PREVALENCE OF TRANSFUSION – TRANSMITTED INFECTIONS AMONG VOLUNTARY BLOOD DONORS IN THE WESTERN REGION OF NORTH MACEDONIA: A RETROSPECTIVE MULTICENTER STUDY (2021-2025)

Fatjona JASHARI[1], Ekrem ISMANI[1,2], Edona ISMANI XHEMAILI [1,2]

[1] Faculty of Medical Sciences, University of Tetovo, North Macedonia
[2] Insitute of Transfusion Medicine – Tetovo, North Macedonia

Abstract

Introduction: Communicable diseases continue to represent a significant public health challenge due to their high transmission potential and impact on population health. Effective control depends on early detection, preventive measures, and continuous epidemiological surveillance, supported by laboratory and field data analysis.
Objective: The aim of this study was to assess the distribution of communicable diseases in the western region of North Macedonia, specifically in Tetovo, Gostivar, Kičevo, and Struga, and to evaluate incidence patterns according to geographic location and type of testing.
Materials and Methods: A descriptive study was conducted using laboratory and field data collected from 2021 to 2025. A total of 151,610 test results were analyzed and categorized by city, age group, type of test, test result, and year. Data were processed using descriptive statistical methods.
Results: Out of 151,610 tests performed, 588 (0.39%) were positive, 150,814 (99.47%) were negative, and 208 (0.14%) were not tested. The highest number of tests was recorded in Tetovo, followed by Gostivar, Kičevo, and Struga. The highest positivity rate was observed in Struga (0.51%), while the lowest was in Kičevo (0.30%). Hepatitis B virus (HBV) accounted for the largest proportion of positive cases, human immunodeficiency virus (HIV), hepatitis C virus (HCV), syphilis, followed by nucleic acid testing (NAT) detections and hepatitis E virus (HEV).
Conclusion: Although overall positivity rates remain low, variations across cities and types of testing highlight the importance of continuous surveillance, early detection, and strengthened preventive strategies to effectively control communicable diseases.

Keywords: Communicable diseases; Epidemiology; Public health; Incidence; Laboratory testing; HBV; HIV; HCV; Syphilis; Surveillance; North Macedonia

Introduction

Transfusion-transmissible infections (TTIs) are infectious agents that can be transmitted through blood and blood components. In transfusion medicine, the control of these infections depends on appropriate donor selection, validated laboratory testing, quality systems, confirmatory testing, documentation, and haemovigilance. Continuous retrospective monitoring of TTI screening results is essential to evaluate whether donor selection and testing protocols remain effective over time, and to identify marker-specific, regional, demographic, or temporal patterns that may require public health or laboratory intervention. This study provides evidence to support blood safety policies, transfusion service planning, laboratory quality improvement, and regional surveillance for Gostivar, Kërçovë, Struga, and Tetovo.
The study covers the period from January 2021 to December 2025 and includes aggregated data for HBV/HBsAg, HCV/anti-HCV, HIV Ag/Ab, HEV, syphilis, NAT, grey-zone results, and confirmatory/supplementary markers, including HBsAg confirmatory, anti-HCV confirmatory, HIV 1/2 confirmatory, dHBV, dHCV, and dHIV. Prevalence refers to the proportion of tested samples that are positive or reactive within a defined denominator, while seropositivity or reactivity describes a positive or reactive screening result for a specific

infectious marker. TTI refers to transfusion-transmissible infections, while NAT represents nucleic acid testing used for the detection of viral genetic material. The markers dHBV, dHCV, and dHIV represent supplementary diagnostic categories analyzed separately from primary screening prevalence.

International blood safety guidelines emphasize the importance of standardized and safe testing of blood donations prior to clinical use, with mandatory screening for HIV, HBV, HCV, and syphilis as core components of blood safety systems, along with confirmatory testing and proper management of donors with reactive results. The data used in this study reflect regional transfusion service activity from four cities in North Macedonia. The inclusion of NAT and additional markers indicates improved diagnostic sensitivity and enhanced laboratory surveillance, although the dataset does not contain individual identifiers, risk factors, or clinical follow-up information.

The main blood-borne diseases included in this study are HBV (primarily screened using HBsAg), HCV (screened using anti-HCV), HEV as a less frequently tested marker, HIV (screened using HIV Ag/Ab syphilis through serological testing, and NAT as a molecular screening method.

This dataset allows descriptive analysis by marker, time, age group, sex, and city. However, due to its aggregated nature, it is not possible to determine whether multiple reactive results belong to the same individual; therefore, true co-infection prevalence cannot be calculated.

The general aim of the study is to assess the prevalence of transfusion-transmissible infections during the period 2021–2025. The specific objectives include determining overall and marker-specific prevalence, comparing prevalence across years, analyzing differences by sex, age group, city, and donor category, and identifying trends over the study period. The research questions focus on overall prevalence, the marker with the highest prevalence, temporal changes, most affected demographic groups, and implications for blood safety, laboratory practice, and surveillance.

Materials And Methods

This study is a retrospective descriptive study. The study area includes data from the Institute of Transfusion Medicine – Skopje, covering the regions of Gostivar, Kërçovë, Struga, and Tetovo. The study period extends from January 2021 to December 2025. The study population consists of aggregated laboratory data from blood donors and donor-related records within regional transfusion services.

The inclusion criteria comprise records within the period 2021–2025, entries with defined city, age group, test marker, result, and count fields. For descriptive purposes, primary screening data, grey-zone results, and confirmatory/supplementary records were included. Exclusion criteria include data outside the study period, if present. Rows marked as “not tested” were excluded from the prevalence denominator but retained for descriptive analysis of testing coverage. Confirmatory and supplementary results were not included in the primary screening prevalence denominator.

The data source is a cleaned Excel workbook: *TTI_Scientific_research CLEANED DATA.xlsx*. The variables included demographic variables such as age group, sex, city, and source category, as well as laboratory variables such as TestStd, TestFamily, TestType, result, number of reactive/positive cases, number of negative cases, and number of not-tested cases. The temporal variable was year.

Laboratory testing methods include standardized marker categories such as HBsAg, anti-HCV, HIV Ag/Ab, anti-syphilis, NAT, HEV, grey-zone markers, and confirmatory/supplementary markers. The study does not independently verify the diagnostic assay type, laboratory platform, or standard operating procedures, as these data were not available in the dataset.
Data collection was performed from the cleaned workbook, and aggregation was conducted by marker, year, city, age group, sex, source, test type, and result category. The analysis was based on frequencies, percentages, and prevalence calculations. Primary screening prevalence was calculated as the proportion of reactive/positive results divided by the total number of tested samples for each marker or group. For donor-related proxy analysis, HBsAg was used as a reference marker to avoid duplication of the same donor population across different markers.
The data are aggregated and do not contain personal identifiers; therefore, the analysis is based on anonymized group-level data. However, all academic or publication outputs must comply with institutional and data protection regulations.
The main limitation of the methodology is that the data are grouped at marker level rather than individual level. Therefore, true individual prevalence cannot be determined, repeat donors cannot be adjusted for, and true co-infections between markers cannot be assessed.

Results

The study data include 7,411 aggregated rows covering the period 2021–2025. Using HBsAg screening data as a proxy for the number of donors, 28,612 tests were identified. This denominator was used solely to describe the donor sample and was not applied for the aggregation of all disease marker tests.

Table 1. Study sample by year (donor-count proxy based on HBsAg screening)

Year	Total tested	Male	Female	HBsAg reactive	HBsAg reactivity %
2021	5,692	5,022	670	38	0.668%
2022	6,276	5,518	758	35	0.558%
2023	5,496	4,796	700	20	0.364%
2024	5,376	4,724	652	20	0.372%
2025	5,772	5,091	681	16	0.277%

Note: HBsAg screening was used as a practical donor-count proxy to avoid multiplying the same donor pool across repeated disease markers.

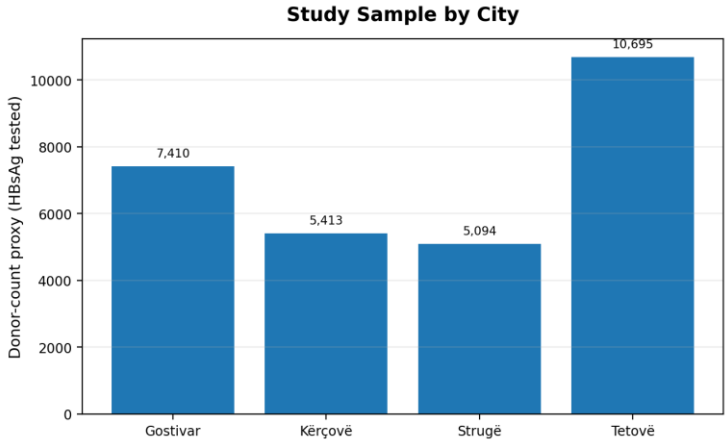

Figure 1. Study sample distribution by city, based on HBsAg screening denominator.

Demographic Characteristics

Table 2. Study sample by city

City	Total tested	Male	Female	HBsAg reactive	HBsAg reactivity %
Gostivar	7,410	6,562	848	27	0.364%
Kicevo	5,413	4,618	795	22	0.406%
Struga	5,094	4,571	523	40	0.785%
Tetovo	10,695	9,400	1,295	40	0.374%

Table 3. Sex distribution of study sample

Sex	Count	Percentage
Male	25,151	87.904%
Female	3,461	12.096%

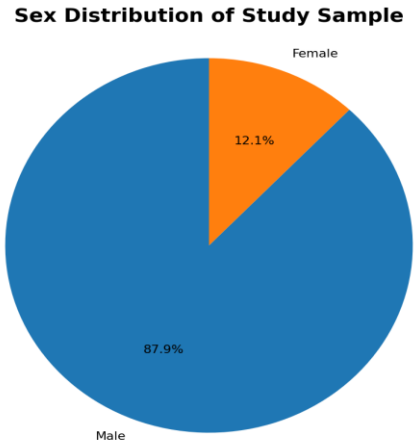

Figure 2. Sex distribution of the study sample. The donor-count proxy shows a male-predominant sample.

Overall Prevalence of Transmissive Blood Diseases

Table 4. Overall marker-level screening prevalence

Total valid screening marker-tests	Reactive/Positive screening results	Negative screening results	Overall marker-level prevalence
151,176	433	150,743	0.286%

Important: This is marker-test-level prevalence. It should not be interpreted as unique donor-level prevalence because individual donor IDs are not available.

Prevalence by Disease Type

Table 5. Prevalence by disease marker

Disease marker	Number tested	Reactive/Positive	Negative	Not tested	Prevalence %
HBV / HBsAg	28,612	129	28,483	0	0.451%
HCV / Anti-HCV	28,557	49	28,508	0	0.172%
HIV Ag/Ab	28,578	54	28,524	0	0.189%
Syphilis / Anti-Syphilis	28,613	40	28,573	0	0.140%
NAT	20,209	150	20,059	107	0.742%
HEV / Hepatitis E	16,607	11	16,596	99	0.066%

NAT showed the highest marker-level prevalence among the analyzed screening markers. HEV and NAT had lower valid denominators because some records were marked as not tested

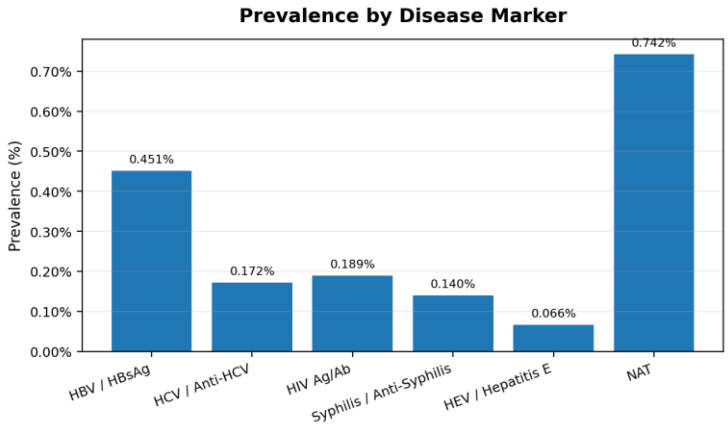

Figure 3. Disease-specific marker-level prevalence, calculated from primary screening rows only.

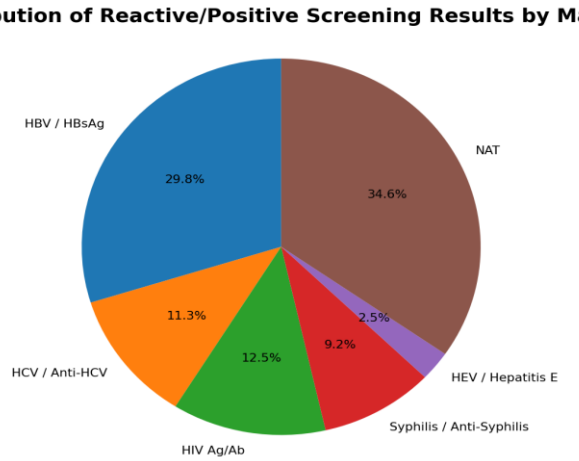

Figure 4. Distribution of reactive/positive primary screening results by marker. This is not a co-infection chart.

Yearly Trend Analysis

Table 6. Yearly trend of screening reactivity

Year	Total valid marker-tests	Reactive/Positive	Negative	Prevalence %
2021	22,788	75	22,713	0.329%
2022	29,094	93	29,001	0.320%
2023	32,914	92	32,822	0.280%
2024	31,837	116	31,721	0.364%
2025	34,543	57	34,486	0.165%

The highest aggregate marker-level prevalence was observed in 2024 (0.364%), while the lowest was observed in 2025 (0.165%).

Figure 5. Yearly trend in marker-level screening prevalence across all primary screening markers.

Prevalence by Age Group

Table 7. Prevalence by age group

Age group	Total valid marker-tests	Reactive/Positive	Negative	Prevalence %
18-22	10,849	37	10,812	0.341%
23-27	13,089	26	13,063	0.199%
28-32	18,090	48	18,042	0.265%
33-37	21,182	60	21,122	0.283%
38-42	25,096	62	25,034	0.247%
43-47	23,533	63	23,470	0.268%

48-52	17,338	65	17,273	0.375%
53-57	13,575	52	13,523	0.383%
58-62	7,036	17	7,019	0.242%
63-65	1,388	3	1,385	0.216%

The highest marker-level prevalence was observed in the 53-57 age group (0.383%).

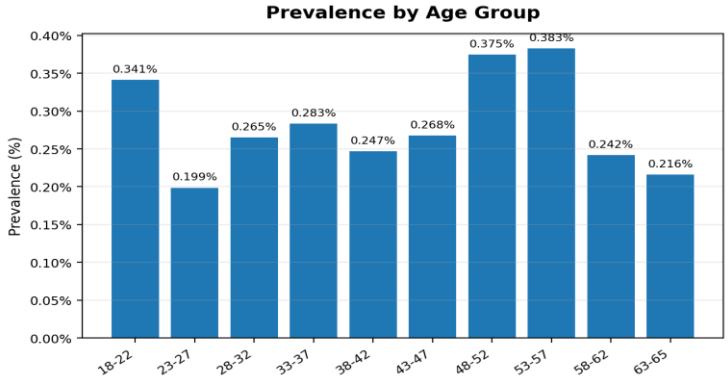

Figure 6. Marker-level prevalence by age group.

Prevalence by Sex

Table 8. Marker-level prevalence by sex

Sex	Total valid marker-tests	Reactive/Positive	Negative	Prevalence %
Male	132,808	382	132,426	0.288%
Female	18,368	51	18,317	0.278%

Sex-specific prevalence is calculated from male/female counts in primary screening rows. The male and female marker-level prevalence estimates are close, but the sample is strongly male-predominant.

Co-Infections, if Available

Table 9. Co-infection assessment

Co-infection type	Number of cases	Percentage	Interpretation
HBV + HCV	Not calculable	Not calculable	Aggregated data do not identify whether markers occurred in the same donor.
HIV + Syphilis	Not calculable	Not calculable	Individual donor IDs are required.

HBV + HEV	Not calculable	Not calculable	Marker-level counts cannot confirm co-infection.

A pie chart was provided for reactive marker distribution, but not for true co-infections.

Confirmatory, Supplementary, and Grey-Zone Findings

Table 10. Confirmatory/supplementary results, including dHBV/dHCV/dHIV

Marker	Number tested	Reactive/Positive	Negative	Positive/Reactive %
Anti-HCV Confirmatory	14	2	12	14.286%
HBsAg Confirmatory	88	84	4	95.455%
HIV 1/2 Confirmatory	4	3	1	75.000%
dHBV	64	52	12	81.250%
dHCV	24	1	23	4.167%
dHIV	25	6	19	24.000%

Confirmatory/supplementary results describe follow-up testing activity and should not be combined with primary screening prevalence denominators.

Table 11. Grey-zone findings

Marker	Number tested	Reactive	Reactive %
Anti-HCV Grey Zone	4	4	100.000%
HBsAg Grey Zone	3	3	100.000%

City and Source-Level Prevalence

Table 12. Marker-level prevalence by city

City	Total valid marker-tests	Reactive/Positive	Negative	Prevalence %
Gostivar	39,781	109	39,672	0.274%
Kicevo	28,651	60	28,591	0.209%
Struga	26,729	103	26,626	0.385%
Tetovo	56,015	161	55,854	0.287%

The highest city-level marker prevalence was observed in Strugë (0.385%).

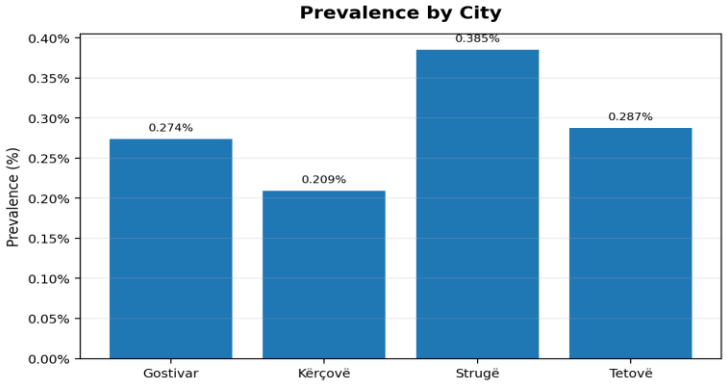

Figure 7. Marker-level prevalence by city.

Key Observations

The overall marker-level screening prevalence was 0.286% across 151,176 valid screening marker-tests.
NAT showed the highest marker-specific prevalence (0.742%), highlighting the importance of molecular screening in detecting reactive results that may not be identified through serological methods alone.
HBV/HBsAg represents a significant burden among serological markers, with 129 reactive results and a prevalence of 0.451%.
The highest annual marker-level prevalence was observed in 2024 (0.364%), followed by a lower value in 2025 (0.165%).
Age-specific prevalence was highest in the 53–57 age group (0.383%), closely followed by the 48–52 age group.
True co-infections cannot be assessed based on the available aggregated dataset.

Discussion

Interpretation of Key Findings: The analysis indicates a low overall marker-level screening prevalence (0.286%); however, reactivity was present across all major marker categories. The persistent presence of reactive results highlights the importance of donor screening, confirmatory testing, and careful documentation.
NAT demonstrated the highest marker-specific prevalence (0.742%). This result should be interpreted with caution, as NAT had a smaller denominator compared to the main serological markers and may reflect targeted implementation or changes in test availability over time.
Comparison with Other Studies and Standards**:** The findings are consistent with the general blood safety principle that even low-prevalence populations require universal standardized screening and confirmatory management of reactive results. The study also supports the continued need for haemovigilance and standardized data systems.
Possible Reasons for Observed Trends: Variations in test availability, particularly the implementation of NAT, may have influenced the number of detected reactive results. Differences in donor composition by year, city, sex, or age group may also affect observed prevalence. Grey-zone and confirmatory categories indicate that screening results require structured follow-up and should not be considered definitive diagnoses without confirmation.

The lower prevalence observed in 2025 may reflect a true reduction, changes in donor composition, incomplete data coverage for the period, or variations in testing volume.
Public Health Implications: The low but persistent reactivity supports the need for donor education, preventive messaging, HBV vaccination promotion where appropriate, continued surveillance, and strong laboratory quality systems. Age groups with higher reactivity may be considered for targeted awareness interventions; however, individual-level risk factor data are required before drawing causal conclusions.
Laboratory and Clinical Implications: Laboratory systems should maintain validated screening algorithms, clear confirmatory pathways, standardized donor notification procedures, and traceable electronic records. NAT and supplementary markers should be consistently documented to ensure that future retrospective analyses can clearly distinguish between screening, confirmation, diagnosis, and donor follow-up outcomes.

Conclusion

Main Conclusion**:** The prevalence of transfusion-transmissible infection markers in the analyzed dataset remained low at the aggregate level, with a total of 433 reactive/positive results among 151,176 valid screening tests (0.286%).
Disease-Specific Conclusion: NAT showed the highest marker-level prevalence, followed by HBV/HBsAg. HIV, HCV, syphilis, and HEV demonstrated lower but still clinically relevant levels of reactivity. Confirmatory and supplementary results, including dHBV/dHCV/dHIV, emphasize the need for a clear distinction between screening and confirmatory testing in reporting.
Trend Conclusion: The overall trend was fluctuating rather than showing a clear linear increase or decrease. The highest annual prevalence was observed in 2024, while the lowest was recorded in 2025. Interpretation of trends should consider testing volume, NAT implementation, and the aggregated nature of the dataset.

Limitations

The retrospective design limits control over missing or inconsistent data. The workbook is aggregated and does not contain individual donor identifiers; therefore, individual-level prevalence and co-infections cannot be determined. Clinical outcomes, risk factors, vaccination status, and donor classification (first-time or repeat donors) are not available. Confirmatory testing may not be recorded for all reactive screening results or may exist in separate registries. NAT and HEV have different denominators compared to serological markers due to “not tested” entries. The results from the included regions cannot be generalized to the entire population without broader national-level data.

Appendices

Appendix A. Data Collection Form / Variables Used

Table A1. Variables available in the cleaned dataset

Variable	Description
Year	Dataset variable
City	Dataset variable
AgeGroup	Dataset variable

TestStd	Dataset variable
TestFamily	Dataset variable
TestType	Dataset variable
Result	Dataset variable
Male	Dataset variable
Female	Dataset variable
SexTotal	Dataset variable
Reactive Positive Count	Dataset variable
Negative Count	Dataset variable
NotTested Count	Dataset variable
Source	Dataset variable

Appendix B. Full source category summary

Table B1. Marker-level prevalence by source category

Source	Total valid marker-tests	Reactive/Positive	Negative	Prevalence %
Gostivar – Field activity	7,573	24	7,549	0.317%
Gostivar – Service	32,208	85	32,123	0.264%
Kičevo – Field activity	3,458	9	3,449	0.260%
Kičevo – Service	25,193	51	25,142	0.202%
Struga – Field activity	1,034	7	1,027	0.677%
Struga – Service	25,695	96	25,599	0.374%
Tetovo – Field activity	13,423	45	13,378	0.335%
Tetovo – Service	42,592	116	42,476	0.272%

Appendix C. Abbreviations

Table C1. List of Abbreviations Used

Abbreviation	Meaning
HBV	Hepatitis B Virus
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus

HIV	Human Immunodeficiency Virus
HEV	Hepatitis E Virus
NAT	Nucleic Acid Testing
TTI	Transfusion-Transmissible Infection
dHBV	Supplementary/diagnostic HBV marker as recorded in dataset
dHCV	Supplementary/diagnostic HCV marker as recorded in dataset
dHIV	Supplementary/diagnostic HIV marker as recorded in dataset

Conclusion

The prevalence of transfusion-transmissible infection markers in the analyzed dataset remained low at the aggregate test level, with a total of 433 reactive/positive results out of 151,176 valid screening tests, representing 0.286%.

At the disease-specific level, NAT showed the highest marker prevalence, followed by HBV/HBsAg. HIV, HCV, syphilis, and HEV demonstrated lower levels of reactivity, although still clinically relevant. Confirmatory and supplementary results, including dHBV/dHCV/dHIV, highlight the need for a clear distinction between screening and confirmatory testing in laboratory reporting and interpretation.

Regarding temporal trends, the overall prevalence did not show a simple linear increase or decrease but rather fluctuations throughout the study period. The highest annual prevalence was recorded in 2024, while the lowest was observed in 2025. Interpretation of these trends should consider variations in testing volume, the implementation of NAT, and the aggregated nature of the dataset.

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