Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 36, issue 3, 2026, pp. 182 – 187
© 2026 Vasile Goldis University Press (www.studiauniversitatis.ro)

EPIDEMIOLOGICAL AND IMMUNOLOGICAL CHARACTERISTICS OF VIRAL HEPATITIS B IN VACCINATED CHILDREN: A CASE STUDY FROM TURKMENISTAN

Amanmyrat SHUKUROV[1], Djuma ANNANUROV[1], Aygozel OVEZOVA[2]

[1]State Medical University of Turkmenistan named after Myrat Garryyev, Ashgabat, Turkmenistan
[2]Institute of International Relations of the Ministry of Foreign Affairs of Turkmenistan, Ashgabat, Turkmenistan

Abstract: Universal childhood immunization against Hepatitis B Virus (HBV) represents a cornerstone of public health and health diplomacy in Turkmenistan. Having introduced standard neonatal vaccination in 2001, Turkmenistan achieved historic reductions in viral transmission, validated by the 2023 WHO Regional Office for Europe certification. This study presents an integrated epidemiological, immunological and molecular evaluation of pediatric HBV prevention and long-term immunogenicity. A prospective and retrospective multi-center study analyzed national pediatric HBV epidemiological trends across pre-vaccination (1993–2000) and post-vaccination (2001–2023) eras in Turkmenistan. Epidemiological data and diagnostic evaluations were compiled across Ashgabat and all five velayats using standardized national surveillance protocols. Vaccine efficacy and disease severity mitigation were quantified using the Chaddock correlation scale. Molecular characterization incorporated quantitative Real-Time PCR and S-gene Sanger sequencing to detect surface determinant variations (G145R). Following the introduction of universal neonatal immunization using monovalent (Hepavax-Gene) and pentavalent (Quinvaxem) vaccines, national pediatric HBsAg incidence sharply declined from 34.2 per 100,000 in 1993 to 0.3 per 100,000 by 2012 (T*dec* = –25%). This reduction was sustained by a mean national immunization coverage of 97.1 ± 0.9%, culminating in official WHO Regional Office for Europe verification of HBV elimination targets in 2023. Among vaccinated pediatric cases presenting with acute manifestations, disease severity demonstrated a striking inverse relationship with vaccine dose completion ($p < 0.001$). Individuals receiving full primary immunization (3–4 doses) achieved high recovery rates (90.9% to 100.0%), with zero severe outcomes observed in the 4-dose subgroup. A strong positive correlation was confirmed between full-dose compliance and syptom mitigation ($r = 0.86$ for mild course; $r = 0.99$ for complete recovery, $p < 0.001$). Long-term anti-HBs seroprotection (≥ 10 mIU/mL) was sustained in 94.0% of children aged 5–8 years. Molecular sequencing revealed a low baseline occurrence of G145R vaccine-escape variants (16.7% of persistent seropositive cases), confirming high national vaccine immunogenicity. Turkmenistan's universal immunization program provides exceptional population-level protection and mitigates health severity even in rare breakthrough instances. Integrating robust antenatal screening, dynamic booster strategies and medical diplomacy infrastructure reinforces sustainable viral elimination across Central Asia.

Keywords: hepatitis B, vaccination efficacy, S-gene variant, medical diplomacy, pediatric epidemiology, Turkmenistan.

INTRODUCTION

Hepatitis B virus (HBV) infection remains a major public health challenge globally due to its potential to progress to hepatic cirrhosis, end-stage liver disease and hepatocellular carcinoma. To mitigate this global disease burden, the World Health Organization (WHO) established target frameworks to eliminate viral hepatitis as a public health threat by reducing new chronic infections and disease-associated mortality (WHO, 2016; WHO, 2024a). Systematic prevention relies on interrupting transmission pathways, particularly mother-to-child transmission (MTCT), which accounts for the vast majority of chronic pediatric cases (WHO, 2020). Operational guidelines emphasize universal maternal HBsAg screening, prompt administration of the birth-dose vaccine and timely completion of the primary series to secure long-term protection (WHO, 2024a; WHO, 2020).

The scientific and structural foundations of Turkmenistan's national healthcare system have prioritized public health protection, preventative medicine and comprehensive disease control strategies (Berdimuhamedov, 2007). Landmark epidemiological assessments established baseline viral transmission characteristics and global immunization monitoring frameworks prior to universal vaccine rollout (WHO, 2000). Following the integration of recombinant hepatitis B vaccination into National Immunization Calendar of Turkmenistan in 2001, sustained nationwide immunization coverage exceeding 95–98% was established across Ashgabat and all five velayats, as validated by independent WHO and UNICEF monitoring estimates (WHO, 2024b).

Achieving consistent birth-dose administration and full primary series completion is critical in national delivery settings to prevent early viral acquisition (Hurie *et al*., 2001; Bascom *et al*., 2005). However, despite universal vaccination coverage, rare breakthrough HBV infections are occasionally observed in vaccinated individuals. Investigating these events requires differentiating host immune factors, non-responsiveness, high maternal viral loads and viral

***Correspondence:** Amanmyrat Shukurov, State Medical University of Turkmenistan named after Myrat Garryyev, Ashgabat, Turkmenistan, email: kakachary777@gmail.com

genetic variations. Mutations within the major hydrophilic region (MHR) of the hepatitis B surface antigen (HBsAg), most notably the classic G145R substitution, can alter conformational epitopes, reducing neutralizing antibody binding affinity and diagnostic sensitivity (Carman *et al*., 1990; Lazarevic, 2014).

Across Central Asia, regional surveillance and observational research have documented historical viral hepatitis patterns, including HBV and hepatitis D virus (HDV) co-infections and varied transmission routes (Nurmanova *et al*., 2024; Tadzhiev *et al*., 2010; Tadzhiev, 2003). Diagnostic and prophylactic frameworks evaluated during early epidemiological studies underscored the necessity of universal active immunoprophylaxis to suppress population-level transmission (Shukurov, 2003, Gorbakov, 2003). Contemporary international management guidelines from EASL and AASLD further reinforce that high population-level immunity is the definitive barrier against chronic liver disease progression (EASL, 2017, Terrault *et al*., 2018).

Furthermore, public health achievements in Turkmenistan are closely interconnected with institutional medical diplomacy (Ovezova, 2024a). By expanding international technical cooperation, aligning national healthcare programs with WHO strategic frameworks and facilitating transparent reporting, medical diplomacy serves as a key mechanism for securing health security and sustainable disease elimination (Ovezova, 2024a; Ovezova, 2024b). To provide a complete scientific evaluation, this study investigates Turkmenistan's pediatric HBV epidemiological trends from pre-vaccination (1993–2000) to post-vaccination (2001–2023) eras, quantifies dose-dependent pathological protection using the Chaddock correlation scale, profiles molecular S-gene variants (G145R) and examines the supporting role of medical diplomacy infrastructure.

MATERIALS AND METHODS

Study design and population

A multi-center, combined prospective and retrospective epidemiological and immunological study was conducted to evaluate national pediatric HBV dynamics. The study covered two distinct chronological eras: the pre-vaccination period (1993–2000) and the post-vaccination period (2001–2023). Observational and epidemiological data were collected from pediatric subjects (born between 2001 and 2013) evaluated across public healthcare institutions in the capital city of Ashgabat and all five velayats (Ahal, Balkan, Dashoguz, Lebap and Mary).

Epidemiological and diagnostic evaluation

Standardized epidemiological questionnaires were administered to collect demographic data, birth history, maternal HBsAg status and documented vaccination history (monovalent Hepavax-Gene birth dose and pentavalent Quinvaxem primary series). Health severity was classified into mild, moderate and severe manifestations based on observational symptoms, bilirubin levels and alanine aminotransferase (ALT/AST) activity according to national diagnostic protocols aligned with international guidelines (EASL, 2017; Terrault *et al*., 2018).

Statistical analysis and correlation scaling

Incidence rates were calculated per 100,000 population. The annual rate of incidence decline was expressed as the mean annual trend percentage (T*dec*). Quantitative relationships between vaccine dose completion and symptom mitigation (recovery rates and symptom attenuation) were evaluated using Pearson's (*r*) and Spearman's (*p*) correlation coefficients. The qualitative strength of the confirmed statistical correlations was interpreted using the established Chaddock correlation scale:

Table 1.

Interpretation Scale for Correlation Coefficients (Chaddock Scale)

0.1 ≤ |r|< 0.3: Weak
0.3 ≤ |r|< 0.5: Moderate
0.5 ≤ |r|< 0.7: Noticeable / Substantial
0.7 ≤ |r|< 0.9: High / Strong
0.9 ≤ |r|< 1.0: Very High / Very Strong

Statistical significance was defined at $p < 0.05$, with high significance set at $p < 0.001$.

Serological and molecular profiling

Serological markers (HBsAg, anti-HBs, HBeAg, and anti-HBc) were measured using commercial enzyme-linked immunosorbent assays (ELISA). Quantitative Real-Time PCR was performed to determine serum HBV DNA viral load in seropositive cases. For molecular characterization of surface antigen escape variants, viral DNA was extracted from persistent seropositive pediatric samples, followed by Sanger sequencing of the S-gene envelope region encompassing the major hydrophilic region (MHR) to detect the G145R point mutation.

RESULTS

Long-term epidemiological dynamics and coverage

The epidemiological efficacy of Hepatitis B Virus (HBV) vaccination in Turkmenistan was systematically evaluated by analyzing population-wide morbidity trends across two main historical phases: the pre-vaccination baseline era (1993–2000) and the post-vaccination universal immunization era (2001–2012) (Berdimuhamedov, 2007, WHO, 2016).

In the pre-vaccination period, pediatric morbidity peaked in 1993, reaching 34.2 per 100,000 population among children aged up to 14 years, alongside an adult baseline incidence of 52.5 per 100,000. In the

© 2026 Vasile Goldis University Press (www.studiauniversitatis.ro)

subsequent years leading up to universal immunization, multi-faceted public health interventions including the strict enforcement of sanitary-epidemiological standards and the universal integration of single-use disposable medical technologies across healthcare facilities THAT facilitated an initial decline in parenteral viral transmission. By the year 2000, pediatric incidence dropped to 12.0 per 100,000 population, while adult incidence reached 14.0 per 100,000.

Following the formal inclusion of recombinant Hepatitis B immunization into the National Immunization Calendar in 2001, viral transmission was interrupted at an accelerated rate. Total national pediatric HBV incidence plummeted from 11.9 per 100,000 population in 2001 to 0.3 per 100,000 population by 2012. This remarkable reduction corresponds to a sustained mean annual trend of decline (T*dec* = –25\%).

This profound epidemiological shift was directly sustained by high national vaccination compliance and universal accessibility. Between 2002 and 2012, mean annual pediatric immunization coverage reached 97.1 ± 0.9%, consistently exceeding the 95% threshold across the capital city of Ashgabat and all five velayats (Ahal, Balkan, Dashoguz, Lebap, and Mary). A key driver behind this high population uptake is the fully state-funded framework, which provides routine childhood immunizations to the public completely free of charge.



Fig. 1. WHO Regional Verification Certificate Awarded to Turkmenistan (2023), confirming the fulfillment of regional viral hepatitis B elimination targets.

These multi-year public health achievements culminated in 2023, when Turkmenistan was officially awarded a verification certificate by the European Technical Advisory Group of Experts (ETAGE) under the WHO Regional Office for Europe, formally certifying Turkmenistan's fulfillment of regional viral hepatitis B elimination targets (WHO, 2016).

Table 2.

Long-term Epidemiological Dynamics and Immunization Performance Indicators in Turkmenistan (1993–2023)

Indicator	Pre-Vaccination Era (1993–2000)	Post-Vaccination Era (2001–2012)	Statistical Trend / Metric
Pediatric HBsAg Incidence	34.2 per 100,000 (1993) 12.0 per 100,000 (2000)	11.9 per 100,000 (2001) 0.3 per 100,000 (2012)	T*dec* = –25\%
Adult HBsAg Incidence	52.5 per 100,000 (1993)	14.0 per 100,000 (2000)	Significant long-term decline
National Vaccine Coverage	<10% (Targeted baseline)	97.1 ± 0.9% (Mean 2002–2012)	State-funded ($p < 0.001$)
WHO Elimination Status	High Baseline Endemicity	Verified Elimination (2023)	WHO Regional Certification

Dose-response protection and symptom mitigation

Analysis of vaccinated children presenting with acute manifestations revealed a strong inverse relationship between vaccine dose completion and disease severity ($p < 0.001$) (WHO, 2024b). Individuals who completed the full primary

© 2026 Vasile Goldis University Press (www.studiauniversitatis.ro)

immunization series (3–4 doses) achieved recovery rates ranging from 90.9% to 100.0%. Notably, in the cohort receiving all 4 doses (including birth dose), zero severe outcomes or fulminant complications were recorded.

Application of the Chaddock correlation scale confirmed strong positive statistical relationships between full immunization compliance and disease mitigation:

Mild Course Trajectory: r = 0.86 ($p < 0.001$), classified as a Strong / High correlation on the Chaddock scale.

Complete Recovery: r = 0.99 ($p < 0.001$), classified as a Very Strong / Very High correlation on the Chaddock scale.

Furthermore, long-term humoral immunity evaluations confirmed that high, protective serum anti-HBs antibody levels (≥ 10 mIU/mL)) were sustained in 94.0% of children evaluated within the younger age bracket of 5 to 8 years (WHO, 2000; WHO, 2024b).

Molecular profiling of S-gene variants

Molecular profiling via Sanger sequencing of persistent seropositive pediatric cases identified a low baseline occurrence of the G145R vaccine-escape substitution (16.7% of persistent seropositive cases; *n*=3) (Bascom *et al*., 2005). The remaining seropositive cases were attributed to perinatal exposure prior to birth-dose completion or non-responsiveness. The low frequency of G145R variants verifies that wild-type HBV suppression remains high and confirms the continued protective efficacy of the current monovalent and pentavalent vaccine strains used in the country.

DISCUSSION

The findings of this multi-center study demonstrate the profound impact of universal neonatal immunization on pediatric hepatitis B epidemiology in Turkmenistan. The decline in pediatric HBsAg incidence from 34.2 to 0.3 per 100,000 population (T*dec* = –25\%) provides empirical evidence of population-level viral suppression achieved through high immunization coverage (97.1 ± 0.9%) (Berdimuhamedov, 2007; WHO, 2024b). These findings align with historic global long-term cohort studies showing that routine infant vaccination virtually abolishes childhood chronic carrier states (WHO, 2020, WHO, 2000).

A critical contribution of this research is the quantitative evaluation of symptom mitigation using the Chaddock correlation scale. The very strong correlation between full-dose immunization compliance and complete recovery ($r = 0.99$, $p < 0.001$) underscores that even in rare breakthrough events, prior vaccination acts as a major disease modifier, preventing severe hepatic complications and acute liver failure (EASL, 2017; Terrault *et al*., 2018). This dose-dependent attenuation reinforces the necessity of enforcing complete primary dose delivery alongside the immediate birth dose (WHO, 2024a, Hurie *et al*., 2001, Bascom *et al*., 2005).

Molecular sequencing revealed that G145R surface gene variants accounted for only 16.7% of persistent seropositive breakthrough cases (Carman *et al*., 1990; Lazarevic, 2014). This demonstrates that immune escape mutations currently pose a minimal threat to national immunization performance in Turkmenistan. Nonetheless, continued molecular surveillance remains essential, however, to ensure that diagnostic immunoassays and recombinant vaccine formulations retain complete sensitivity (Carman *et al*., 1990, Lazarevic, 2014).

Finally, these public health milestones are inseparable from Turkmenistan's strategic application of medical diplomacy. Through active collaboration with the World Health Organization, UNICEF and international scientific forums, Turkmenistan has established resilient vaccine cold chains, standardized surveillance protocols, and transparent epidemiological reporting (Ovezova, 2024a; Ovezova, 2024b). Integrating medical diplomacy into public health governance not only secured the 2023 WHO verification certificate for HBV elimination but also provides a sustainable model for regional health security and disease elimination across Central Asia (Ovezova, 2024a; Ovezova, 2024b).

CONCLUSIONS

The comprehensive evaluation of multi-year epidemiological, immunological and molecular data demonstrates that Turkmenistan's national strategy for universal hepatitis B immunization has achieved profound success, fundamentally transforming the landscape of public health across the country. By systematically transitioning from pre-vaccination high endemicity where pediatric incidence peaked at 34.2 per 100,000 in 1993 to a state of sustained viral suppression with current incidence dropping to 0.3 per 100,000, the national immunization program has effectively interrupted vertical and horizontal transmission chains among children and young populations.

A pivotal finding of this research is that high population-level impact is directly underpinned by exceptional programmatic performance. Specifically, the maintenance of a mean annual vaccination coverage rate of 97.1 ± 0.9% across the capital and all regions, backed by continuous state funding and free public access, ensures robust and equitable protection. Furthermore, the quantitative assessment utilizing the Chaddock correlation scale provides definitive evidence that complete primary series adherence goes beyond mere prevention of infection; it acts as a powerful health modifier. The strong and very strong statistical correlations established between full immunization compliance and mild trajectories ($r = 0.86$) as well as complete recovery ($r = 0.99$) confirm that breakthrough cases, when they occur, are successfully mitigated against severe hepatic complications, acute failure, or chronic progression.

From a molecular perspective, the low baseline detection frequency of the classic G145R escape mutation (16.7%) among persistent seropositive cases indicates that viral populations remain predominantly wild-type, thereby validating the ongoing structural compatibility, immunogenicity, and protective efficacy

© 2026 Vasile Goldis University Press (www.studiauniversitatis.ro)

of the monovalent and pentavalent vaccine strains utilized in the national calendar. This finding underscores the absence of widespread immune selection pressure and confirms the stability of current prophylactic regimens.

Ultimately, these multi-faceted achievements culminated in the prestigious 2023 WHO regional verification certification, recognizing Turkmenistan's fulfillment of viral hepatitis elimination targets. By harmonizing rigorous epidemiological surveillance, high-standard health management, molecular monitoring, and proactive medical diplomacy, Turkmenistan has established a resilient, sustainable model for infectious disease control. This integrated public health framework not only secures long-term generational health security within the nation but also offers valuable empirical insights and benchmarks for regional and global health initiatives striving toward the complete elimination of viral hepatitis.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the staff and specialists of the National Center for Infectious Disease Control and Prevention and the regional hospitals for their invaluable assistance in data collection. We also thank the State Medical University of Turkmenistan for providing the infrastructure for molecular analysis.

AUTHORS' CONTRIBUTIONS

Conceptualization, A.S. and D.A.; methodology, A.S.; software, A.O.; validation, A.S., D.A., and A.O.; formal analysis, D.A.; investigation, A.S. and D.A.; resources, A.O.; data curation, D.A.; writing—original draft preparation, A.S.; writing—review and editing, D.A. and A.O.; visualization, A.O.; supervision, D.A. All authors have read and agreed to the published version of the manuscript.

FUNDING

This research received no external funding.

CONFLICT Of INTEREST

The authors declare no conflict of interest.

REFERENCES

Berdimuhamedov G. Scientific Foundations of Development of Healthcare in Turkmenistan. Ashgabat; 2007.

World Health Organization. Global health sector strategy on viral hepatitis 2016–2021: towards ending viral hepatitis. Geneva: World Health Organization; 2016. (WHO/HIV/2016.06).

World Health Organization. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. Geneva: World Health Organization; 2024a.

World Health Organization. Prevention of mother-to-child transmission of hepatitis B virus: guidelines on antiviral prophylaxis in pregnancy. Geneva: World Health Organization; 2020.

World Health Organization. WHO vaccine-preventable diseases monitoring system: 2000 global summary. Geneva: World Health Organization; 2000. (WHO/V&B/2000.32).

World Health Organization, UNICEF. Turkmenistan: WHO and UNICEF estimates of immunization coverage: 2023 revision. Geneva: WHO/UNICEF; 2024b.

Hurie MB, Saari TN, Davis JP. Impact of the joint statement by the American Academy of Pediatrics, US Public Health Service on thimerosal in vaccines on hospital infant hepatitis B vaccination practices. Pediatrics. 2001;107(4):755-758.

Bascom S, Miller S, Greenblatt J. Assessment of prenatal hepatitis B and rubella prevention in New Hampshire delivery hospitals. Pediatrics. 2005;115(Suppl):594-599.

Carman WF, Zanetti AR, Karayiannis P, Waters J, Thomas HC, Zuckerman AJ, et al. Vaccine-induced escape mutant of hepatitis B virus. The Lancet. 1990;336(8711):325-329.

Lazarevic I. Post-translational modifications of the hepatitis B virus surface antigen and their impact on hepatitis B virus immunogenicity and pathogenesis. World J Gastroenterol. 2014;20(32):11297-11310.

Nurmanova A, et al. Reporting of progress toward eliminating viral hepatitis B and C in the Central Asian region. Adv Gut Microbiome Res. 2024;2024:4818435.

Tadzhiev BT, Annanurov ZH, Tedjenova ZA. Clinical and laboratory features of B- and D-hepatitis depending on routes of transmission. In: Collection of materials of the international scientific conference "Era of the Great Revival of Turkmenistan and changes in the healthcare system — 2010". Ashgabat; 2010. p. 206, 366, 545.

Tadzhiev BT. Current state of the problem of viral hepatitis B and D in Turkmenistan. In: Materials of the scientific-practical conference "Topical Issues of Gastroenterology". Ashgabat; 2003. p. 9.

Shukurov A. Viral hepatitis — epidemiology, diagnosis, treatment. In: Materials of the scientific-practical conference "Topical Issues of Gastroenterology". Ashgabat; 2003. p. 9.

Gorbakov VV. Possibilities of vaccine prophylaxis of viral hepatitis A and B. In: Topical problems of hepatology: epidemiology of viral hepatitis. Moscow; 2003. p. 73–81.

European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol. 2017;67(2):370-398.

Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018;67(4):1560-1599.

Ovezova A. Türkmenistanyň daşary syýasatynda lukmançylyk diplomatiýasynyň esasy ugurlary [The main directions of medical diplomacy in the foreign policy of Turkmenistan]. Science

© 2026 Vasile Goldis University Press (www.studiauniversitatis.ro)

and Technology in Turkmenistan. 2024a;(2):116-119.

Ovezova A. Turkmenistan on the world health arena: cooperation with WHO. Int J Humanit Nat Sci. 2024b;6-3(93):148-151.

© 2026 Vasile Goldis University Press (www.studiauniversitatis.ro)