et al.</i> , 2016 ⁹²	Lahore, Punjab	29,522	368 (1.24%)	CLIA
67.	Arshad <i>et al.</i> , 2016 ⁹³	Karachi, Sindh	16,602	305 (1.84%)	CLIA
68.	Ahmed <i>et al.</i> , 2016 ⁹⁴	Peshawar, KP	8,439	92 (1.1%)	CLIA
69.	Zameer <i>et al.</i> , 2017 ⁹⁵	Lahore, Punjab	10,048	159 (1.59%)	RDT
70.	Farooq <i>et al.</i> , 2017 ⁹⁶	ICT	4,500	119 (2.64%)	ELISA
71.	Israr <i>et al.</i> , 2017 ⁹⁷	Peshawar, KP	460	12 (2.60%)	ELISA
72.	Memon <i>et al.</i> , 2017 ⁹⁸	Hyderabad, Sindh	4,683	66 (1.40%)	ELISA
73.	Harris <i>et al.</i> , 2017 ⁹⁹ (a)	Rawalpindi, Punjab	267,918	3,886 (1.45%)	ELISA
74.	Harris <i>et al.</i> , 2017 ⁹⁹ (b)	Karachi, Sindh	438,657	10,528 (2.4)	ELISA
75.	Sultan <i>et al.</i> , 2017 ¹⁰⁰	Karachi, Sindh	16,957	291 (1.71%)	CLIA
76.	Saeed <i>et al.</i> , 2017 ¹⁰¹	Lahore, Punjab	18,274	210 (1.10%)	RDT
77.	Butt <i>et al.</i> , 2017 ¹⁰²	Rawalpindi, Punjab	10,516	177 (1.68%)	CLIA
78.	Batool <i>et al.</i> , 2017 ¹⁰³	Peshawar, KP	41,033	961 (2.30%)	CLIA
79.	Ahmed <i>et al.</i> , 2017 ¹⁰⁴	Muzaffarabad, AJK	10,519	39 (0.37%)	RDT
80.	Saba <i>et al.</i> , 2018 ¹⁰⁵	Peshawar, KP	3,211	24 (0.75%)	CLIA
81.	Billah <i>et al.</i> , 2018 ¹⁰⁶	Multan, Punjab	1,195	18 (1.5 %),	ELISA
82.	Awan <i>et al.</i> , 2018 ¹⁰⁷	ICT	30,470	322 (1.06%)	NAT
83.	Akhtar <i>et al.</i> , 2018 ¹⁰⁸	Multan, Punjab	7,270	91 (1.26%)	RDT
84.	Azeem <i>et al.</i> , 2018 ¹⁰⁹	Peshawar, KP	204,942	4,253 (2.07%)	ELISA
85.	Abdullah <i>et al.</i> , 2019 ¹¹⁰	Lahore, Punjab	76,530	1,262 (1.65%)	ELISA
86.	Jahan <i>et al.</i> , 2019 ¹¹¹	Faisalabad, Punjab	1,942	10 (0.5%)	ELISA
87.	Siddiqui <i>et al.</i> , 2019 ¹¹²	ICT	847	11 (1.29%)	CLIA
88.	Sadiq <i>et al.</i> , 2019 ¹¹³	Nawabshah, Sindh	37,845	2,467 (6.52%)	ELISA
89.	Jiskani <i>et al.</i> , 2019 ¹¹⁴	Tando M. Khan, Sindh	3,028	41 (1.38)	ELISA
90.	Ahmad <i>et al.</i> , 2019 ¹¹⁵	Lahore, Punjab	79,774	792 (0.9%)	RDT
91.	Rauf & Cheema 2019 ¹¹⁶	Faisalabad, Punjab	6,594	74 (1.12%)	CLIA
92.	Mengal <i>et al.</i> , 2019 ¹¹⁷	Quetta, Balochistan	23,814	105 (0.44%)	CLIA
93.	Ahmed <i>et al.</i> , 2019 ¹¹⁸	Muzaffarabad, AJK	10,632	37 (0.35%)	CLIA
94.	Zaheer <i>et al.</i> , 2019 ¹¹⁹	ICT	75,348	807 (1.07%)	CLIA
95.	Zahoor <i>et al.</i> , 2019 ¹²⁰	Peshawar, KP	11,760	184 (1.6%)	CLIA
96.	Khan <i>et al.</i> , 2020 ¹²¹	Abbottabad, KP	23,596	928 (3.93%)	ELISA
97.	Rehm <i>et al.</i> , 2020 ¹²²	Sahiwal, Punjab	39,114	467 (1.19%)	RDT
98.	Iqbal <i>et al.</i> , 2020 ¹²³	Multan, Punjab	3,348	49 (11.3)	ELISA
99.	Samo <i>et al.</i> , 2021 ¹²⁴	Hyderabad, Sindh	353	5 (1.3%)	ELISA
100.	Saba <i>et al.</i> , 2021 ¹²⁵	Peshawar, KP	41,817	815 (1.95%)	CLIA
101.	Khan <i>et al.</i> , 2021 ¹²⁶	Peshawar, KP	119,263	1,693 (1.42%)	CLIA
102.	Qadir <i>et al.</i> , 2021 ¹²⁷	Karachi, Sindh	29,732	887 (2.9%)	CLIA
103.	Ghazanfer <i>et al.</i> , 2021 ¹²⁸	Sialkot, Punjab	84,305	682 (1.25%)	RDT
104.	Saeed and Ullah 2021 ¹²⁹	ICT	312,320	5,752 (1.84%)	CLIA
105.	Bhatti <i>et al.</i> , 2021 ¹³⁰	Sialkot, Punjab	400	5 (1.25%)	RDT
106.	Arshad <i>et al.</i> , 2021 ¹³¹	Lahore, Punjab	200	21 (10.5%)	RDT
107.	Bibi <i>et al.</i> , 2021 ¹³²	Swat, KP	1,292	17 (1.31%)	ELISA
108.	Sijjeel <i>et al.</i> , 2021 ¹³³	ICT	143,053	2452 (1.71%)	CLIA
109.	Ahmed <i>et al.</i> , 2022 ¹³⁴	Karachi, Sindh	23,656	365 (1.54%)	ELISA
110.	Ghazanfar <i>et al.</i> , 2022 ¹³⁵	Rawalpindi, Punjab	223,242	2,410 (1.080%)	RDT
111.	Bhatti <i>et al.</i> , 2022 ¹³⁶	ICT	120,968	3 (0.01%)	NAT
112.	Shah <i>et al.</i> , 2023 ¹³⁷	Peshawar, KP	6,311	54 (0.855%)	ELISA
113.	Ullah <i>et al.</i> , 2022 ¹³⁸	Multan, Punjab	7,858	63 (0.8%)	ELISA
114.	Zorob <i>et al.</i> , 2023 ¹³⁹	Rawalpindi, Punjab	15,405	164 (1.06%)	CLIA
115.	Raza <i>et al.</i> , 2023 ¹⁴⁰	Faisalabad, Punjab	1,500	42 (2.8%)	RDT

116.	Sabir <i>et al.</i> , 2023 ¹⁴¹	Rawalpindi, Punjab	308,767	2,085 (0.68%)	NAT
117.	Aslam <i>et al.</i> , 2023 ¹⁴²	Lahore, Punjab	6,380	53 (0.83 %)	ELISA
118.	Amjed <i>et al.</i> , 2023 ¹⁴³	Lahore, Punjab	2,236	13 (0.6%)	RDT
119.	Jan <i>et al.</i> , 2023 ¹⁴⁴	Peshawar, KP	463	12 (2.6%)	ELISA
120.	Khan <i>et al.</i> , 2023 ¹⁴⁵	Peshawar, KP	300	11 (3.7%)	ELISA
121.	Qadir <i>et al.</i> , 2023 ¹⁴⁶	DI Khan, KP	3,329	72 (2.2%)	CLIA
122.	Jadoon <i>et al.</i> , 2023 ¹⁴⁷	Lakki Marwat, KP	100	4 (4%)	CLIA
123.	Zeeshan <i>et al.</i> , 2023 ¹⁴⁸	Peshawar, KP	6,378	83 (1.3%)	CLIA
124.	Jamal <i>et al.</i> , 2023(a) ¹⁴⁹	Sindh	185,781	4,030 (2.17%)	CLIA
125.	Jamal <i>et al.</i> , 2023(b) ¹⁴⁹	Punjab	406,039	10,610 (2.61%)	CLIA
126.	Naz <i>et al.</i> , 2024 ¹⁵⁰	DI Khan, KP	25,000	405 (1.62%)	ELISA
127.	Saba <i>et al.</i> , 2024 ¹⁵¹	Peshawar, KP	62,185	1,154 (1.86%)	CLIA
128.	Saeed <i>et al.</i> , 2024 ¹⁵²	Lahore, Punjab	6,616	51 (18.8%)	CLIA
129.	Waheed <i>et al.</i> , 2024 ¹⁵³	Karachi, Sindh	56,732	1,280 (2.30%)	CLIA
130.	Hassni <i>et al.</i> , 2024 ¹⁵⁴	ICT	523	39 (7.9%)	CLIA
131.	Babar <i>et al.</i> , 2024 ¹⁵⁵	Swabi, KP	10,939	234 (2.13%)	ELISA
132.	Wazeer <i>et al.</i> , 2024 ¹⁵⁶	Mirpur, AJK	11,255	16 (0.14%)	CLIA
133.	Ikram <i>et al.</i> , 2024 ¹⁵⁷	Rawalpindi, Punjab	298	38 (12.8%)	RDT
134.	Haleem <i>et al.</i> , 2024 ¹⁵⁸	Quetta, Balochistan	21,815	12 (0.06%)	CLIA
135.	Jamal <i>et al.</i> , 2024 ¹⁵⁹	Sindh	4,199,195	112 (0.0027%)	ELISA, CLIA
136.	Hussain <i>et al.</i> , 2025 ¹⁶⁰	Skardu, GB	1,400	7 (0.5%)	ELISA
137.	Kousar <i>et al.</i> , 2025 ¹⁶¹	Karachi, Sindh	46,455	19 (0.04%)	NAT
138.	Akbar <i>et al.</i> , 2025 ¹⁶²	Quetta, Balochistan	1,680	42 (2.50%)	ELISA
139.	Ashraf <i>et al.</i> , 2025 ¹⁶³	Faisalabad, Punjab	16,206	213 (1.31%)	ELISA
Total			10,015,962	110,126 (1.10%)	
RDT: Rapid Diagnostic Test; ELISA: Enzyme Linked Immunosorbent Assay; CLIA: Chemiluminescence Immunoassay; NAT: Nucleic Acid Testing; AJK: Azad Jammu & Kashmir; ICT: Islamabad Capital Territory					

Analysis across decades indicated a pronounced decline in HBV seropositivity. From 1991-2000, the average was 3.8% (range 2.0-6.5%), reflecting limited screening coverage and predominance of replacement donors. During 2001-2010, the mean was 2.4%, concurrent with wider adoption of ELISA-based testing. From 2011-2020, the mean prevalence was 1.7%. Over the last five years, (2021-2025), the mean was 1.0%, consistent with implementation of Regional Blood Centres, national donor deferral criteria, and enhanced quality management systems.

Figure 3 displays a Forest plot showing the regional prevalence of HBV among blood donors in Pakistan while the figure 4 shows a temporal trend line chart indicating a decline in HBV prevalence among blood donors in Pakistan. Figure 5 shows the funnel plot to assess the publication bias. Each point represents a region's study precision (1/SE) plotted against its prevalence estimate. The red dashed line indicates the pooled prevalence (1.10%), while the degree of symmetry around this line reflects publication bias, the distribution here appears relatively symmetrical, suggesting minimal small-study bias.

Discussion

Infection with the HBV is a major threat to blood transfusion safety, particularly in developing countries.¹⁶⁴ The majority of blood donations (~ 82%) in Pakistan are collected through family or replacement donations, rather than the recommended from voluntary non-remunerated donors,^{165,166} which is the gold standard recommended by WHO and national blood safety legislations for ensuring blood safety.^{23,167} Family or replacement donations occur when patients' relatives or friends donate blood in exchange for transfusions, a practice that increases the risk of TTIs like HBV, as these donors are generally under pressure to donate even when they are not fully aware of their health status.¹⁶⁸

According to the last national data collection exercise under the auspices of national Safe Blood Transfusion Programme, Pakistan's blood banking network comprised ~ 700 blood banks across the public, private, and NGO sectors. These blood banks varied widely in size, with some collecting fewer than 500 units annually, while others exceeded 150,000 units per year. In the same year, around 2.7 million units of blood were collected nationwide.¹⁶⁹

Table 2: Province-wise pooled prevalence of HBV

Province/Region	Studies (n)	Sample size	HBV Positive (%)
Azad Jammu and Kashmir	5	50,260	392 (0.78%)
Balochistan	4	48,783	230 (0.47%)
Gilgit Baltistan	3	11,199	406 (3.63%)
Islamabad Capital Territory	14	805,454	13,427 (1.66%)
Khyber Pakhtunkhwa	25	782,766	16,237 (2.07%)
Punjab	55	2,432,118	43,737 (1.79%)
Sindh	33	5,786,505	35,697 (0.62%)
Total	139	10,015,962	110,126 (1.10%)

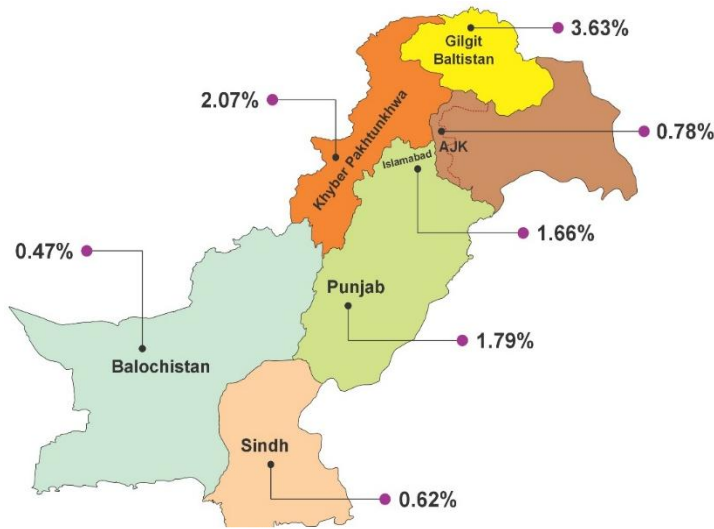


Fig. 2: Geographical distribution of HBV prevalence across Pakistan

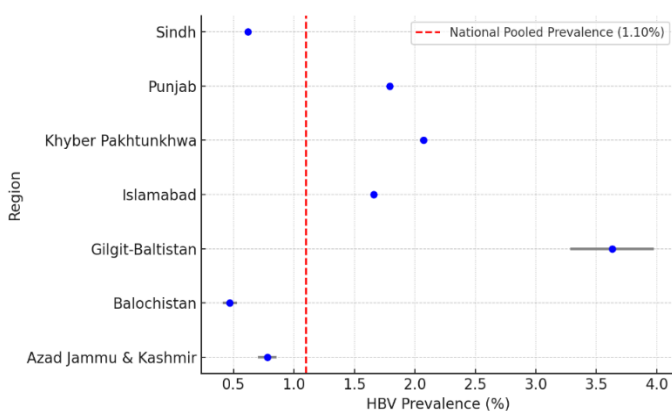


Fig. 3: Forest plot showing the regional prevalence of Hepatitis B Virus (HBV) among blood donors in Pakistan. Each blue marker represents the mean prevalence (%) for a province or region, with gray horizontal lines showing 95% confidence intervals. The red dashed line indicates the national pooled prevalence (1.10%)

The findings of this study, encompassing 139 studies conducted between 1991 and 2025, reveal a national pooled prevalence of 1.10% for Hepatitis B virus (HBV) among blood donors in Pakistan. This places Pakistan within the low-to-intermediate endemicity range as per WHO classification. The consistent decline in HBV seroprevalence from above 4% in the 1990s to around 1% in recent years (2021-2025) demonstrates the cumulative impact of national blood safety reforms, mandatory donor screening, and expanded hepatitis B immunization coverage.

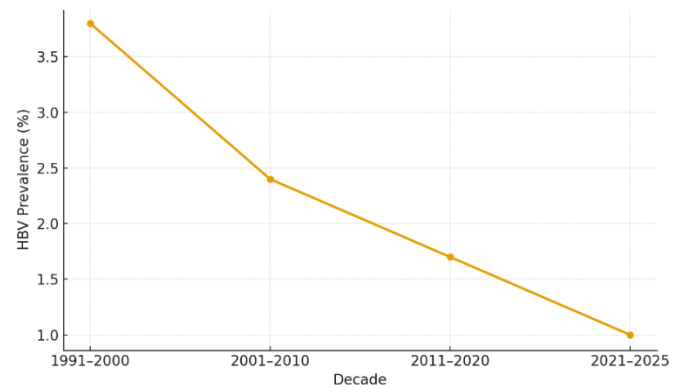


Fig. 4: Temporal Trend Line Chart showing the decline in HBV prevalence among blood donors in Pakistan

The downward trajectory is temporally aligned with policy milestones such as the National Health Vision, National Hepatitis Strategic Framework, and the National Blood Policy.

The progressive shift from serology-based assays such as RDTs and ELISAs to chemiluminescence immunoassay (CLIA) and nucleic acid testing (NAT) has enhanced diagnostic sensitivity,

reducing false negatives and reflecting a more accurate burden estimate across provinces.

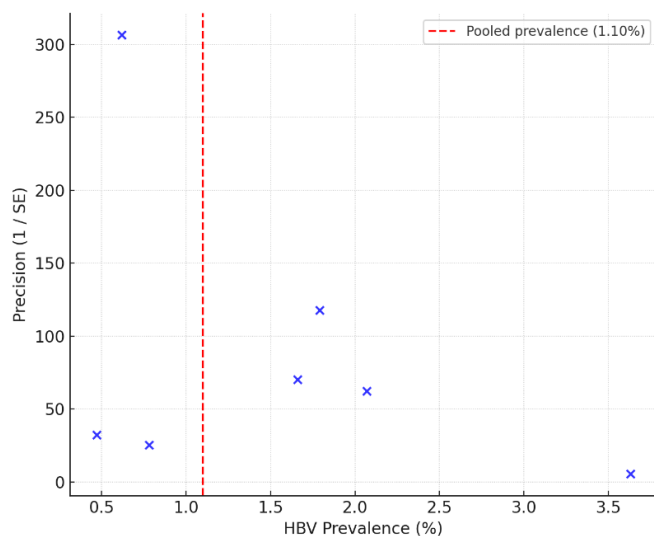


Fig. 5: Funnel Plot for HBV prevalence among blood donors in Pakistan

Marked regional disparities persist across Pakistan, with Gilgit-Baltistan (3.63%) and Khyber Pakhtunkhwa (2.07%) exhibiting higher HBV prevalence, likely influenced by socioeconomic barriers, suboptimal vaccination outreach, and reliance on replacement donations. In contrast, Sindh (0.62%) and Azad Jammu & Kashmir (0.78%) reported lower rates, indicating better donor selection and laboratory infrastructure in urban centers such as Karachi

and Mirpur. The large-scale blood donor data from Sindh and Punjab, collectively representing more than 80% of the total donor pool, provide strong epidemiological evidence supporting provincial blood transfusion authorities' progress since the establishment of Regional Blood Centres and Quality Management Systems under international collaborations (i.e., World Health Organization and German partners including GIZ and KfW).

Conclusion

The collective evidence from 139 studies and 10.01 million donations indicates a steady epidemiological transition from intermediate toward low endemicity of HBV among Pakistan's donor population. This improvement parallels national immunization efforts, modernization of blood-screening infrastructure, and regulatory enforcement by provincial blood transfusion authorities. Nevertheless, persistent heterogeneity and occasional high-prevalence clusters underscore the need for continued surveillance, full NAT adoption, and equitable vaccine outreach in underserved regions.

Overall, these results not only highlight the substantial progress made in blood safety in Pakistan but also provide an essential baseline for post-2025 HBV elimination targets, aligning with the WHO's goal of reducing new hepatitis infections by 90% by 2030.

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