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1504 Sofia; 26, Yanko Sakazov Blvd.
Tel.: +359 2/ 846 83 07, Fax: +359 2/ 943 30 75
e-mail: infovita@ncipd.org

PROBLEMS OF INFECTIOUS AND PARASITIC DISEASES
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CONTENTS

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ACKNOWLEDGEMENTS

Individuals who supplied facilities, strains or reagents, or gave advice may be acknowledged. Also, supporting grants may be mentioned.

REFERENCES

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FOREWORD

Surveillance systems have evolved and continue to evolve in response to the changing HIV epidemic. An effective surveillance system is essential for health professionals, national governments and for an adequate national response to HIV. This supplement is devoted to the bio-behavior component of the Bulgarian HIV Surveillance System. In Bulgaria, epidemiological case-based data and CD4 counts have been collected since the first diagnosed HIV case in 1986. After the introduction of antiretroviral therapy (ART) in 1997, patient-based monitoring was started, including viral load. Later on, data on viral resistance was added to monitoring (2000), and in 2008 molecular HIV epidemiology was introduced. Bulgaria is a low-prevalence country for HIV, with the epidemic being concentrated among hard-to-reach populations. In this regard, the implementation of a National biological and behavior HIV surveillance system among key populations [injecting drug users (PWID), men having sex with men (MSM), sex workers, Roma men at higher risk and prisoners] in 2004 was a significant step in the development of HIV surveillance in Bulgaria. In order to monitor the effectiveness of the National Program interventions, program products recommended by UNAIDS for modeling of HIV epidemic were adopted in 2007 with the All these steps have helped the building of a comprehensive HIV epidemiological surveillance system, comprising the components of integrated biological and behavioral surveillance. A detailed description of the National HIV Surveillance System is provided in the article "*Bulgarian HIV Surveillance System in the context of continuum of care 90-90-90 strategy."*

The article "*National HIV biological and behavioral surveillance among hard-to-reach populations in Bulgaria (2004 -2016*)" in three parts describes in detail the formative research, methodology and ethical issues of the National bio-behavioral survey carried out in the period 2004-2016 (NBBS-04-16). Within five cycles, a detailed assessment of HIV risk at the municipality level was made, with estimations of the size of hard-to-reach populations and mapping of 2,276 venues for contact with group representatives. Based on this assessment, nine cycles of survey (NBBS-04-16) were conducted, and information from 27,210 participants was collected in the selected municipalities.

The second part of the article describes in details NBBS-04-16 methodology: cross-sectional surveys, with two-stage clusters and probability time-location sampling, fully aligned with the WHO recommendations and the current best practices for bio-behavioral surveys in high-risk groups. The developed protocols, questionnaires, standard operating procedures, and other documents are a valuable manual not only for conducting HIV NBBS but also for other studies among hard-to-reach populations.

The third part is devoted to a very important issue - the ethics of vulnerable population's research. It should be emphasized that NBBS-04-16 follows the guidelines of all international and national documents concerning research on human subjects. Strict compliance with these guidelines has enabled NBBS-04-16 to be successful amongst stigmatized and criminalized groups, contributing to their widespread recruitment and enrollment, as well as to avoiding the risks arising from the study itself and from legislations.

NBBS-04-16 is the first major national study on the spread of HIV and sexually transmitted infections among key populations, which simultaneously provides a characterization of their risk behaviors. The results from NBBS-04-16 study were used to assess the effectiveness of the National HIV prevention and control programs in 2001-2007 and 2008-2015, as well as of the Global Fund grant. They served as a scientific basis for the development of National HIV Programs for 2008-2015 and 2017-2020, as well as for the reprogramming of their activities.

In this supplement, the results from NBBS surveys carried among MSM and PWID in 2016 are presented. Results from previous NBBS-04-16 cycles are published elsewhere (1-5)

The modeling of epidemic for assessment of the current situation and prognosis is an important element of the National HIV Surveillance System. Estimations of the number of HIV-infected individuals and new HIV infections is essential for both antiretroviral therapy planning and for monitoring the cascade of continuum for care in the light of 90-90-90 strategy. In Bulgaria, the modeling of HIV epidemic began in 2007, following the recommendations and methodology of UNAIDS and ECDC. The article *"Using case surveillance data for estimation and projection of HIV infection in PWID and MSM population by combining ECDC-HIV modeling tool and Spectrum-Estimation and projection package*" explores modeling problems in terms of low HIV prevalence and concentrated epidemic. An original algorithm allowing to derive epidemiological parameters for assessment and prognosis of the epidemic among MSM and PWID is provided. This algorithm can be used in other subpopulations, as well.

In conclusion, the above shows that a robust HIV epidemiological surveillance system exists in Bulgaria that treats the infection in all its aspects. The implementation of this system, together with the successful interventions of the national programs, has contributed to keep the low HIV prevalence in the country.

Professor Hristo Taskov, MD, DSc.
National Center of Infectious and Parasitic Diseases, Sofia

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ABBREVIATONS

AIDS	Acquired Immuno-Deficiency Syndrome
ART	Anti-retroviral Therapy
BBS	Bio-behaviour surveillance
CBBSU	Central Bio-behaviour survey Unit
CCS	Conventional Cluster Sampling
CDC	Centers for Disease Control and Prevent
CDSD	Communicable Diseases Surveillance Directorate
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CSRE	Commission of Scientific Research Ethics
ECDC	European Centre of Diseases Control
EMCCDA	European Monitoring Centre for Drugs and Drug Addiction
EMIS	The European MSM Internet Survey
EQAS	External Quality Assurance System
FPC	Finite population correction
FSW	Female Sex Worker
GARPR	Global AIDS response report
GFATM	Global Fund to Fight AIDS, Tuberculosis and Malaria
HBV	Hepatitis B virus
HCT	HIV Counseling and Testing
HCV	Hepatitis C virus
HIS	HIV incidence surveillance
HIV	Human Immuno-deficiency Virus
HRG	High Risk Group
HTRP	Hard-to-reach- populations
IBBS	Integrated Biological and Behavioural Surveillance
KP	Key populations
LTHTS	Low threshold HIV testing site (former Voluntary counselling and testing site)
MMC	Mobile medical cabinet
MoH	Ministry of Health
MOS	Measure of size
MSM	Men who have Sex with Men
MSW	Male sex worker
NBBS	National Biological and Behavioral Surveillance
NCIPD	National Center of Infectious and Parasitic Diseases

NGO	Non-Governmental Organization
NHID	national HIV identification number
NHP	National program for prevention and control of HIV and STI
NHSS	National HIV Surveillance System
NRCL of HIV	National Reference Confirmatory Laboratory of HIV
NRLI	National Reference Laboratory of Immunology
NSE	Needles and syringes exchange
NSI	National Statistical Institute
PLH	People living with HIV
PPS	Probability proportional to size
PWID	People who inject drugs
RHI	Regional Health Inspectorate
RMHR	Roma men at higher risk
SEC	Scientific Ethics Committee
SID	Survey Identification number
SOP	standard operating procedure
STI	Sexual transmitted infection
SW	Sex workers
TLS	Time-location sampling
TPHA	Treponema pallidum hemagglutination assay
UNGASS	United Nation General Assembly Special Session
VCT	Voluntary and Counselling and Testing
YRMHR	Young Roma at higher risk

BULGARIA HIV SURVEILLANCE SYSTEM IN THE CONTEXT OF CONTINUUM OF CARE 90-90-90 STRATEGY

T. Varleva[1], M. Zamfirova[1], V. Georgieva[1], M. Nikolova[2], I. Alexiev[2] and H. Taskov[2]

[1]Ministry of Health
[2]National Center of Infectious and Parasitic Diseases, Sofia.

ABSTRACT

The burden of HIV disease in Bulgaria is monitored by using a comprehensive surveillance system which includes case surveillance, incidence surveillance, and bio-behaviour surveillance. Data from this system are used for describing epidemics trends and risk behaviours associated with HIV acquisition, which are essential for effective public health interventions for HIV, to plan, implement, and evaluate public health policies and programs and monitoring of 90-90-90 strategy.

In this article we describe the elements of National HIV surveillance system.

KEYWORDS

HIV case surveillance, incidence surveillance, bio-behaviour surveillance.

INTRODUCTION

Bulgaria's public health surveillance system for HIV was established in the late 1980s. It has evolved significantly during the past three decades in parallel with the implementation of antiretroviral therapy monitoring (1, 2), tests for recent infection (3, 4), HIV modeling tools (5, 6) and monitoring of HIV cascade to reach the 90-90-90 goals (7). All this requires high-quality usable surveillance data. To maximize the ability to monitor trends in HIV and HIV cascade, the Ministry of Health (MoH) has developed a comprehensive National HIV Surveillance System (NHSS) that guides data collection and reporting. Surveillance data for HIV infection have been used for many years to monitor the spread of HIV, plan prevention programs and health-care services, and allocate funding for prevention and care (8-10).

ADDRESS FOR CORRESPONDENCE:
Prof. Dr. Hristo Taskov MD, PhD, DSc,
National Center Infectious and Parasitic Diseases,
Sofia 1504, Bul. Yanko Sakazov 26,
E-mail: hristotaskov@gmail.com

SURVEILLANCE METHODS

HIV case surveillance

Conducting HIV case surveillance. HIV infection is one of many nationally notifiable diseases regulated by an ordinance of Ministry of Health (11). The country has adopted the ECDC/CDC/WHO HIV surveillance case definition (12-14). which incorporats a staging system that categorizes Acquired immunodeficiency syndrome (AIDS) as HIV infection, stage 3, and underscores that AIDS is late-stage HIV infection, rather than a separate disease. The case definition will continue to be updated, as needed, to ensure the most accurate monitoring of HIV disease (e.g., to incorporate new diagnostic testing algorithms or monitor acute HIV infection [stage 0]).

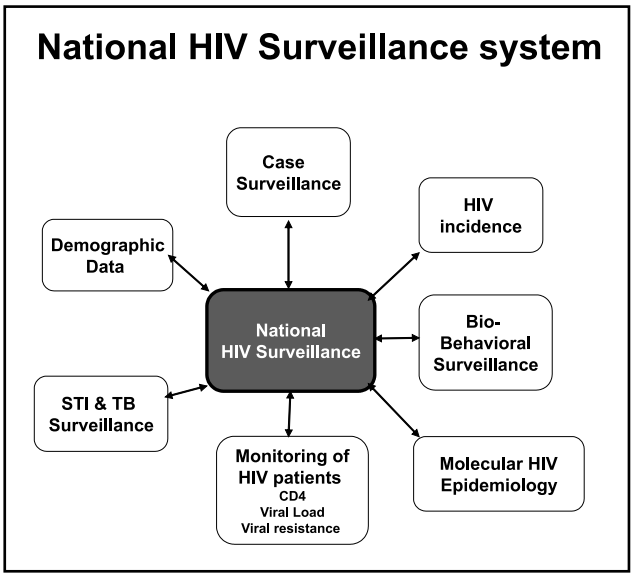

Fig.1. Components of Bulgaria HIV surveillance system

The Ministry of Health, National Center of Infectious and Parasitic Diseases (NCIPD) and Regional Health Inspectorates (RHI) hold the legal authority for public health surveillance.

New cases of HIV infection are typically identified passively through laboratory reports of HIV-specific tests based on antibody, antibody/antigen, or viral nucleic acid detection. Case

information is collected on standardized case report form (15), which is completed by providers or through active follow-up with reporting entities (e.g., clinics and hospitals). According to the centralized HIV diagnosis system in the country, all HIV cases are confirmed at the National Reference Confirmatory Laboratory of HIV (NRCL of HIV) at NCIPD. NRCL of HIV collects the necessary epidemiological information and assigns a national HIV identification number (NHID) for each HIV case (16). It serves to anonymize any further follow-up testing and reporting data for people living with HIV (PLH). NRCL of HIV also performs the determination of HIV viral load (VL) and HIV drug resistance in all cases with HIV before the start and in the course of antiretroviral treatment (ART) The determination of CD4 counts is performed at the National Reference Laboratory of Immunology (NRLI) at NCIPD, and in several other laboratories reporting their results to NRLI. At present, all HIV cases and laboratory test results from diagnosis and monitoring of HIV infection (e.g., CD4, VL and HIV drug resistance) are reported by NCIPD to the MoH where they are collected in an anonymous database. This allows a nationwide aggregation and monitoring of data concerning the continuum of HIV care, the burden of disease and implementation of 90-90-90 strategy. Once a year, aggregated and individual data are reported to the European surveillance system (TESSy) (17).

For each HIV case, hospitals and laboratories use document-based surveillance methods including collection, storage, and management of all case reports, laboratory test results, and other original documents, and allowing longitudinal monitoring of cases. Hospitals and laboratories collect demographic, clinical, vital status, and risk data for use at the local level and submission to MoH.

Monitoring the spectrum of disease. HIV may be detected at various points along the spectrum of disease, and reportable events range from reporting HIV exposure among infants, and HIV infection in asymptomatic people, to late-stage disease (HIV infection, stage 3 [AIDS]), and death (Figure 1). In addition to these key events, the increased demand to use HIV surveillance data for public health action (e.g., linkage to and engagement in HIV medical care) has resulted in expanding data collection to include all CD4 and VL test results.

National HIV surveillance cascade includes the following stages:

a) The exposure of an infant to HIV infection through his/her HIV-infected mother. Only applicable for mother-to-child transmission (MTCT). Data are used to monitor MTCT elimination.

b) The time of first HIV infection. Because incident infections cannot be directly measured, diagnosed cases are categorized as recent or longstanding infections using an immunoassay for recency. ECDC “HIV-modelng tool” (5), Spectrum-EPP and Spectrum-ECDC (6) are also used for inderect estimation HIV incidence and number of new infections.

c) HIV is detected through testing; some diagnosed cases are new (incident) infections, while others are longstanding infections. Some people may be tested for HIV during the acute stage of infection; that is, during the time immediately after being infected and before antibodies have developed (window period). Acute HIV infection can result in an indeterminate or negative result when using conventional HIV antibody tests, such as enzyme immunoassays or Western blots. In this scenario, nucleic acid testing, which detects the presence of the virus itself, may be necessary to determine acute infection (18, 19).

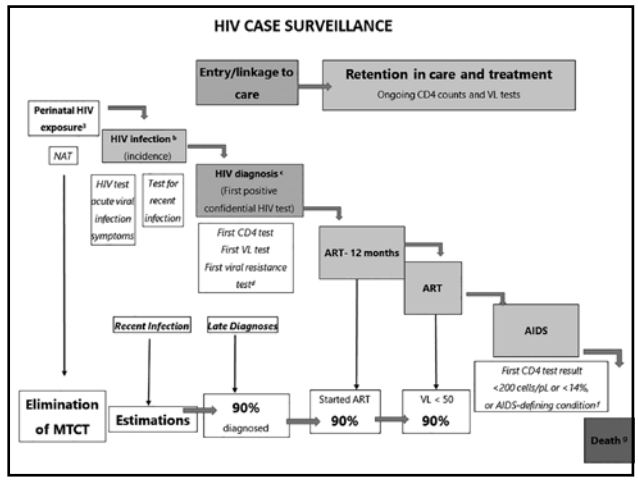

Figure 2. HIV continuum. Reportable events in HIV surveillance and definitions to for longitudinal monitoring of HIV infection in Bulgaria.

d) After HIV diagnosis, the first laboratory test results for CD4 count (or percentage) and VL serve to establish the degree of current immunosuppression. Drug resistance testing is a part of the standard of care for people infected with HIV and is recommended at entry into care,

prior to initiation of antiretroviral therapy, upon treatment failure, and when clinically indicated (20).

e) HIV surveillance data are used to monitor, linkage to and retention in HIV care and viral suppression among people with HIV. Therefore, all results from of CD4 and VL testing are continuously collected (1, 2).

f) Using information included in surveillance case reports and laboratory results, cases of HIV infection can be monitored longitudinally on a population basis for progression to stage 3 (AIDS) determined by CD4 <200 cells/μL or <14%, or the occurrence of an AIDS-defining condition (11).

Laboratory reporting. MoH provides technical support to HIV surveillance entities at RHI, NRCL of HIV and NRLI to enhance laboratory reporting. Areas of focus include regulations to require reporting of all HIV-related laboratory test results and other laboratory reporting activities.

All RHI require reporting of HIV-related laboratory results from the medical laboratories in the region. Because CD4 and VL test results are key components for monitoring and management of HIV disease (21), measuring the outcomes, and planning of public health actions, the national HIV surveillance system requires that all results from CD4 testing are reported monthly to the NRLI, which reports collected and validated data biannually to MoH. HIV VL and drug resistance are also reported monthly by NRCL of HIV, which currently carries out these testing across the country.

HIV Incidence Surveillance

Screening and confirmatory HIV tests can detect the infection at any point after infection. As a result, HIV diagnoses do not necessarily represent recent infections. To provide reliable and scientifically valid national estimates of the number of newly acquired infections (diagnosed and undiagnosed), NHSS conduct HIV incidence surveillance (HIS). All reactive samples for HIV from the country are sent to the NRCL of HIV for further confirmatory tests. This allows remnant diagnostic HIV positive blood to be further investigated for recent infection using an immunoassay for recency to distinguish between recent and longstanding infections (3, 4). HIS data are used in combination with the estimates from HIV modeling to describe the characteristics of newly infected populations and subgroups; monitor trends in transmission; and monitor the outcomes of HIV prevention programs and strategies.

Bio-behaviour surveillance BBS

In 2004, MoH developed a System for National Biological and Behavioral Surveillance (NBBS) to conduct behavioral surveillance in hard-to-reach-populations for HIV infection aligned with WHO guidelines (22-24). NBBS is carried out periodically in the following subpopulations at increased risk for HIV:1) Gay, bisexual and other men who have sex with men (MSM); 2) Persons who inject drugs (PWID); 3) Marginalised population (MP) at increased risk for HIV infection; 4) Male and female sex workers (FSW and MSW) including transgenders and 5) Prisoners.

The surveillance places are selected according to the following criteria: HIV prevalence, rate of new diagnoses and size of populations (25).

Before each NBBS cycle, formative assessment is conducted to learn more about each local population and to inform operational procedures. *Venue-based, Time-Space Sampling* (TLS) is used among target groups and RHI staff with NGOs assiatence identify venues frequented by them (e.g., bars, clubs, parksand street locations) as well as days/time intervals when those venues are frequented. In next NBBS cycles *Respondent-Driven Sampling (RDS)* is planed for PWID, and MP. RHI staff and NGOs working with respective population recruit participants. Enrolment and interviewing then continue until the target sample size is reached (25).

Trained interviewers in all NBBS project areas use a standardized, anonymous questionnaire to collect information on HIV-related risk behaviors, HIV testing, and the use of HIV prevention services. HIV testing is offered to all participants. During each cycle, a minimum of 300-400 eligible persons from each participating project area are interviewed and offered HIV testing. Detailed information for all NBBS is provided elsewhere (25-27). Data collected includes behavioral risk factors for HIV (e.g. sexual behaviors, drug use), HIV testing behaviors, the receipt of prevention services, and use of prevention strategies (e.g. condoms, NSE). In addition to these interview data, all NBBS participants are offered an HIV and STI tests (28).

NBBS data are used to provide a behavioral context for trends seen in HIV surveillance data. They also describe populations at increased risk for HIV infection and thus provide an indication of the leading edge of the epidemic (28-33). Through systematic surveillance in groups at high risk for HIV infection, NBBS is essential for monitoring the impact of the National HIV Strategy and Programs, which focuses on decreasing HIV incidence, improving linkage to care, and reducing disparities.

Other activities

Additional activities under HIV case surveillance, including molecular HIV surveillance and perinatal HIV exposure reporting (34, 35). Given the observed dynamic of the HIV-1 epidemic in Bulgaria with regard to vulnerable population, and the specific geographic location of Bulgaria at the intersection of Western Europe, Eastern Europe and the Middle East, defining the diversity of HIV genotypes in Bulgaria in different populations is epidemiologically important (36). To analyze genetic characterization, origin and spatiotemporal evolutionary history as well as rapidly evolving phylogenetic clusters of the introduced and disseminated HIV-1 strains, phylogenetic and population genetic (phylodynamic) analysis methods are implemented in the NRCL of HIV. The phylogenetic analysis aims to characterize the epidemic on the basis of the genetic interrelatedness of HIV viral sequences, capturing the underlying structure of transmission networks or clusters within a given population. Molecular epidemiological research provides valuable information on HIV-1-infected individuals in Bulgaria and highlights the importance of sustained molecular surveillance to better understand and control the epidemic in the country.

DATA ANALYSIS AND DISSEMINATION.

HIV surveillance data are used for estimation and projection of trends of HIV infection in Bulgaria. Estimations and projections of HIV infection started in 2006 with Workbook and Spectrum-EPP (37), using data from NBBS. Since 2016 case-surveillance data are used for modeling with ECDC-HIV-Modeling-Tool (5) and Spectrum-ECDC (6), in parallel to Spectrum-EPP. Annually modeling is performed for estimation and projection of HIV infection, which is important for understanding the spread of HIV infection and its dynamics. Results of estimations and projections are used for evident based preparation, monitoring and evaluation of National HIV Programs.

National surveillance data are used for the preparation of an annual surveillance report and an epidemiologic country profile. The annual surveillance report describes the epidemiology of HIV (e.g., case counts, rates, and trends) in terms of demographic and risk characteristics. The epidemiologic profile describes the burden of HIV disease in terms of sociodemographic, geographic, behavioral, and clinical characteristics. These reports complement one another and are used.

National surveillance data are also used to plan and evaluate programs and policies, to monitor the goals of National HIV Strategy, plan HIV prevention programs, and allocate funding for HIV prevention and care (8, 9). Improved surveillance methods allow us to better direct our programs and resources to the populations most affected.

CONCLUSIONS

In summary, the completeness and quality of HIV surveillance data are critical for monitoring progress toward the goals of the National HIV/AIDS Strategy. HIV surveillance data provide accurate and timely information necessary to (1) monitor and characterize the trends and burden of HIV infection; (2) measure reliably the number of people in need of HIV prevention and care services; (3) plan, prioritize, and allocate resources for HIV prevention, intervention, and care programs; and (4) monitor and evaluate the impact of HIV prevention, intervention, and care programs on the key outcome indicators for implementation 90-90-90 strategy.

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NATIONAL HIV BIOLOGICAL AND BEHAVIORAL SURVELLANCE IN HARD-TO-REACH POPULATIONS IN BULGARIA (2004 -2016). PART 1: FORMATIVE RESEARCH

Tonka Varleva[1], Hristo Taskov[2], Emilia Naseva[3], Vyara Georgieva[1], Elena Kabakchieva[4], P. Tsintsarski[5], Tsveta Raycheva[6], Stefka Boneva[5] and B. Petrunov[2]

1 – Ministry of Health, Sofia
2 – National Center Infectious and Parasitic Diseases, Sofia
3 – Medical University of Sofia, Faculty of Public Health
4 – Health and Social Development Foundation (HESED)
5 – Consultant at Ministry of Health
6 –State psychiatric hospital for drug and alcohol users

ABSTRACT

Between 2004 and 2016, in Bulgaria nine rounds of National bio-behavioural survey were conducted within the framework of the National programs for the prevention and control of HIV and STIs. Surveys were performed in five key populations: men who have sex with men (MSM), people who inject drugs (PWID), sex workers (SW), prisoners and young Roma at higher risk (YYRMHR). The total number of participant was 27,210.

All municipalities were included in the pre-assessment cycle. Based on criteria for HIV risk ten municipalities were selected for the first two rounds of the survey. Thereafter four formative assessments were performed in 19 municipalities in the country. The venues frequented by target population were listed and their number estimated. Total 2,276 venues were identified: 812 venues for PWID; 540 - for MSM; 817 for FSW and 229 for MSW. In 747 of the venues, mixing of the groups was associated with an increased risk of HIV infection. Municipalities with at least 800 high-risk group members were selected for surveys.

In this article, we describe the outcomes of five formative research cycles. The methodology and ethical issues of the survey are described in Part 2 and 3 of the article.

KEYWORDS:

HIV surveillance, key populations, Bulgaria

ADDRESS FOR CORRESPONDENCE:

Prof. Dr. Hristo Taskov DSc,
National Center Infectious and Parasitic Diseases,
Sofia 1504, Bul. Yanko Sakazov 26,
Tel.: +359887304381;
E-mail: hristotaskov@gmail.com

INTRODUCTION

Bulgarian HIV epidemic has been best described as concentrated mainly in people who inject drugs (PWID) and men having sex with men (MSM) (1, 2). In 2006 HIV transmission due to injection drug use was responsible for 37.4% of all newly diagnosed HIV cases. In 2015 about 49.6% of newly diagnosed HIV cases came from MSM population (3). In 2016, PWID and MCM contributed for 88% of new HIV diagnoses. Three other subpopulations are also involved in the spread of HIV infection in the country. In 2011 the HIV prevalence rate in sex workers (FSW) was 0.82%, in young Roma men at higher risk (YRMHR) -2.06% and prisoners - 0.98% (4). The concentrated nature of the Bulgarian HIV epidemic necessitates a strong surveillance among key populations (KP) to understand HIV dynamics and facilitate an effective national response. With a view to strengthen the surveillance activities among hard-to-reach-populations (HTRP) Ministry of Health (MoH) has implemented National Biological and Behavioural Surveillance (NBBS) to generate evidence on risk behaviours among key populations to support planning and prioritization of prevention programmes interventions at the district and national levels.

In line with WHO guidelines (5) in 2004 Bulgaria started NBBS among five key populations (MSM, PWID, SW, YRMHR and prisoners) with strategic focus to strengthen the HIV surveillance within them. Having in mind the value of conducting formative research prior to implementing epidemiologic surveys (6, 7) in 2003, a comprehensive formative assessment was carried prior the first round of NBBS in the period 2004-2016 (NBBS_04-16). This coincided

with the preparatory phase of the implementation of the Global Fund grant (8) co-financing the National Program for Prevention and Control of HIV and STI, 2001-2007 (NHP-01-07) (9). The data collected during this preparatory phase provided contextual information about HIV risk behaviors within the populations of interest and help the project sites to make decisions about field operations and other logistical issues. The information laid the foundation for collection of relevant NBBS data. During next years, four formative assessments were performed with detailed mapping of the studied populations, their locations and size.

In summary, the overall purpose of the formative researcher component of BBS-04-16 was to provide Ministry of Health and NHP-01-16 with information to help the HIV surveillance authorities and NHP managers: (1) describe the populations of interest, and their characteristics; (2) gain preliminary insight into the context of HIV risk behavior within the community of study; (3) garner the support of community stakeholders for the behavioral survey; (4) develop questions to measure access to local HIV prevention services, and (5) finalize decisions about BBS-04-16 field operations, such as the best locations and hours for data collection and the selection of appropriate interview staff.

This article describes outcomes of above-mentioned five cycles of formative research in 2003, 2010, 2011, 2012 and 2014. The methodology and ethical issues of NBBS-04-16 are described in Part 2 and Part 3 of the article (10, 11) and the results are described elsewhere (1, 2, 12-15) .

PRE-SURVEY FORMATIVE ASSESSMENT

During the first two years of the implementation of the NHP-2001-2007, NGOs collected initial information on the size of key populations and their locations. However, in 2003, during preparatory phase of the implementation of the Global Fund grant (8), a formative assessment was carried out. Its aim was to collect comprehensive information on the hard-to- reach-populations (HTRP), and to inform the development and conduct of the survey itself. It comprised the determination of size of HTRP; overlapping risk behaviours (sexual and injecting drug use); mixing of HTRP with other risk behaviours and practices (prostituted, drug addicts) in the rest of the population; presence of main transport network (port, highways and railways) and tourist flow.

Methodology of the assessment and data collection

The research was initiated in June 2003 and completed in September 2003 in all municipalities to assess their vulnerability to HIV.

The following criteria were used to assess the municipalities: 1) Size of the target groups; 2) Combined risk behaviors (e.g. risky sexual behavior and intravenous drug use); 3) Mixing of target groups with other groups with risk behavior and practices (prostitution, drug addicts); 4) Mixing of target groups and the general population; 5) Existence of a main transport network (highways and railways) near the place of residence; 6) Availability of a port; 7) Presence of tourist flow; 8) Existence of higher and upper secondary schools; 9) Existence of secondary schools with a large number of students from other settlements.

These criteria met the objectives of the survey: 1) to reach as many people as possible within the target groups; 2) to select settlements with high concentration of target groups and risk factors; 3) to survey of exposure of population to a higher risk factors and likelihood of HIV infection.

Based on those criteria, 35 indicators describing the vulnerability of municipalities to HIV and STIs were developed. Based on those indicators a questionnaire was elaborated and send to the mayors of all municipalities - district centers. Collecting the baseline data in this way was necessary due to the scarcity of data from the health statistics at the municipal level on the type and size of the populations at risk as well as on the presence of risk factors. On the other hand, engaging municipal authorities early in the process was particularly important for the program's performance. Data from all 27 municipalities - district centers for the years 2001 and 2002 were collected.

For quantitative analysis, ten key variables from 35 were selected to allow a comparative analysis based on the most important risk factors for HIV and for which information was provided by a maximum number of municipalities.

The *baseline indicators* of the quantitative analysis were as follows: a) *"Intravenous drug*

users" - total number of intravenous drug addicts; b) *"sex workers*" - number of sex workers and number of newly discovered cases of syphilis (total); c) *"Roma"* - population with Roma ethnic identity (number); d) *"young people"* - Population aged 10 - 19 years (number); total number of students; total number of students and dropouts (school-age young people not attending school); e) "*risky sexual behavior*" - Number of newly diagnosed cases of syphilis (up to 19 years of age) and abortions (up to 19 years of age).

For qualitative analysis, the following indicators were selected: 1) Availability of major transport networks (highways and railways); 2) Port availability; 3) Existence of large tourist flows; 4) Existence of discrete, communities of young people from other settlements (students, soldiers etc.).

Data analysis

The following approaches were applied in the analysis of the collected data: 1) Statistical procedures for processing the data from the quantitative indicators and 2) Expert analysis of the qualitative indicators and 3) comparison and superposition of the qualitative ones with the quantitative indicators.

Statistical procedures

The statistical classification of municipalities had to meet two important requirements: 1) it should take into account the vulnerability (e.g. number of PWID, number of SW in the municipality etc.) and 2) take into account the degree of concentration of risk factors in a given municipality (e.g. the proportion of PWID in the total population, proportion of SW, etc.).

In order to meet the first requirement ranking was performed based on absolute values of the indicators, and in the second case – based on proportions. The method of ranking of municipalities combines these requiremen ts in the following way:

1. Missing data values were replaced by values from similar municipalities. This procedure allows for subsequent operations to average hierarchies of the same size (from 1 to 27);
2. The municipalities were ranked according to the absolute values of each of the output variables. Each of the municipalities receives a rank of 1 to 27 for each of the 10 selected indicators, where 1 corresponds to a low vulnerability and 27 to a high vulnerability;
3. The absolute values of each of the output variables were transformed into proportion relative to the total population of the municipality. Municipalities were again assigned a rank of 1 to 27 for each of the indicators according to which 1 corresponds to low vulnerability and 27 to high vulnerability (step 1);
4. The variables were weighted. The weight of the each variable for vulnerability to HIV infection was assigned according experts opinion as follows: PWID wt = 5; SW wt = 3; Roma wt = 2; Syphilis all age wt = 3; Abortions wt = 2; Young people wt = 1; Students wt = 1; High school students wt = 1; Leaved school wt = 1.;
5. The arithmetic mean of the scores for all variable (from step 4) was taken as the final assessment of the vulnerability of the municipality. The municipality were ranked according the score.

Results

The results are presented on Table 1 and Figure 1. The statistical method used for the selection of municipalities is appropriate from an epidemiological point of view and complies with the criteria recommended by UNAIDS. It takes into account the need to combine "scale" and "concentration" of interventions.

Expert analysis

Expert analysis was needed due to the existing difficulties in quantifying the qualitative indicators and the degree of the risk factors. Expert analysis has taken into account the important circumstances relating to the mixing of risk groups both with each other and with the community as a whole, the existence of large transport networks and ports; flows of tourists and travelers; mobility of young people (secondary school students, higher school students, soldiers).

Selection of municipalities

To ensure that the required sample size could be reached, it was stipulated that each potential domain/ district should be estimated to have at least 800 high-risk group members: PWID -300, YRMHR -300 and FSW- 300. There was no quantitative requirement for the number of

TABLE 1. Ranking of municipalities according the score of vulnerability variables.

Municipality	Average wt	PWID wt =5	SW wt = 3	Roma wt = 2	Syph. wt = 3	Abort. wt = 2	Young wt = 1	Stud. wt = 1	HS stud. wt = 1	Leaved school wt = 1
Varna	45	130	100	36	57	40	16	23	22	10
Sliven	41	118	100	49	24	43	21	18	8	20
Pleven	41	118	74	36	53	36	17	18	10	19
Burgas	39	70	100	30	30	46	25	22	18	23
Pazardzhik	37	118	60	49	35	46	18	13	6	25
Stara Zagora	36	85	92	48	30	38	18	20	16	16
Sofia	35	83	74	21	41	35	17	14	25	18
Ruse	33	100	86	33	32	19	12	12	18	19
Plovdiv	33	95	64	29	26	44	18	26	23	9
Blagoevgrad	31	118	16	29	36	26	16	19	25	1
Dobrich	29	75	44	32	41	25	22	22	16	21
Haskovo	28	68	33	13	42	37	14	15	13	15
Veliko Tarnovo	28	28	70	13	68	5	7	6	24	5
Shumen	27	35	64	35	54	29	19	18	22	8
Gabrovo	27	46	54	9	78	13	4	4	19	2
Targovishte	26	28	72	29	68	12	14	5	2	20
Vratsa	25	25	35	29	56	13	9	8	14	6
Montana	24	55	16	40	60	41	10	15	2	13
Pernik	23	85	26	25	24	17	11	14	22	9
Kyustendil	23	98	16	29	17	20	6	9	4	3
Lovech	22	49	40	11	26	16	7	9	10	16
Vidin	21	68	8	29	12	43	4	12	6	24
Smolyan	17	8	52	2	62	31	12	4	13	15
Razgrad	17	8	74	20	6	20	17	17	2	20
Silistra	15	35	30	13	30	6	8	9	8	9
Kardzhali	13	15	4	13	51	2	15	6	10	11

Abbreviations: **wt** stands for weight; **PWID** stands for people who inject drugs; **SW** stands for Sex workers; **Roma** stands for Roma population; **Syph** stands for syphilis; **Abort** stands for abortion; **Young** stands for young people; **Stud** stands for secondary school students and **HS stud** stands for high school students.

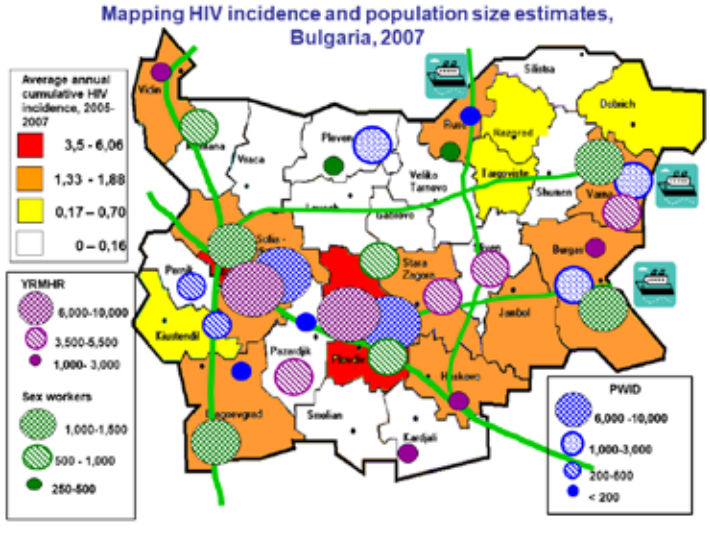

Fig. 1. Overly of the main transport network, average rate of new diagnosed HIV cases per 100 000 population, localizations and the size of PWID, sex workers and Young Roma man at higher risk (YRMHR)

MSM, assuming that they were 3% of the adult male population. There was no requirement for MSW as they are extremely difficult to reach population in Bulgaria that is commonly surveyed along with FSW. According to the ranking and expert opinion, the following municipalities were selected for implementation of the BBS-04-16: Varna, Pleven, Burgas, Pazardzhik, Stara Zagora, Sofia, Ruse, Plovdiv and Blagoevgrad (Table 1). Blagoevgrad was selected instead of Sliven because of its geographical location on the international road to Greece.

It should be noted that selected municipalities account for 37% of the total population of a country. In these municipalities are concentrated majority of the target groups: 90% of PWID, 67% of SW, 50% of Roma population, 67% of young people, 66% of secondary school students and 82% of higher school students. Finally yet importantly, these data show that the BBS_04-16 was implemented in municipalities with significant mix of target groups. It is also important to note that the majority of activities were planned for implementation in the selected municipalities during NHP-04-07 and the Global Fund grant (Figure 2). By combining NHP-01-07 activities with epidemiological surveillance of HIV, the effect of prevention interventions among the target groups could be monitored well. In the course of BBS-04-16 and NHP-01-07, it was demonstrated that the selected municipalities have actually the highest risk for the spread of HIV in the country.

Reassessment of venues and size of the target populations (2010 -2012)

Six years after the initial preliminary assessment, the regular formative research was carried out before each BBS cycle. The main purpose was to map the locations where the groups frequented, as well as to determine their size. The study included: a) Estimations on the size of groups, sub-groups; their geographical location in terms of public appearances, meeting and entertainment venues, sex search and offer, meeting venues for procurement drugs and drug injection and others, and b) Identifying the locations where target groups representatives can be reached with BBS activities.

Study methodology.

Geographical coverages of the study

In 2010, 2011 and 2012, three assessments were carried out in 20 municipalities, out of 28 (74%), thus covering the main part of the country.

In 2010 the study was performed in nine municipalities namely Blagoevgrad (included Sandanski), Burgas, Varna, Pazardzhik (including Peshtera), Pleven, Plovdiv, Rousse, Stara Zagora and Sofia. The reason for this was that the first two rounds of BBS-04-16 were conducted in these municipalities. In addition, geographically they cover all main regions of the country. Most of the target populations are concentrated in them and there are overlapping risk factors. Peshtera was included due to the demonstrated higher number of new HIV diagnoses in PWID and Sandanski as a place for sex tourism.

In 2011, the survey was conducted in the municipalities of Vidin, Haskovo and Yambol. Fifteen area were selected in Vidin, including the ring roads. In the municipality of Haskovo the survey covered the cities of Haskovo,

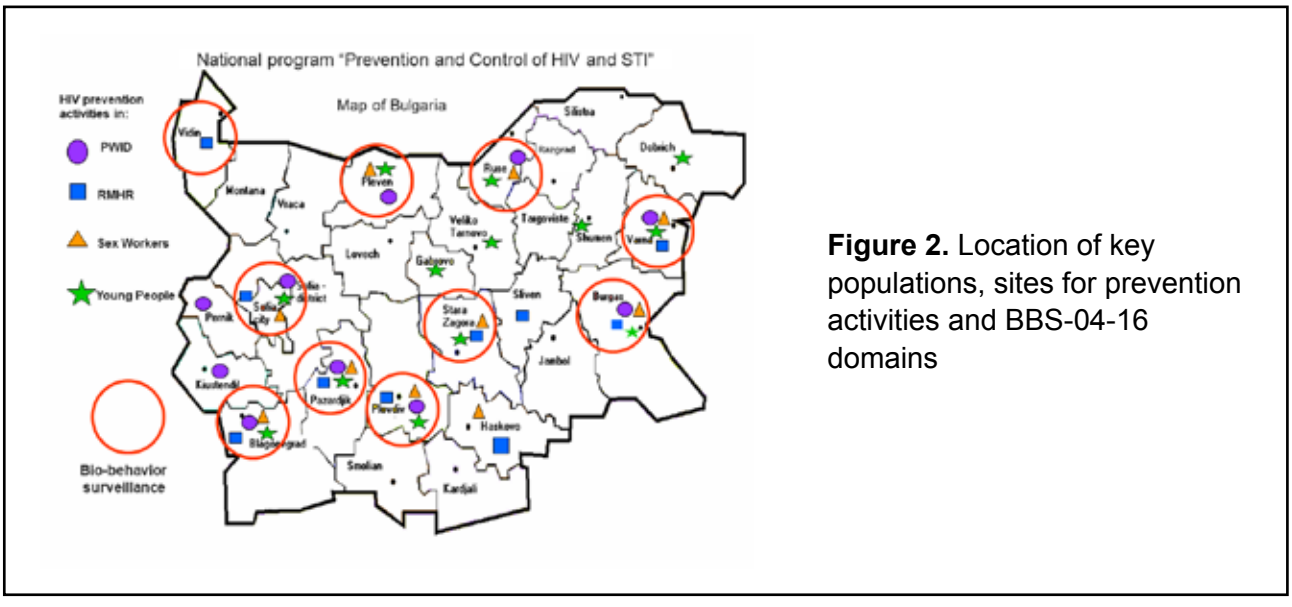

Figure 2. Location of key populations, sites for prevention activities and BBS-04-16 domains

Dimitrovgrad, Harmanli, Svilengrad and the international road E80. In the municipality of Yambol 14 area were selected which are frequented by HTRP according to NGO data.
In 2012 the survey was conducted in the municipalities of Montana, Sofia-district, Kyustendil, Smolyan, Sliven, Razgrad and Silistra. In municipality Montana, the towns of Montana, Lom, Berkovitsa and Varshets were selected, including ring roads and places close to the cities that the target groups can be found. There are no areas excluded from the survey. The two largest municipalities - Samokov and Botevgrad - were included in the Sofia region. In the municipality of Kyustendil the survey included Kyustendil, Dupnitsa and Bobov Dol. In Sliven municipality, the survey was conducted in the following places: the towns of Sliven, Kotel and Tvarditsa and the villages of Samuilovo, Chintulovo, Seliminovo, Shivachevo,
All studies were managed by the Central BBS Unit (CBBSU) at Ministry of Health (MoH) at country level and by Regional Health Inspectorates (RHI) at municipality level. Local NGOs providing prevention activities among study populations were hired for fieldwork.
Before starting of assessment, on-site meetings with RHI and NGOs were organized by CBBSU staff for planning of activities and assignment of tasks. Based on existing information from NHP and NGOs, in each city areas were identified where the target groups are most likely to be found.

Collection of quantitative and qualitative data
Information was collected mainly through NGO staff, key informants, community representatives and outreach workers. RNIs collected the available official information.
As a key informants, individuals were selected which are knowledgeable about the study populations. Criteria for selection were to have direct supervision on the target group and to have reliable information. The main key informants used were district police officers, general practitioners and emergency doctors, NGO staff and others. After identifying the locations and depending on the target group, community representatives and other key informants were used to collect information: bartenders and waiters; 24-hour shops; pawn shops; hobos and homeless people; taxi drivers and NGO staff that provided prevention services to the study population. The latter have a long-standing experience of working with the studied populations; they know well the venues and have established contacts with bars and locals, gatekeepers, police and others.

Field Survey
Field survey started by anonymous interviews with key informants and representatives from the community and target groups. Experienced NGO staff held the interviews. They recorded all the places, venues and events reported by key informants and community representatives. The data were send to the local RHI where an epidemiologist processed and entered the collected information in electronic spreadsheet. Than the data were send to CBBSU for evaluation and further processing. There a list of established unique sites for each city was drawn up. This information was sent back to RIHs where the sites were allocated to the relevant NGO for organizing site visits. NGO assistants visited these sites, described and mapped the venues where target group's representatives could come in contact. Information was collected on name of the venue, its accessibility, attendance, the time of its visit by the target groups, pick-time and the mixing of the groups. Together with the mapping, an enumeration of target group's individuals was carried out at each visited site. The coverage of the venues was done by two visits in each site. The data were entered into spreadsheets and sent to the local RHI**.**

Processing, analysis and storage of data.
The information received in RHI was summarized and analyzed by an epidemiologist, and then analytical summary report was prepare with a map of the identified venues, number of target populations and mixing of risks. The report was sent to CBBSU together with the questionnaires and electronic spreadsheets. Questionnaires from informants' interviews were kept in a locked cabinet, with only those working in the study accessing them.

Results
CBBS staff combined spreadsheets in a database and cleared it of errors and duplicates. The results of the analysis are presented in Tables 2 -5.

In 2010 total 2276 venues were identified in nine municipalities (Table 2), with 2207 (97%) of them being visited twice. There are 812 PWID's venues; 540 MSM's venues; 817 for FSW and 229 for MSW. In 747 of the venues, mixing of the groups was associated with an increased risk of HIV infection. As can be expected, most venues are located in capital Sofia (471), followed by big cities like Plovdiv (336), Burgas (326) and Varna (267). Although in size, the FSW group is several times smaller than that of MSM, it has the largest number of venues. It is interesting to note that one third of the venues are at high risk (747 sites out of total 2,276) due to the mixing of the groups in them. On average, in 52% of venues, more than five representatives of the target groups were registered and these venues were determined as appropriate for BBS clusters.

TABLE 2. Number of identified venues and events frequented by the target groups.

City	Venues identified	Venues visited	PWID		MSM		FSW		MSW		Venues with mixed populations
			Total venues	Venues > 5 PWID	Total venues	Venues > 5 MSM	Total venues	Venues > 5 FSW	Total venues	Venues > 5 MSW	
Blagoevgrad	197	193	81	21	4	0	82	42	1	0	33
Burgas	326	326	118	32	99	20	166	33	14	1	133
Varna	267	267	63	46	96	92	211	97	75	44	109
Pazardzhik	251	250	157	92	44	7	82	23	9	0	64
Pleven	197	200	125	114	8	1	84	54	0	0	20
Plovdiv	336	263	67	34	55	44	10	6	16	9	77
Ruse	93	94	16	7	5	3	21	9	0	0	19
Sofia	471	476	156	86	159	133	104	69	82	30	223
Stara Zagora	138	138	29	13	70	39	57	38	32	17	69
Total	**2276**	**2207**	**812**	**445**	**540**	**339**	**817**	**371**	**229**	**101**	**747**

Size of studied populations

Enumeration of hard-to-reach populations is challenging. In our case, we used four sources of information: (a) a two-fold enumeration of populations during venue visits (unadjusted and corrected); b) information from a key informant and c) information from outreach workers. The results from the survey in 2010 are presented Table 3. It was found that the number of persons reported by the key informant and outreach workers were about twice as large as the number registered by enumerations. For this reason, the final number of persons in the groups was calculated as arithmetic mean of the four estimates. As shown on Table 2 all municipalities have the required numbers of PWID and female and sex workers. For MSM and male sex workers the requirements was met by four municipalities. Surprisingly only eight people of MSM group were enumerated in Blagoevgrad. This findings do not corresponds to the estimated number of 1,000 MSM by NGOs in 2014 and 2,745 MSM as 3% of adult men in the city (Table 6).

TABLE 3. Formative assessment 2010. Number of PWID, MSM, female and male sex workers distributed by location and source of information.

	Total number of PWID					**Total number of MSM**				
City	After two enumerations	After two enumerations -adjusted	According to key informant	According to outreach workers	**Average**	After two enumerations	After two enumerations -adjusted	According to key informant	According to outreach workers	**Average**
Blagoevgrad	293	296	685	612	**472**	8	8	8	9	**8**
Burgas	402	434	760	960	**639**	330	376	614	601	**480**
Varna	734	750	1401	1354	**1060**	2634	2699	3846	3908	**3272**
Pazardzhik	810	934	1285	941	**993**	75	140	147	120	**121**
Pleven	1770	1785	2964	3029	**2387**	14	14	17	17	**16**
Plovdiv	426	489	640	696	**563**	588	622	805	783	**700**
Ruse	100	103	90	105	**100**	51	52	64	104	**68**
Sofia	1109	1232	2221	1923	**1621**	1449	2262	4286	4848	**3211**
Stara Zagora	138	138	214	177	**167**	379	379	354	362	**369**
	Total number of female sex worker					**Total number male sex workers**				
Blagoevgrad	450	450	450	528	**470**	2	2	2	2	**2**
Burgas	452	552	951	959	**729**	44	45	99	83	**68**
Varna	2048	2211	2893	2867	**2505**	505	525	787	734	**638**
Pazardzhik	177	308	330	265	**270**	6	19	38	15	**20**
Pleven	586	611	598	605	**600**	ND	ND	ND	ND	
Plovdiv	350	362	436	409	**389**	152	159	141	235	**172**
Ruse	169	172	288	337	**242**	ND	ND	ND	ND	
Sofia	1377	1635	2094	1874	**1745**	708	742	895	1094	**860**
Stara Zagora	579	585	558	541	**566**	173	176	145	144	**160**

The results from the assessment in 2011 are presented in Table 3. In Vidin and Yambol the number of target groups are below the required. Hskovo is eligible only for female sex workers. The figures of enumerated MSM in Vidin, Hskovo and Yambol are far below the expected 3% from adult men population.

The results from the assessment in 2012 are presented in Table 4. Five of eight municipalities are eligible for BBS within PWID and two within SWs. In Kyustendil total 255 SWs were enumerated and also was considered for survey among this subpopulation. In this assessment, the results for MSM were controversial as in previous.

TABLE 4. Formative assessment 2011. Number of PWID, MSM, female and male sex workers distributed by location and source of information.

	Total number of PWID					**Total number of MSM**				
City	RHI report	After two enumerations -adjusted	According to key informant	According to outreach workers	**Average**	RHI report	After two enumerations -adjusted	According to key informant	According to outreach workers	**Average**
Vidin	118	118	133	116	**121**	7	8	8	7	**7.5**
Haskovo	0	0	3	1	**1**	75	75	87	77	**78.5**
Yambol	158	162	197	168	**171**	62	62	86	62	**68**
	Total number of female sex worker					**Total number male sex workers**				
Vidin	68	68	78	68	**71**	ND	ND	ND	ND	
Haskovo	366	366	414	411	**389**	ND	ND	ND	ND	
Yambol	137	140	184	135	**149**	10	10	10	10	**10**

REASSESSMENT THE SIZE OF PWID AND MSM POPULATIONS (2014).

MSM and PWID are the main groups driving the HIV epidemic in Bulgaria. The unsatisfactory results in previous surveys for enumeration of MSM lead us to carry out a special study of the group size in 2014. This option was used to perform parallel estimates of PWID size as well. MSM were studied in 19 major cities in the country (Table 4.), and PWID in 18 cities (Table 5.) CBBSU has gathered information from various sources: NGOs providing preventive services to PWID and MSM; actually reached individual clients by NGOs in 2013 by prevention activities of NHP; RHI reports; assessment of outreach workers or key informant and published data.

National Center for Drugs and Drug Addictions have estimated in 2009 the number of PWID in the country at 21,100 and respective prevalence rate at 0.27% in the general population (16, 17). On this basis, an estimate of the number of PWID in cities was made in current study.

There are no direct studies about the number of MSM in Bulgaria. In three articles, MSM's share in Bulgaria is estimated to be between 2.3% and 3.3% (an average of 3%) of the male population aged 16-54 (18). In another publication, the number of MSM was estimated to be 26,341 (19).

TABLE 5. Formative assessment 2012. Number of PWID, MSM, female and male sex workers distributed by location and source of information.

	Total number of PWID					**Total number of MSM**				
City	RHI report	After two enumerations -adjusted	According to key informant	According to outreach workers	**Average**	RHI report	After two enumerations -adjusted	According to key informant	According to outreach workers	**Average**
Montana	309	317	529	423	**395**	ND	ND	ND	ND	
Sofia Region	85	91	92	101	**92**	ND	ND	ND	ND	
Kyustendil	278	278	446	417	**355**	263	263	530	389	**361**
Smolyan	108	46	46	108	**77**	5	ND	ND	5	**5**
Sliven	510	430	1316	1015	**818**	367	113	339	411	**308**
Nova Zagora	350	645	803	797	**649**	400	276	354	341	**343**
Razgrad	82	82	82	82	**82**	37	37	37	37	**37**
Silistra	298	298	384	238	**305**	ND	ND	3	ND	**3**
	Total number of female sex worker					**Total number male sex workers**				
Montana	43	43	64	76	**57**	ND	ND	ND	ND	
Sofia Region	26	69	70	70	**59**	ND	ND	ND	ND	
Kyustendil	140	140	208	181	**167**	75	75	112	88	**88**
Smolyan	45	ND	ND	48	**47**	ND	ND	ND	ND	
Sliven	433	143	543	468	**397**	54	63	116	119	**88**
Nova Zagora	200	296	460	433	**347**	80	44	61	54	**60**
Razgrad	98	98	98	98	**98**	24	24	24	24	**24**
Silistra	56	56	111	69	**73**	ND	ND	ND	ND	

The result for PWID are presented in Table 5. The values obtained by different sources for PWID size are similar. Largely, they coincide with the numbers of PWID calculated by the prevalence rate (16, 17). Due to the high uncertainty in this group, the experts have indicated not a specific number but the range of estimated PWID size in the studied cities.

The MSM results are presented in Table 6. For this group, the data provided by the different sources differs significantly compared to values calculated on the basis of 3% MSM prevalence. One explanation is that NGOs had reached only a part of MSMs, which visited the studied venues. It is important to note that not all MSM are subject to active prevention. According to Aghaizu, A. et al., unprotected anal sex by MSM is between 43% and 53% (20). This raises the question of which MSM group should be studied when considering the risk of HIV infection, the whole population or only those at high risk. For this reason, based on the information collected and the NGO reports on MSM, the experts have indicated values that lie between the two endpoints. Further studies are needed for estimation the size of the MSM group in the studied municipalities.

TABLE 6. Estimated number of PWID in the studied cities by different source of information.

City	NGO estimations#	Individual contacts by NGO$	Outreach worker's opinion	Key informant	Report of RHI	Prevalence rate (0.27%) &	Expert estimations*
Blagoevgrad	220	300	296 -612	685	288	831	400-600
Burgas	600-650	760	960	-	800	1111	800
Varna	1300-1700	960	1354	-	700-1354	1275	1300-1600
Vidin	-	-	118	133	118	235	100-200
Kyustendil	400	170	417	-	278	327	300-400
Nova Zagora	600		645-797		350	352	350-600
Pazardzhik	420	400	941		260-940	697	600-800
Pernik	300-310	200	-	-	-	352	300-400
Pleven	720	660	3029	-	1777	659	700-1000
Plovdiv	1530	750-800	696		480 -700	1808	1500-2000
Ruse	600	290	105		100	598	400-600
Sliven	40	40	430-1015	-	510	509	400-500
Sofia **	1540-1575	900	1923		1074-1923	3579	7 000 -8000
Stara Zagora	-		138-177	-	177	861	200-250
Yambol	60	-	162	-	158	325	150-300

Estimation by NGO of whole size of the group in the city.
$ Number of individual clients contacted by NGO during 2013.
& Prevalence rate 0.27% from adult population [ ]
***** Estimation by expert of MoH- Program "Prevention and control of HIV and STI" based on provided information and experience.
** The estimated number for Sofia is low because the assessment is performed only on certain venues in the wide center of Sofia.

TABLE 7. Estimated number of MSM in the studied cities by different source of information.

City	NGO estimations#	Report of RHI	From Table 2@	Outreach worker's opinion	Key informant	Prevalence rate (3%)&	Expert estimations*
Blagoevgrad	1000	8	8	9	8	2745	918
Burgas	2000	452	376	601	614	4513	1504
Varna	3000	2698	2699	3908	3846	5803	1934
Vidin		7	8	7	8	895	298
Kyustendil		263	263	389	530	1312	437
Nova Zagora		400	276	341	354	320	106
Pazardzhik		60	140	120	147	2458	819
Pleven		14	14	17	17	2532	844
Plovdiv	1000	410	622	783	805	7287	2429
Razgrad		37	37	37	37	818	273
Ruse		51	52	104	64	2591	863
Sliven		367	113	411	339	1858	619
Smolyan		5	0	5	0	930	310
Sofia	5000	1449	2262	4848	4286	17879	5959
Sofia Region		0	0	0	0	2168	722
St. Zagora		362	379	362	354	3428	1142
Haskovo		75	75	77	87	2529	843
Yambol		62	62	62	86	1306	435

Estimation by NGO of whole size of the group in the city.
@ Table 2. Column "After two enumerations –adjusted".
& Prevalence rate 3% from adult man population [ ]
***** Estimation by expert from MoH- Program "Prevention and control of HIV and STI based on provided information and experience.

It should be borne in mind that the expert assessment of group's size is approximate for the survey populations. There is a lot of mobility especially at MSM and SW. People often travel in the country and abroad in search of work, education, places for social and sexual contacts.

CONCLUSIONS

Much has been written about the value of incorporating both qualitative and quantitative data collection methods into public health research (21, 22). This article provides a detailed description of the formative research process in preparation of BBS-04-16 and highlights the role of data collection for the implementation of the BBS-04-16. In addition to providing BBS sites with information about issues associated with HIV risk within their area, the data collected during this assessment help BBS managers to make decisions about field operations and other logistical issues related to the BBS-04-16. The findings of these formative assessments suggest that there are defined clusters of high-risk groups in the biggest cities in Bulgaria. This was essential for development of survey design and calculating the survey budget. Although our study provides deep insight into the distribution and size of target groups, some of the study limitations should be noted in order to place our findings in the proper context. The common challenge that researchers usually face for probability sampling is to estimate the size of studied populations. The results of this formative study suggest that there is a need for determining more precisely the size of the target groups especially of MSM. During the surveys, we used information from NGOs, key informants and official institutions. Currently methods are available that are more reliable for enumeration hard-to-reach populations and they should be used in futures rounds of NBBS (23, 24). We hope that the process and outcome of this formative research will help to inform similar work in the future.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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NATIONAL HIV BIOLOGICAL AND BEHAVIOURAL SURVEY AMONG HARD-TO-REACH POPULATIONS IN BULGARIA (2004 -2016). PART 2: METHODOLOGY OF THE SURVEY

Tonka Varleva[1], Hristo Taskov[2], Emilia Naseva[3], Vyara Georgieva[1], Elena Kabakchieva[4], P. Tsintsarski[5], Tsveta Raycheva[6], Stefka Boneva[7] and B. Petrunov[2]

1 – Ministry of Health, Sofia
2 – National Centre Infectious and Parasitic Diseases, Sofia
3 – Medical University of Sofia, Faculty of Public Health
4 – Health and Social Development Foundation
5 – Consultant at Ministry of Health
6 – State Psychiatric Hospital for Drug and Alcohol Users

ABSTRACT

Between 2004 and 2016, in Bulgaria 9 rounds of bio-behavioural surveillance were conducted within the framework of the National Programs for the Prevention and Control of HIV and STIs. Surveys were performed in the major cities among five key populations: men who have sex with men (MSM), people who inject drugs (PWID), sex workers (SW), prisoners and young Roma at higher risk (YRMHR). The surveys were cross-sectional with a two-step complex cluster design with a time location sampling (TLS). The total number of participants in the NBBS-04-16 was 27,210 disaggregated by studied groups as follows: MSM (4,725); PWID (8,626); SW (4,013); prisoners (4,557) and YRMHR (5,289).
In this publication, we describe the survey methodology. The formative research and ethical issues are described in Part 1 and Part 3.

ADDRESS FOR CORRESPONDENCE:
Prof. Dr. Hristo Taskov MD, DSc,
National Center Infectious and Parasitic Diseases,
Sofia 1504, Bul. Yanko Sakazov 26,
E-mail: hristotaskov@gmail.com

KEYWORDS:
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INTRODUCTION

Bulgarian HIV epidemic has been best described as concentrated epidemic. HIV infection is concentrated mainly among people who inject drugs (PWID) and men having sex with men (MSM) (1, 2). In 2006 HIV transmission due to injection drug use was responsible for 37.4% of all newly diagnosed HIV cases. In 2015, about 49.6% of the newly diagnosed HIV cases came from MSM population (3). In 2016, PWID and MCM contributed for 88% of new diagnoses. Three other subpopulations contributes to the spread of HIV in the country. In 2011, the HIV prevalence rate for female sex workers (FSW) was 0.82%, for YRMHR-2.06% and prisoners - 0.98% (4).The concentrated nature of the Bulgarian HIV epidemic necessitates strong surveillance among the key populations (KP) to understand their epidemic dynamics and facilitate an effective national response. In order to strengthen the surveillance activities among hard-to-reach populations (HTRP), the Ministry of Health (MoH) implemented National Biological and Behavioural Surveillance (NBBS) to generate evidence on risk behaviours among HTRP and to support planning and prioritization of prevention programmes interventions at the district and national levels. A key feature of NBBS was the inclusion of behavioral studies designed to provide a better understanding of the epidemic dynamics in low HIV prevalence and a concentrated type of epidemic.

In line with WHO guidelines (5) in 2004 Bulgaria started the implementation of NBBS among five KP (MSM, PWID, SW, YRMHR and prisoners) with a strategic focus to strengthen the HIV surveillance among them. NBBS is integral part of the comprehensive Bulgarian HIV Surveillance system (6) to obtain extensive data about the status, changing trends and progress of their HIV epidemic. Unlike conventional surveillance systems, NBBS helps identifying vulnerability to infection and ascertains the main determinants of the HIV epidemic. NBBS collects information on many key parameters of programmatic importance (7). It included knowledge indicators related to HIV prevention, STI, condoms, HIV/AIDS services, risk profile and practices, HIV testing, stigma and discrimination as

well as exposure to HIV/AIDS services and community mobilization. NBBS also provides basic information for focusing and designing interventions, and for monitoring the impact of the interventions proposed within the strategic planning process.

Between 2004 and 2016, nine rounds of the NBBS were held in Bulgaria. In this paper, we describe the methodological aspects of NBBS-04-16. The ethical issues and results of NBBS are described elsewhere (1, 2, 8-11).

ORGANIZATION AND MANAGEMENT OF THE NBBS

The NBBS-04-16 was organized and conducted by the MoH in the framework of the National Program for Prevention and Control of HIV and STIs (NHP) (12, 13) with the support of the Global Fund to Fight (14) AIDS, Tuberculosis and Malaria (GFATM) (13). NBBS-04-16 was managed by a principal investigator, who is director of the NHP. A specifically designated objective of the NHP targeted the epidemiological studies, in particular in this NBBS (13). Within this objective, a Central BBS Unit (CBBSU) was established at the MoH. It was situated at the National Centre of Infectious and Parasitic Diseases (NCIPD, www.ncipd.org) and provided epidemiologist, sociologist, data manager and consultants.

At municipal level, the Communicable Diseases Surveillance Directorate (CDSD) at the Regional Health Inspectorates (RHIs) were managing the NBBS with experienced epidemiologists. CDSD structure includes Laboratory of Virology and Low Threshold HIV Testing Sites (LTHTS) (3) providing free of charge HIV testing and counselling.

Fieldwork was carried out by non-governmental organizations (NGOs) carrying out prevention services with target groups. These NGOs have been working with HTRP for many years, have received especially designed upgraded training to meet the updated standards for outreach services, have a good knowledge of the local situation, enjoying the trust of key populations and have established contacts with gatekeepers and local authorities.

The NBBS-04-16 was conducted in four main phases: a) planning and pre-surveillance assessment; b) development of sampling frame; c) collection of behavioural and biological data and (d) data analysis and dissemination.

PLANNING AND PRE-SURVEILLANCE ASSESSMENT

The methodology adopted for the NBBS was community based cross-sectional survey design using a probability-based two-stage complex sampling.

Respondent Groups

In the NHPs 2004-2007 and NHP 2008-2015, five key high-risk populations were identified: MCM, PWID, SW, YRMHR and prisoners (15, 16).

Men who have sex with men

There are no direct studies to determine the number of MSM in Bulgaria. In three publications, MSM's proportion in Bulgaria is estimated to be between 2.3% and 3.3% (an average of 3%) of the male population aged 16-64 (17). According to the data published in 2009, the number of MSM in Bulgaria is estimated at 26,341 (18, 19). According to the report of Dublin Declaration monitoring in 2016, the number of MSMs was set at 57,826 people for the country, and 44, 122 people in the cities. An assessment of NGOs in the 18 largest Bulgarian cities reported the number of 21,153 MSM (20). Assuming that the proportion of MSM is an average of 3% of the male population, there are around 70,000 people in the country. It should be borne in mind that not all MSM are at high risk for HIV infection. Having unprotected anal sex is between 43% and 53% (21). Therefore, it can be assumed that the size of the group of MSM at high risk for HIV and STIs prevention in Bulgaria is about 30,000 persons.

People who inject drugs

In Bulgaria, the estimated PWID population size decreased from 21,100 in 2009 to 12,520 in 2016 (20, 21). The number of problem opioid users in 2016 was 16,694 (22). The rate of the PWID for the country was estimated of 0.27% of general population (23).

Sex workers

The group of sex workers in Bulgaria ranges between 12-15,000 on the basis of expert judgment and observation of the NGOs working on the terrains in the cities where the NHP activities are carried out (16). In the framework of NBBS-04-16 the surveys were performed in two group of sex workers:

- *SWs working in outdoor* - includes those who provide services on major roads, ring roads of major cities, near railway and bus stations, certain neighborhoods, streets, squares, etc .

- *SWs working indoors* - includes those who offer paid sexual services in clubs, bars, apartments, massage studios, etc.;

Each of the above-mentioned sub-groups of providers occupies about 60-70% of the whole group, e.g. about 7,000 to 9,000 SWs. One third of them are of Roma origin, with an average of about 5% injecting drugs.

Due to the high mobility caused by various factors - seasonality of business /tourism, frequent police actions, opportunities for work outside Bulgaria, discussed future law governing prostitution, etc., outdoor workers are about 60% of the actual target group size - e.g. from 4200 to 5400. The NBBS-04-16 targeted the latter group SWs.

Yung Roma men at higher risk

Another target group identified in the NHP are young Roma men at higher risk for HIV infection (9). According to data from the National Statistical Institute from the 2011 Population and Housing Census, the Roma remain the third largest ethnic group in Bulgaria. As Roma, they identified 325,343 people or 4.9% of Bulgarian citizens. YRMHR are in the 18-24 age group, practice anal sex, inject drugs and sell sex. YRMHR are concentrated mainly in the cities of Sofia, Plovdiv, Pazardjik, Varna, Burgas, Stara Zagora, Sliven, Haskovo, Kyustendil, Yambol, Blagoevgrad, Sofia - district. According to experts' estimations, the size of the YRMHR in the above-mentioned areas is about 26,500 (16).

Prisoners

The number of prisons in Bulgaria is 13. According to data from the National Statistical Institute (NSI), the number of prisoners between 2010 and 2015 was between 9,379 and 7,408 per year, with the tendency of decreasing their number.

Survey's risks

Vulnerability is the central core of HIV-related research in key populations who are more likely to be socially and economically vulnerable due to historical attitudes and discrimination. Therefore, when planning and implementing the NBBS-04-16, special attention was paid to the vulnerability and steps were taken to protect target populations and the survey teams in all stages of the research. Considering security concerns special safety procedures have been developed to reduce the chances of placing staff and participants in danger, to reduce the harm that could be done to the staff and/or participants, and to respond quickly and appropriately in case a safety issue occurs.

The procedures were specifically aimed at ensuring that the participants in the study lack a significantly increased risk of arrest, detention and physical harm, unwanted disclosure of risky behavior or loss of access to healthcare. In this regard, an overview of national and local laws and policies was performed before the implementation of the survey, as well as information from the relevant stakeholders (NGOs, health and municipal authorities and police) was collected.

In keeping with confidentiality and trust, special attention was paid to developing agreements with the local authorities not to interfere on NBBS-04-16 sites and participants so that they are not exposed to increased risk of arrest or detention. These include copies of the letter of support from the Ministry of Health and notifying local authorities about the locations and timing of the survey. For the surveys in prisons, an agreement was signed between the Ministry of Health and the Ministry of Justice and each year a special permission was requested.

Formative assessment

During the first two years of the implementation of the NHP-2001-2007, information on the size of the key populations and their localization was collected. However, in 2003, a formative assessment (24) was carried out to collect initial information on the target populations, and to inform the development and conduct of the survey itself (20). It comprised the determination of the size of the vulnerable groups; overlapping risk behaviours (risky sexual behaviour and injecting drug use); mixing vulnerable groups with other risk behaviours and practices (commercial sex, drug addiction) and/or the rest of the population; presence of main transport network (port, highways and railways) and tourist flow. Based on those criteria, a total of 35 indicators describing the vulnerability of municipalities to HIV and STIs were developed. Based on the collected information, a map of the country was created with the localization of the key populations, geographic and social risk factors (24). For each key population, the locations where prevention interventions will be conducted were identified.

Development and adaptation of the questionnaires

The sociological and behavioral information was a collection of paper-based questionnaires for face-to-face interviews. In the beginning, an individual questionnaire was developed for each of the target groups. During the development of the questionnaires, essential variables for the monitoring of HIV related behaviors, recall periods, logical order of questions, length of interviews, validation rules and mandatory fields were taken into account. Started at 2008 the questionnaires were formatted for machine reading. In 2012 the questionnaires were reformatted for male and female participants.

The questionnaires were pretested in focus groups with the interviewing team and representatives of the key populations. The objectives of the evaluation were to assess how respondents interpreted survey questions and to identify potential response problems that could introduce errors in the survey data (25). Questions were redesigned if necessary, based on the results of this evaluation.

Testing for biomarkers

All respondents were tested for antibodies to HIV, HSB, HBsAg and syphilis according the national guidelines (26). Blood sample of 3 ml venous blood was withdrawn from each respondent. If not possible, peripheral blood was taken in a microtainer. The blood samples were labelled with the patient's code and sent to the RHI laboratory for testing. The ELISA method was used for screening. Positive HIV samples were sent to the National Reference Confirmatory Laboratory of HIV (NRCL of HIV) to confirm the result with immunoblot. Positive Syphilis Tests were confirmed by Treponema pallidum hemagglutination assay (TPHA).

Study protocol and standard operating procedures

Detailed study protocol and algorithms comprising each step of the NBBS-04-16 were developed in four parts: pre-survey, field survey, RHI and CBBSU activities.

Detailed standard operating procedures (SOPs) and forms were developed for recruitment, enrolment, consent, data collection, counselling and testing, samples field storage, transportation, laboratory testing, data handling and analysis. Before each NBBS-04-16 round the study protocol and the SOPs were reviewed and updated.

Confidentiality of information

The confidentiality of data with the NBBS was of utmost importance, as the questionnaire contained highly sensitive information. The study was conducted among the most HIV-vulnerable groups and the information collected focuses on their behaviour in relation to HIV transmission patterns through sexual contacts and injecting drug use.

In the NBBS-04-16 CIOMS Guidelines for Epidemiological Studies were applied, collecting only the minimum amount of identifiable information Survey Identification (SID) code was used which included the first letter of the respondent's name, the month of birth, the date of birth and the first letter of the mother's name of the respondent. This code was adopted for all activities of the NHP-04-16 since its launch in order to keep the client's anonymity and confidentiality.

Compensations for participants

The responders expect to be compensated for their time and effort when they participate in a project. In the NBBS-04-16 the compensations were tangible and intangible. Material compensation was specific for each study group. In the beginning of NBBS-04-16 PWID received small wages in the amount of 2-6 Bulgarian Leva(BGN) equivalent to 1-3 Euro. Since it was inapropriate to use money for a compensation, especially for economically weak persons (27) after 2009 the participants received a gift set (28) Nonmaterial compensation consisted of comprehensive counselling on HIV prevention, testing for HIV and STIs followed by referral to other services including treatment.

Conflict of interests

Measures have been taken to minimize conflicting interests. They included an open discussion of plans and protocols for the NBBS conducted as part of the NHP (15) and the Global Fund grant (14). The National Committee for the Prevention and Control of HIV/AIDS[1] approved the study.

[1] Council of Ministers Decree No. 61 / 26.03.1996, prom. SG No. 29 of 05.04.1996, amend. 3 of 11.01.2000

Adherence to the ethical standards

The study design of NBBS and the accompanying documents were developed and conducted in line with current national (29, 30) and international laws and ethical standards (5, 31-33). In this case, the documents for each round of the IBPN-04-16 were considered for approval by the Committee for Ethics in Research at the National Center for Infectious and Parasitic Diseases.

Staff selection and training

As mentioned above, the IBPN-04-16 was managed at 3 levels: CBBSU at the MoH, CDSD at the RHIs and NGOs. At each level, the staff was selected according to previous experience with working on other health surveys; knowledge of the topics and issues related to the survey; experience with data collection; and experience with specimen testing and counselling. Outreach workers from NGOs were used for field staff, who know where to find or contact members of the target population; these workers enjoy more trust and have better rapport with the target population.

Prior to the implementation of the IBPN-04-16, the CBBSU carried out a training of the RHI's staff for survey protocols and SOPs. They, in turn, provided training of NGO field staff. The training covered the following topics: steps in the survey implementation process and related tasks; roles and responsibilities of each staff member involved in the survey; sampling methodology; laboratory procedures; data collection and management; ethics and confidentiality; and safety and security procedures.

Particular attention was paid to the interviewing in order to increase staff comfort in interacting with the participants and discussing sensitive topics in the NBBS questionnaire. The interviewers were thoroughly trained in nonjudgmentally questioning techniques and accurate recording of responses.

The IBPN-04-16 was planned as a repeated study for 12 years. Consequently, the final meetings and seminars following after each round of the NBBS were used to improve staff capacity. Besides the achieved results, the lessons learned and the weaknesses of the given round were discussed. This contributed to adjustments and improvements of the methodology and the procedures for the next round.

CONDUCTING THE SURVEY

Bio-Behavioural Data Collection

Using the sampling frame of time location clusters respondents were sampled by convenience from these selected in these sampling units. Usually Mobile Medical Cabinets (MMC) were set up as a temporary venue near the vicinity of hotspots for interview and specimen collection. The recruited respondents were invited and accompanied to the MMC where questionnaires were administered in utmost privacy and blood samples were collected after getting informed consent. In some cases, data collection and sample collection were performed in drop-in centres, LTHTS for MSM and special room for prisoners.

Recruitment and enrolment of participants

Respondents were recruited mainly by NGOs, which provided prevention services among the target populations. The outreach workers met the participants on the places they frequent as bars, clubs, parks, selected streets etc. Another part of the respondents were called directly at the service delivery sites or office as NSE settings, drop-in centres for PWID, low-threshold centres and MSM and Roma health centres. The prisoners were recruited in prison by the staff of LTHTS at RHI.

Prior to enrolment, participants were screened for eligibility. Common inclusion criteria were: age over 16 years old, who not participated in a survey the same year and who agree with the conditions of survey described in the informed consent form.

Group-specific inclusion criteria were as follow:

a) for PWID - have used injecting drug substances over the last 1 month;

b) for MSM - have had anal sex with a male in the last 6 months;

c) Male or female SW - have provided sexual services for remuneration or in exchange for drugs, food or shelter during the last 1 month;

d) for YRMHR: between 16 and 25 years of age, injecting drug use or practicing anal sex with men.

Grounds for exclusion were: under age 16, refused to give informed consent and who participated in the survey that same year.

Informed consent and interview

The participants were provided with a confidential environment for data and blood sample collecting,

such as a MMC, drop-in centers, LTHTS or a special room for prisoners. The enrolment of participants was after obtaining written informed consent. They were informed about the nature of the study, that their participation is voluntary and confidential and that their consent or refusal had no effect to receive prevention services or treatment. After obtaining informed consent, Survey Identification number (SID) was assigned to each respondent. Then a face-to-face interview was held. Trained interviewers collected behavioural data using structured paper based questionnaires. Individuals declined to participate in the study, but willing a test for HIV were referred to LTHTS at RHI.

Testing for HIV, HCV, HBV and syphilis

All participants received a pre-test consultation to enable him/her to make an informed decision for HIV testing. At this stage, the respondent may refuse the testing.

From the respondents 3 ml. venous blood was drawn, and when this was not possible, peripheral blood was taken in a microtainer. Blood samples were labelled with the patient's SID code and sent to the RHI laboratory of virology.

All measures have been taken to protect personnel from blood-borne infections. The staff was acquainted with the guidelines and procedures for processing biological fluids (34). In the case of incidence, the national guidelines for post-exposure prophylaxis were followed. In order to meet the ethical requirement that "survey participants should share the benefits of research," it should be ensured that they will be informed about HIV status (35). The respondents were motivated to know the result from testing and they received an identification card to obtain the result.

The communication of result was accompanied by post-test counselling. Particular attention was paid to the participants with a HIV positive result. They received psychological support and were referred to HIV care facilities to initiate antiretroviral treatment.

After the completion of data and blood collection, the respondents were provided with HIV/STIs prevention materials (e.g., information pamphlets, condoms, lubricant and clean injection kit). As a compensation, they received small presents.

Processing of Questionnaires and blood samples

The completed questionnaires and blood samples were transported to the RHI in the PSU where a link was made between the questionnaire and blood sample by participant's SID. A trained epidemiologist checked the questionnaires for identification and correcting data errors. If necessary, the interviewer was contacted for clarifications and corrections. Questionnaires filled in below 70% were considered invalid.

The blood samples were processed in the laboratory of virology at the RHI. Plasma HIV antibodies were tested by ELISA, and positive results were verified by Western blotting assay in the NRCL of HIV. Syphilis seropositivity was determined by ELISA and a confirmatory Treponema pallidum hemagglutination assay (TPHA). The presence of HCV antibody and HbsAg were tested by ELISA. The results of biomarker were recorded in the questionnaires and the latter were send to the CBBSU.

The questionnaires were stored in locked cabinets in the CBBSU and only authorized personnel had access to them. They were once again checked for completeness and mistakes. The completed paper-based questionnaires were scanned by FlexiCapture-8 (ABBYY Europe Global) and then transferred in electronic form to SPSS version 18.0 for pre-analysis data processing.

DATA MANAGEMENT, WEIGHTING AND ANALYSIS

Preparing a dataset for analysis

Creating new variables

In order to address the specific survey objectives the dataset was additionally processed by creating new variables, collapsing response categories and categorizing continuous variable. Since the missing or nonresponse data may influence estimates, they were evaluated by SPSS 18.0 before analysis.

Creating sampling weights

The NBBS-04-16 is a two-stage complex survey with time location sampling with first stage - selection of clusters with probability proportional to size (PPS) and second stage: subsampling. The sampling probability for each cluster was calculate by the equitation:

$$P_i=(m*M_i/M)*(n_i/N_i) \quad (36)$$

where

P_i = probability that an individual in cluster i was selected for the survey

m = number of sample clusters selected

M_i = expected measure of population size for cluster i = number of population of study group in cluster i

M = total measure of size for the survey universe = total number of population of study group
n*i* = number of subpopulation members selected in cluster *i*
N*i* = total number of subpopulation members in the cluster *i*

The sampling probability for each survey unit was converted to a sampling weight as follows:

W*i*=1/P*i*

where

W*i* = sampling weight in the i_{th} cluster
P*i* = probability of selection in the i_{th} cluster.

Finite population correction (FPC) was calculated by the formula *"sqrt[(N - n) / (N - 1)]"* where *(N)* is total population of study group and *(n)* is the sample size.

Analytical methods

For analysis of the data, the latter were transferred from SPSS to STATA and additionally the sample units, the sample weight and FPC entered in STATA "Survey analysis module" for further analysis. The flow chart in Figure 1 shows the steps of the analysis.

For each study, a univariate analysis was used to describe the variables and the distribution of data in the case of continuous variables. The association between two variables was first established with a Chi-square test. Variables with $p < 0.1$ were than subjected to Pearson's R test for collinearity between two variables. For multivariate analysis, variables with $p > 0.05$ were selected.

Qualitative variables of two years of surveys were compared using the equation (37):

$$z_1 = \frac{p_1 - p_2}{\sqrt{\left(\frac{p_1(100-p_1)}{n_1} + \frac{p_2(100-p_2)}{n_2}\right)}}$$

Quantitative variables were compared by Mann-Whitney U test.

The trend over the years was analyzed by logistic regression.

For all analyses, we set the significance level for P values at 0.05.

The estimations and projections of HIV infection for each population was performed by SPECTRUM software (Avenir Health/UNAIDS) (38).

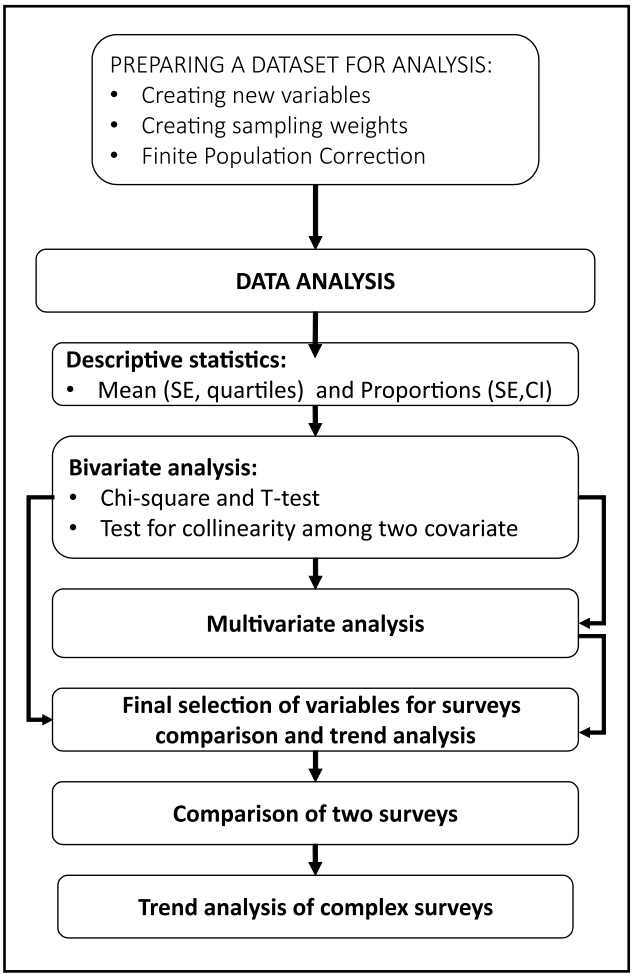

Fig. 1. Flowchart of data analysis

DISCUSSION

Countries that implement repeated NBBS can monitor changes in their populations' risks for HIV, determinants of those risks, and access to prevention over time. There is a long tradition of surveillance in Europe. In response to the HIV epidemic, some countries in Europe established studies to monitor HIV and/or related risk behaviours among PWID (39). The high burden due to infections among PWID resulted in developing drug-related infectious disease key indicator by European Monitoring Centre for Drugs and Drug Addiction (EMCCDA) [13]. This indicator has collected data on the prevalence of HIV and hepatitis B and C since the late 1990s, and more recently has collected behavioural data.

At European level, several studies were performed among MSM, SIALON (37) and EMIS (38). The NBBS-04-16, was the first long-standing nationwide community-based bio-behavioural survey among KP in Bulgaria, which gave the opportunity to collect valuable information on

many key parameters for HIV determinants of programmatic importance. It included knowledge indicators related to HIV prevention, HIV/STI prevalence, condoms use, HIV/AIDS services, risk profile and practices of KP, HIV testing, as well as exposure to HIV/AIDS services.

The NBBS-04-16 is a key milestone for the National AIDS Program. The learning from NBBS are two fold: data from NBBS will contribute to an increased knowledge base understanding of the HIV epidemiology among KP in the context of concentrated epidemics and subsequently more informed decision making. Successful implementation of the NBBS-04-16 at such a large scale offers tremendous opportunity to learn lessons on a spectrum of issues including, technical/ methodological, planning, executing and monitoring.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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NATIONAL HIV BIOLOGICAL AND BEHAVIORAL SURVEY AMONG HARD-TO-REACH POPULATIONS IN BULGARIA (2004 -2016). PART 3: ETHICAL ASPECTS OF THE SURVEY

Tonka Varleva[1], Hristo Taskov[2], Emilia Naseva[3], Vyara Georgieva[1], Elena Kabakchieva[6], P. Tsintsarski[5], Tsveta Raycheva[4], Stefka Boneva[5] and B. Petrunov[2]

1 – Ministry of Health, Sofia
2 – National Center Infectious and Parasitic Diseases, Sofia
3 – Medical University of Sofia, Faculty of Public Health
4 – State psychiatric hospital for drug and alcohol users
5 – Consultant at Ministry of Health
6 –Health and Social Development Foundation (HESED)

ABSTRACT

Between 2004 and 2016, in Bulgaria nine rounds of bio-behavioral survey were conducted among hard-to-reach populations within the framework of the National Programs for Prevention and Control of HIV and STIs. The surveys were performed in ten of 28 municipalities were selected according their HIV risk. The total number of participant in the survey was 27,210 disaggregated by groups as follow: MSM (4,725); PWID (8,626); SW (4,013); prisoners (4,557) and Roma population at higher risk (5,289).

In Part 1 and 2 of the article, we described in detail the methodological aspect of survey. This article describes the ethical issues faced in all phases of the study. The results are published elsewhere.

ADDRESS FOR CORRESPONDENCE

Prof. Dr. Hristo Taskov MD, PhD, DSc,
National Center Infectious and Parasitic Diseases,
Sofia 1504, Bul. Yanko Sakazov 26
E-mail: hristotaskov@gmail.com

KEYWORDS:

vulnerable groups**,** MSM, PWID, SW, prisoners, Roma population, key populations, surveillance.

INTRODUCTION

Ethical principles for human research are based on the United Nations Declaration on Human Rights (1). For the first time, they were formulated in Nuremberg (2, 3) and Helsinki Declaration of 1964 (4). Later, they were further developed in the European Convention for the Protection of Human Rights (EU Charter of Human Rights 1991) (5) and the revised CIOMS 2009 epidemiological guidelines (6). In Bulgaria, all medical information is generally considered confidential and protected by law. This is regulated by the Health low (2004) (7) and Ordinance No 31 of the Ministry of Health on the Determination of Good Clinical Practice (2007) (8).

There are three broadly recognized principles in bioethics, which refer to both clinical and scientific ethics: respect for personality, prosperity and justice (9). *Respect for the person* implies respect for the decisions of autonomous persons and protection of persons who have no decision-making power and are therefore not autonomous. It also imposes an obligation to treat individuals with respect by preserving trust and keeping promises. *Prosperity* imposes an obligation to act in the best interests of patients or participants in research. It is often understood that research risks should be minimized so that the risks are acceptable in the light of the potential benefits of research. Finally, *justice* requires people to be treated fairly. It is often understood that benefits and burdens must be distributed fairly in society. Bio-behavioral research, especially among vulnerable people, obeys the above-mentioned principles and norms.

At 2004, National Biological and Behavioral Survey of HIV (NBBS-04-16) has been launched among key populations as part of the National Program for Prevention and Control HIV and STIs (NHP-01-07) (10), financially supported by a GFATM Grant (11). NBBS-04-16 continued to be carried out within the next National HIV/AIDS Prevention and Control Program (NHP-08-16) (12). NBBS-04-16 was conducted among men who have sex with men (MSM), people who inject drugs (PWID), sex workers (SW), prisoners and Roma population at higher risk

(RMHR) (13-24). In the survey cycles between 2004 and 2016, total 27,210 participants were enrolled, disaggregated by surveyed groups as follow: MSM (4,725); PWID (8,626); SW (4,013); prisoners (4,557) and RMHR (5,289).

NBBS-04-16 faced particularly challenges, dictated by the need to address complex ethical and practical issues related to the vulnerability of these key populations and the existence of great stigma, restrictive norms and legal policies. Ethical challenges (both expected and unexpected) have been accompanying each stage of the NBBS-04-16: planning, implementation and completion. They will be described in turn. The methodology and results of NBBS-04-16 are described in details elsewhere (13-19, 25, 26).

Planning the survey

It is not ethical to expose people to the risks of participating in a study. People who may be at higher risk in research are considered vulnerable and require special protection from research risks. Such vulnerability must be taken into account when planning the study (27, 28).

The World Medical Association in the 2013 Helsinki Declaration recommends that medical research with a vulnerable group will be justified only if the study meets the health needs or priorities of that group and the research cannot be carried out in a non-vulnerable group. In addition, this group should take advantage of the knowledge, practices or interventions that are the result of the study (29).

Key ethical issues in the area of HIV/AIDS research are prioritized as follows: (a) vulnerability; (b) engaging the community; (c) informed consent; (d) additional care obligations, including prevention and treatment, and (f) sharing of results and resources.

Vulnerability is the central core of HIV-related research. People living with HIV (PLH) are medically vulnerable due to their infection. In addition, MSM, PWID, minorities and women at higher risk of HIV infection are more likely to be socially and economically vulnerable due to historical attitudes and discrimination. Therefore, HIV-related research needs to pay special attention to vulnerability and take steps to protect potentially vulnerable participants in all stages of research.

Due to the nature of the spread of HIV in Bulgaria, the main targets of the NBBS-04-16 were the vulnerable groups (MSM, PWID, SW, RMHR and prisoners). All aspects of the vulnerability of these groups have been taken into account when planning NBBS-04-16. These are discussed in detail below.

Risks of the study

With regard to possible risks for participants in NBBS-04-16, an assessment of national and local laws/policies as well as information collected from relevant stakeholders (community, health and municipal authorities, police etc.) was carried out. In line with the study protocol, an evaluation was specifically aimed at ensuring that there was not a significantly increased risk of arrest, detention, physical harm, unwanted disclosure of risky behavior, or loss of access to healthcare for participants.

In keeping with confidentiality and trust, special attention was paid to developing agreements with local authorities not to focus on NBBS-04-16 locations and participants so that they are not exposed to an increased risk of arrest or detention. For the prisoners an agreement between the Ministry of Health and the Ministry of Justice was signet and Ordinance 2 on the Terms and Procedure for the Medical Services in Prison implemented [21]. From all participants, information was collected on problems related to police/legal issues; social problems; problems with friends, family, pimps, drug vendors, and others to cause concern. All this was taken into account for development of NBBS-04-16 protocol, which was specifically aimed at ensuring that respondents' participation in survey is unlikely to expose them to any additional risk.

Ethical Issues in methodology of NBBS-04-16

Selection of target groups and venues for NBBS-04-16

There is low prevalence of HIV infection in Bulgaria (rate of diagnosis 2.8 per 100 000 population in 2016) (30) and is mainly concentrated among MSM, PWID (Table 1) (13, 14). In addition, young men living in compact Roma communities(18-25 years) have a high potential risk of HIV infection (15), prisoners and SW are traditionally groups for HIV surveillance and prevention (16, 17). The activities of the NHP-01-16 was mainly focused on prevention of these five groups. Therefore, epidemiological studies among these key populations were ethically justified.

TABLE 1. Average HIV prevalence rate in Key populations (2006-2011) [14-18]

	IDUs	MSM (2007-2016)	Roma men 15-25 year	Prisoners	FSW
Prevalence rate	5.73%	2.95%	2.06%	0.98%	0.82%

In order to meet ethical research commitments, the size of the study should be adequate and the appropriate study endpoints should be selected (31). The survey included a minimum number of respondents from each group (13-17). BS-04-16 was initially conducted on a yearly basis in order to accumulate sufficient data to assess and plan preventive interventions. It took place within 4 months. From 2012, the NBBS was held in 5 years to track the dynamics of biological and behavioral indicators to evaluate the outcomes of the activities of NHP-01-07 and NHP-08-15.

The selection of NBBS-04-16 sites took place within the process of identifying locations for the implementation of NHP-01-07. The choice was based on a detailed study and analysis of a number of factors such as: size and concentration of vulnerable groups; the spread of HIV and STIs; overlapping risk behaviors (risky sexual behavior and intravenous drug use); availability of main transport corridors and spots for mass tourism (32). This approach was ethically justified because the study focuses on the places and populations with the highest risk of HIV infection without unnecessarily engaging the entire community (33).

Engaging the community

One of the ethical requirements of the study is engagement of people in the community and involving them in the process of preparing, implementation and closing the survey (33). Initial community engagement activities for NBBS-04-16 consisted of community consultations, on-site visits and meetings with local services. This included representatives of the local health services, public security bodies and criminal justice, representatives of key populations and local NGOs. Initial community consultations were focused on providing information on the survey process, its individual and public benefits, the risks and potential barriers to participation. After the NBBS-04-16 protocol was finalized, community engagement continued with a discussion of its execution, which facilitated the joint implementation of the project.

These discussions have clarified that a major threat to participants in NBBS-04-16 would be the fears among PWID and the SWs for increased risk of detention from local authorities because of participation in the survey.

Behavioral and sociological information. Questionnaires.

For the success of behavioral studies of paramount importance are the kind and amount of collected information, as well as the adequate formulation of the questions. It is not ethical to collect information about the information itself. In the majority of cases in the groups we studied, it was difficult to conduct a lengthy interview because of the low social and educational status of participants. In the first rounds of the NBBS-04-16 the questionnaires were unnecessarily long (contained over 300 questions), which took excessive attention and unnecessary time for the respondents to answer in full. The analysis demonstrated that a significant part of the information was not essential for assessing the behavioral risk associated with HIV. Therefore, in the following rounds of NBBS-04-16 the number of questions was reduced. In parallel, the questionnaires for each group were transformed to unify questionnaires for male and female respondents. This facilitated both the conducting of the interview as well the processing of the questionnaires and the analysis of the information.

From an ethical point of view, another important condition was that the questions are appropriately formulated to study sensitive topics and to be in a language that the respondents are able to understand. For the adaptation of the questionnaires, an important role played the involvement of outreach workers in the process of their development and testing, as they are familiar with the language culture and attitudes of the respondents.

Specific issues for HIV testing

HIV testing presents a particular ethical issue (34). At the onset of the AIDS epidemic, the HIV testing was perceived as different from other blood tests, as it could lead to serious psychosocial risks such as family rejection; discrimination with regards to employment; and/or limited access to healthcare (35). Studies in this area have demonstrated that concerns about the impact of stigma and discrimination on individuals, their families and their communities can determine the decision for HIV testing (34). In the late 1990s, due to the lack of proven treatment at that time, the benefits of early diagnosis of HIV patients were uncertain. Recognizing these circumstances, the promotion of HIV testing, was accompanied by specific procedures, such as special informed consent and pre-test counselling (36).

In Bulgaria since 1998, care and antiretroviral treatment is provided for all HIV-positive individuals, regardless of their social and health insurance status (37). However, because of the vulnerability of the participants, HIV testing in NBBS-04-16 was accompanied by strict compliance with the national guidelines for informed consent and HIV pre- and post- test counseling (38, 39).

Currently, medical ethics dictates that HIV testing programs provide sufficient funding and case management to ensure that anyone with an HIV positive outcome will be linked to care as an integral part of the survey (40). In NBBS-04-16, all participants were provided with free access to care and antiretroviral treatment according to the National guidelines (41).

Confidentiality of information

Other important ethical issues in bio-behavioral studies of human subjects concern privacy and confidentiality. Therefore, it is the duty of the researchers to maintain the confidentiality of the participant's data.

In the course of NBBS-04-16, the confidentiality of the data was of paramount importance, as highly sensitive information was collected, which concerns the behavior related to HIV transmission through sexual contacts and injecting drug use. Therefore in NBBS-04-16 the CIOMS Guidelines for Epidemiological Research were applied, collecting only the required amount of identifiable information (42). It is required that the data be encrypted anonymously and cannot be able to be linked to the respondent (43). However, there are circumstances in which identifiable entries are required (44) and an encoded identifier is added to each record and the link between that code and a person is stored in a highly secure file (34). In NBBS-04-16 respondents were enrolled with a code that included the date of birth of the participant (excluding the year), the first letter of the participant's small name and the first letter of the participant's mother's first name (13-17, 25, 26).

Compensations

Usually the participants expect to be compensated for their time and effort when they participate in a survey (33). Inappropriate compensation can attract in the study people who would not agree without compensation. For NBBS-04-16, the type of compensation was discussed before each round to choose the type that would reward participants for their time, but not to encourage people to participate in the survey. They were tangible and intangible. In the first rounds of NBBS-04-16, respondents received small wages ranging between two and six Bulgarian leva (equivalent to 1-3 euro). For ethical reasons, it is inappropriate to use money as a compensatory instrument, especially for economically weak persons (45). Therefore, after 2009, in addition to the kits for safe sex, participants were given a gift set specific to each study group. PWID responders received packet of chocolate crispy waffles and a t-shirt; MSM respondents - a t-shirt and a cap; SW responders - a foldable umbrella and a packet of multivitamins; respondents from the Roma community – a packet of instant coffee and a packet of disposable shaving razors and prisoners - packet of biscuits, a packet of chocolate crispy waffles and a packet of coffee.

Intangible compensation consisted of comprehensive counseling on the prevention of HIV infection, a medical checkup of sexually transmitted infections and referral to treatment.

The experience in the course of NBBS-04-16 has shown that material compensations in the form of gifts were very appropriate for all surveyed groups. They were well accepted by respondents and, on the other hand, and unlike the monetary compensation, they facilitated the efforts of the researchers and organizers.

Study protocol and standard operating procedures

To ensure that all appropriate safety conditions are in place for the participants, detailed protocols and standard operating procedures (SOP) for NBBS-04-16 have been developed. The protocols and SOP for each round of NBBS-04-16 were discussed with all stakeholders: CNBBSU, RHI, NGOs and representatives of the key populations. The latter provided invaluable help in adapting the procedures to the norms and customs of each of the groups surveyed. This significantly facilitated the contacting and recruitment of respondents as well as conducting the field survey itself.

Selection and training of investigators

From an ethical point of view, it is very important to select the NBBS team for research among the specialists working with the key populations. He/she needs to be familiar with specifics of the vulnerable groups, have a positive attitude towards them and have experience in achieving contacts and gaining confidence.

For the participation in the NBBS-04-16 of the RHI staff, people with experience in the field of HIV prevention were selected. All of them were epidemiologists and sociologists with expertise especially in HIV testing and risk reduction counseling. Prior to the launch of the NBBS-04-16, they underwent detailed HIV counseling and testing training by WHO Technical Assistance (26, 46). Subsequently, their qualifications were maintained by work at LTHTS (VCT) and annual seminars for exchange of experience.

NGOs working with key populations played a particularly important role in the NBBS-04-16. The outreach workers (most of them psychologists, social workers and nurses) were selected with proven experience in work with the target groups. The outreach workers enjoyed trust and implementing prevention activities. Their participation in NBBS-04-16 falls within the field of research on public health issues, therefore NGO activities were considered as research and were managed accordingly (40). Ministry of Health hired NGOs with clearly defined responsibilities and the controls were carried out under the supervision of qualified public health officials of the CNBBSU and RHI.

For interviewers, experienced outreach workers were selected, which implemented HIV prevention activities for NHP among the surveyed key populations. They have the advantage of knowing the communities, respecting cultural and ethical norms, having experience in communication and interviewing. In the early stages of the interviewing RMHR, experienced external sociologists were hired, who used outreach workers to translate or clarify the questions. After a time when outreach workers gained the necessary experience to interview, they were employed in the next NBBS-04-16 rounds.

RHI medical staff or medical professionals from mobile medical cabinets with experience in HIV counseling and testing conducted HIV testing. This medical staff was recognized by the target groups and was well acquainted with the specifics of working with them. From an ethical point of view, this created confidence in the respondents, which is of great importance for accepting HIV testing and collection of sensitive behavioral information.

Before each cycle of NBBS-04-16, all participants were trained on ethical aspects, protocols, questionnaires, and HIV testing. Each participant was issued a certificate. After each cycle, seminars were held to report results, analyze problems, share best practices etc.

Conflict of interests

Conflicts of interests are inherent in research. For example, healthcare providers receive prestige, grants and promotions by exploring and publishing their work. Accordingly, they have a personal interest in recruiting and maintaining participants in their research (47, 48). Some contradictory interests, especially financial ones, create ethical problems and they can influence the many of the decisions that researchers make during the study. In the case of NBBS-04-16, steps have been taken to minimize conflicting interests. They included an open broad discussion of NBBS plans and protocols. National Committee for Prevention and Control of HIV/AIDS and Tuberculosis approved the study. In addition, the Commission of Scientific Research Ethics (CSRE) at the National Center for Infectious and Parasitic Diseases (NCIPD) approved the ethical aspects of each round of NBBS-04-16.

Commission of scientific research ethics

The requirement to consider and approve all documents related to NBBS-04-16 is regulated by international documents (49) and national regulations (7, 8).

In this case, CSRE at NCIPD reviewed for approval all documents related to each round of NBBS-04-16. From an ethical point of view, particular attention was paid to the safety and protection of vulnerable people; the importance and benefits of the study; the relevance of the methods; the balance of risks and benefits; ensuring/preserving confidentiality and well-being; type and amount of compensation; conditions for making informed consent by the participants; compliance with the legal framework.

CONDUCTING THE SURVEY

Recruitment and enrolment of respondents

Respondents from the key populations were recruited by NGOs working amongst them. During the outreach work, they informed the study groups about the place and time of the interview and blood sampling (25).

The enrolment of respondents was by defined inclusion and exclusion criteria in accordance with national regulations. In general, they included a willingness to participate, a minimal age and risky behavior. The inclusion and exclusion criteria were specific for each group (25).

Informed consent

Informed consent is one of the most important ethical aspects of human research. Ethical norms require people to agree to participate after informed consent. They must be assured that their decisions will not endanger access to care or services that the person would otherwise be entitled to (33). In NBBS-04-16, outreach workers informed prospective participants in advance of both the survey's benefits for the public health and the nature of the psychological stress to which the responders may be subjected.

A special form for informed consent was created for NBBS-04-16. It provided a comprehensive information on the nature of the study in a language consistent with the cultural features of the studied populations. It clarified that the participation was voluntary and would not affect the care that the respondents received, that protection is provided against stigma and risk, and the preservation of the confidentiality of the information is guaranteed. Particular attention was paid to the respondents' right to opt-out at any point of the survey. By reading the informed consent text, the interviewer was following whether the respondents understood the key aspects of the survey nature to clarify possible misunderstandings. Documentation of the consent required a signature of the respondent.

Conducting the interview

Interviews allow researchers to deepen questions of interest and follow conversations in a way to obtain the most accurate and honest answers to the questions. Through the interviews within NBBS-04-16, lot of valuable information on the behavioral characteristics of key populations was obtained. However, the respondent is in a vulnerable position and the researcher should be careful not to harm him/her in the context of his/her community, traditions and values (45). In most cases, social behavioral studies do not result in the risk of physical harm, but many psychological and/or social harm can occur.

When conducting NBBS-04-16 during the interview, special attention was paid to protecting the participants. The survey was conducted at specific locations in a secure confidential environment e.g. MMU or special room. Interviewers observed the cultural peculiarities of the groups to avoid psychological trauma, breach of confidentiality and stigmatization due to insufficient attention to the questions about risky behavior. In the interviews, the focus was on assisting in understanding issues, helping with delicate questions and patience.

Testing for HIV, Hepatitis B/C and syphilis.

Adhering to the ethical requirements and taking into account the existing stigma regarding HIV testing, blood samples were taken only after informed consent. In NBBS-04-16, the participants was given a pre-test consultation by experienced medical professionals to enable him/her to make an informed choice for HIV testing (25). At this point, the respondent could refuse a survey.

In order to meet the ethical requirement that "survey participants should share the benefits of research," it should be ensured that respondents will be informed about their HIV status (50) participants in NBBS-04-16 were motivated to know the testing results. The data show that an average over 97% was given the opportunity to know the result. This was accompanied by post-test counseling. Particular attention was paid to the participants with a HIV positive result.

According to ethical requirements, they received psychological support and referred to HIV care facilities to initiate antiretroviral treatment.

Supply of additional services and referral

During the HIV interview and HIV testing, it can be seen that a participant is at risk due to lack of information on how HIV is transmitted, limited access to preventive services or psychosocial stress. Ethical standards require such persons to be protected by appropriate services (33). In this respect, it is important to emphasize that NBBS-04-16 has been fully integrated into NHP-04-16. The studies were conducted jointly with the teams providing preventive services to the populations under study. In case of necessity, outreach workers took the „hard cases" for longer preventive interventions and/or providing psychological and social support. If appropriate, such participants were further referred for special services (social, psychological, medical, including HIV and STI treatment). In this sense, NBBS-04-16 fulfilled the expectations of the groups surveyed for the continuous improvement of the care and support for them.

CLOSING THE SURVEY. PUBLICATION AND USE OF DATA.

There is an international consensus that research in a given population is ethically justified only if the results of the studies are beneficial for this population (51). When collecting information about a population, the study has a responsibility to share the results with this population (51). The Nuffield Bioethics Council came to the conclusion that the obligation to disseminate data includes an obligation to explain "the effects of the results for future healthcare "and" prevention of community diseases ". The form of dissemination of information may vary, but it must be ensured that the survey responds to all community questions (43).

The results of NBBS-04-16 were distributed in various forms. At regular annual seminars with all stakeholders, results were presented and future actions were discussed to better target prevention activities, including new sites and study groups. National and local campaigns were regularly used as a channel for disseminating information from NBBS-04-16.

Another form was the Annual Reports on the implementation of NHP-01-16. It is important to emphasize that the results of NBBS-04-16 were used for the regular preparation of the country Global AIDS Response Reports (GARPR) and the reports of monitoring of Dublin Declaration. Last but not least, there are NBBS-04-016 a series of publications in scientific journals (13-17). Thus, the results of NBBS-04-016 are broadly presented not only to interested populations, but also to the broader audience at national and international level.

CIOMS's international ethical guidelines for biomedical research involving people pay great attention to the application of the results of an epidemiological study, and point out that "it is not enough to sate simply that the disease is predominant in the population and that new or more researches are needed. The ethical requirement for "responsiveness" can only be met if populations are given advocacy, successful interventions, effective resource allocation, or other benefits" (52). In this respect, it should be emphasized the main objective of NBBS-04-16 was to collect periodically bio-behavioral information in order to evaluate the effectiveness of prevention interventions under NHP-01-15 and to plan the next steps to improve care, prevention and control of HIV. Data from NBBS-04-16 served for the annual modeling of the epidemic in Bulgaria with SPECTRUM_EPP of UNAIDS [35]. As a results of the estimates and projections for the number of people living with HIV and the number of new infections among surveyed populations, served both to reprogram ongoing interventions and to develop new programs. Based on the results of NBBS-04-07, the NHP-08-16 (10) was developed and a grant of the Global Fund (RCC, 2009-2016) was received (11). In 2007, it was found that the infection in Bulgaria is spreading rapidly among the MSM population. As a result, a new objective for HIV prevention among MSM was included in NHP-08-15 (12) and Global Fund's RCC grant for 2009 - 2016. An analysis of the results of the NBBS-08-16 also served as a ground to develop NHP-17-20 on scientifically justified bases (53).

CONCLUSIONS

Like many bio-behavioral HIV surveys, NBBS-04-16 has encountered a number of ethical challenges that need to be resolved in the course of the study. Descriptions of encountered, whether solved or unsolved, issues are intended to provide a model for future research. In this

case, conducting NBBS-04-16 has shown that conducting a new survey among key populations is unlikely to harm the rights and well-being of the participants.

- A sustained system of biological and behavioral HIV surveillance has been established with appropriate human resources documentation and capacities that conform to ethical principles and norms for human research.
- NBBS-04-16 has evolved as an integral part of the prevention and control of HIV among the key populations. The results served to assess the effect of prevention and the need to reprogram the activities.
- Through the survey, key populations have been involved as part of the process of identifying the leading risk and spread of HIV among them in order to take action to reduce the risk of infection.
- The involvement of NGOs in field research and their very good collaboration at the local health services played a particularly useful role. This cooperation and coordination has been established not only for the implementation of the NBBS-04-16 but also for providing prevention interventions for tuberculosis and other communicable diseases.
- The result of this survey demonstrated that it is possible to conduct reliable behavioral and biological research among vulnerable populations such as PWID, MSM, SW, RMHR and prisoners most affected by the HIV epidemic in Bulgaria.
- The resulting scientific information from NBBS-04-16 allowed national resources to target areas where they would be most effective in reducing HIV incidence and providing care for those affected.
- The results of this study enabled a scientifically validated measure of the effect of the interventions set out in the NHP-01-07 to be made and detailed objectives, tasks and indicators for monitoring the NHP-08-12 and NHP-17-20 to be developed.
- Data analysis has shown that longer time is needed to account for changes in biological and behavioral indicators. Therefore, after the first cycles of accumulated reliable information, NBBS can take place over several years.
- When designing such a survey, they approaches have to be adopted that ensure that research is ethical from the outset and that the rights and well-being of the participants are protected.
- It is important to consider ethical issues throughout the life cycle of the survey. Particularly useful for updating protocols and procedures were their broad discussion after each cycle with local health authorities, NGOs and representatives of key populations.
- Regardless of the ethical challenges encountered, an important finding is that the NBBS-04-16 did not adversely affect the rights and well-being of the participants and did not lead to significant additional risk or social harm.
- The experience gained from the NBBS-04-16 will contribute to improving the next cycles of BSS and to finding adequate HIV prevention approaches among key populations that can be safely and appropriately surveyed. The obtained knowledge and experience from survey in surveyed groups can be used under similar conditions and with similar vulnerable populations.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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INJECTION RISK BEHAVIORS AND HIV/STI PREVALENCE AMONG PEOPLE WHO INJECT DRUGS IN BULGARIA IN YEARS 2012 AND 2016: DATA FROM A CROSS-SECTIONAL BIO-BEHAVIOR STUDY

T. Varleva[1], Ts. Raycheva[2], E. Naseva[3], V. Georgieva[1], H. Taskov[4], B. Petrunov[4]

1 – Ministry of Health, Sofia
2 – State psychiatric hospital for drug and alcohol users
3 – Medical University of Sofia, Faculty of Public Health
4 – National Center Infectious and Parasitic Diseases, Sofia

ABSTRACT

Introduction: The aim of this study was to assess injecting and sexual risk behaviors and measure HIV and STI prevalence among people who inject drugs (PWID) in Bulgaria in 2016.

Methods: We conducted a cross-sectional study from April to September 2016 in five major cities of Bulgaria. The time location sampling was used to recruit PWID. Behavioral data were collected by interviewer-administered questionnaires. HIV and STIs status was assess by ELISA blood test.

Results: A total 421 PWID were enrolled in this study. The most commonly used illicit drugs injected in the last month were heroin (75.9%) and amphetamines (51.5%), followed by methadone hydrochloride (46.8%) and heroin&amphetamines (39.8%). PWID were engaged in various high-risk injection practices in the last month including injection with used needle/syringe (50.9%), selling used needle (57.4%), using pre-filled syringes (34.0%) or common equipment for sharing/preparation (46.3%). Casual sexual partner in last 12 months was declared by 64.0% of PWID and 57.6% had used condoms in last sexual intercourse. Almost one fifth of participants (17.5%) had had sex for money or gift in past 12 months and 90.3% had used condoms in this case. HIV prevalence rate for HIV, HVB, HCV and syphilis was respectively 1.6%, 9.1%, 65.5% and 0.8%.

Conclusions: This study confirms the observed trend of reduction of new HIV diagnoses among the PWID in Bulgaria started by 2012. Nevertheless, PWID continue to be one of the main subpopulation for transmission of HIV and HCV in Bulgaria because of persistent high risk of injection and sexual behaviours. Developing innovative strategies that can improve accessibility of current harm reduction services and incorporate prevention that is more comprehensive are needed.

KEYWORDS:

HBV, HCV, syphilis, surveillance

ADDRESS FOR CORRESPONDENCE:

Prof. Dr. Hristo Taskov MD, DSc,
National Center Infectious and Parasitic Diseases,
Sofia 1504, Bul. Yanko Sakazov 26,
E-mail: hristotaskov@gmail.com

INTRODUCTION

Injecting drug use is considered as an international public health threat that can lead to serious health-related outcomes such as the transmission of blood-borne viruses resulting from risky behaviors among people who inject drugs (PWID). PWID are at increased risk of HIV infection due to both high-risk injecting and sexual practices (1). This pattern of behavior allows HIV infection to spread within both injection and sexual networks (2, 3).

Bulgaria is a country with an HIV epidemic concentrated mainly among people who inject drugs (PWID) and men having sex with men (MSM) (4, 5). In Bulgaria, the estimated PWID population size decrease from 21,100 in 2009 to 12,520 in 2016 (6, 7). In 2006, HIV transmission due to injection drug use was responsible for 37.4% of all newly diagnosed HIV cases. During the last decade, Bulgaria has implemented a number of HIV prevention programs for PWIDs using an evidence-based harm reduction services.(8, 9) Since than there have been significant falls of the PWID's share of newly diagnosed HIV cases from 43.3% % in 2009 to 12% in 2015. Although injection drug use is no longer the primary mode of HIV transmission in Bulgaria, PWIDs remain a group at high risk (5). At the end of 2012, of the 157 new HIV diagnosis,

25.5% were attributed to injection drug use. The reported national HIV prevalence rate among injection drug users (IDUs) in 2011 was 7.5% (5). Monitoring of HIV and other blood-borne or sexually transmitted infections (STI) and related risky behaviors among PWID is very important because they are considered as a key bridge population for the spread of these diseases from high-risk groups to the general population (10). The spread of HCV in PWID is usually high (11). In previous survey the reported HCV prevalence rate was 78.6% (12).

In order to monitor and manage HIV infection in PWID in 2004 national biological and behavior surveillance (BBS-04-12) was implemented (12). This study represents the ninth round of NBBS-04-16 survey to estimate the trends of HIV prevalence and injection risk behavior of PWID in Bulgaria. Additionally, the study was aimed to determine whether an expansion of prevention programs among PWIDs in Bulgaria was associated with decreases in risky injection-related behaviors.

MATERIALS AND METHODS

Study design

The study was cross-sectional, with two-stage clusters and probability time-location sampling (13, 14) which is described elsewhere in details (15). Five cities were selected as primary sampling units namely Sofia, Pazardjik, Varna, Burgas and Blagoevgrad. These cities were selected based on probability to size of PWID, on the rate of newly diagnosed PWID between 2010 and 2015 and where the previous rounds of NBBS-04-16 were conducted. The study combined a blood test and face-to-face interview. Outreach workers from local non-governmental organizations (NGO) working with PWID in these cities were hired to reach the target population. Experienced interviewers conducted face-to-face interviews. The questionnaire was an adapted version of the questionnaire previously used among PWID in NBBS-12 (4) and was based on GARPR indicators (16). Experienced medical workers provided blood collection for biological markers after the interview for all participants.

Recruitment and enrollment procedures

Time location sampling was used for recruitment of participants (17). Mobile medical cabinets (MMC) were used primary as recruitment sites. They provide preventive interventions in the PWID and are well trusted by them. Outreach workers were referring or bringing participants to MMC situated in a previously announced places where prevention activities are usually born among PWID. The participants were also recruited during their visits at low threshold drop-in centers for PWID. Recruitment and interviewing then continue until the target sample size was achieved.

PWID were enrolled in NBBS-16 following preliminary screening based on the following criteria: at least 16 years old and injecting drug use in the last 30 days. Prior to enrollment into the study, the respondents were provided with comprehensive information about the study and signed a consent form.

HIV and STIs testing

All participants were tested for anti-HIV, anti-HCV and anti-Syphilis antibodies and HBsAg as well. Qualified well-trained counsellors from voluntary and counselling and testing (VCT) sites provided pre-test counselling. A venous blood sample (3 mL) was obtained from each participant by a trained laboratory technician. Plasma HIV antibodies were tested by ELISA, and positive results were verified by Western blotting assay. Syphilis seropositivity was determined using ELISA and a confirmatory Treponema pallidum hemagglutination assay (TPHA). The presence of HCV antibody and HbsAg was tested by ELISA.

Post-test counselling was provided for each participant regardless of their HIV test result by the same counsellor who conducted the pre-test counselling. Participants who were HIV reactive and did not know their HIV status were referred by the counsellor for confirmatory testing. The quality of HIV tests was monitored using external quality control system.

Data entry and analysis

The completed paper-based questionnaires were scanned by FlexiCapture-8 (ABBYY Europe Global) and transferred in electronic form to SPSS version 19.0 for pre-analysis data processing. Than data were transferred to STATA 14.2 and analyzed with “Survey analysis” module. Sample weights and finite population coefficients (FPC) were calculated as described (14). Qualitative variables of

NBBS-12 and NBBS-16 were compared using the equation (18):

$$z_1 = \frac{p_1 - p_2}{\sqrt{\left(\frac{p_1(100-p_1)}{n_1} + \frac{p_2(100-p_2)}{n_2}\right)}}$$

Quantitative variables of NBBS-12 and NBBS-16 were compared by Mann-Whitney U test.
For all analyses, we set the significance level for P values at 0.05.

Ethical considerations

The ethical issues of NBBS-04-16 are described elsewhere in details (19). In brief, the study documents were reviewed and approved by the Committee on Medical Ethics at the National Center of Infectious and Parasitic Diseases, Sofia (20, 21). Participation in this study was voluntary, and a written informed consent was obtained from each study participant after a detailed description and explanation of the study objective and procedures. Participants were informed that they could stop responding to questions and discontinue their participation at any time. Interviews were conducted at a private place, and confidentiality was enhanced by assigning a unique and anonymous code to each participant (20, 21).

RESULTS

Socio-demographic characteristics

The study included 421 participants with median age 34 years (interquartile range 28 - 36 years). In terms of educational status, data have remained similar over the years, namely: a major part of the participants has completed primary and elementary education (52%) followed by a group of PWID with secondary education (40%). The group of tertiary graduates is negligible (3%). The prevailing ethnicity among the participants is Bulgarian (67%) followed by Roma ethnic groups (25%). The interviewed representatives of Turkish ethnicity decreased significantly during the years from 10% in 2004 to 3% in 2016.

TABLE 1. Demographic characteristics of PWIDs by year 2012 and 2016.

Variables	NBBS 2012, N = 742				NBBS 2016, N = 421				
	Median	SE	Q_{25}	Q_{75}	Median	SE	Q_{25}	Q_{75}	P-value
Age	30	2.50	26	33	34	4.33	28	36	p > 0.05

	Prop %	SE	CI95% LI	CI95% UI	Prop %	SE	CI95% LI	CI95% UI	P-value
Education									
primary education	13.0%	0.019	0.085	0.193	15%	0.003	0.140	0.154	p > 0.05
elementary education	36.3%	0.059	0.219	0.536	37%	0.006	0.353	0.383	p > 0.05
secondary education	37.2%	0.072	0.202	0.581	40%	0.010	0.371	0.420	p > 0.05
higher education	2.3%	0.006	0.010	0.049	3%	0.002	0.028	0.037	p > 0.05
Incomplete primary education	6.2%	0.008	0.043	0.088	3%	0.002	0.027	0.036	p > 0.05
He has not attended school	4.0%	0.010	0.020	0.077	2%	0.001	0.020	0.026	p > 0.05
Ethnicity									p > 0.05
Mixed Origin	0.5%	0.001	0.003	0.010	1%	0.001	0.012	0.015	p > 0.05
Bulgarian	72.1%	0.025	0.647	0.785	67%	0.006	0.656	0.686	p > 0.05
Roma	23.1%	0.022	0.175	0.297	25%	0.004	0.244	0.265	p > 0.05
Turkish	3.5%	0.008	0.019	0.066	3%	0.001	0.030	0.038	p > 0.05
Other	0.3%	0.001	0.002	0.007	2%	0.002	0.018	0.026	p > 0.05

Services received and knowledge of HIV transmission

Most of participants had received prevention services by the outreach workers. Overall 61.1% reported receiving free confidential HIV testing and counselling, needle and syringes exchange (84.4%), condom distribution (76.7%) and information materials about HIV infection (51.1%). Details are shown in Table 2.

The majority of participants were aware of the main risk factors for HIV transmission. They were significantly more likely to disprove (61.1%) the misconception that HIV could be transmitted by dining together with HIV carriers or with patients with AIDS. When asked if HIV can be transmitted via a mosquito bite, 65.3 % of the participants chose the correct transmission route. Almost 85% of participant approve the statements that the risk of getting HIV can be reduced the by: "Using clean injecting materials every time" (87.6 %) "Using a condom every time having sex" (70.2 %) and that "Healthy-looking person can have the AIDS virus" (84.4%).

TABLE 2. Services received and knowledge of HIV transmission

Variables	NBBS-2012 N = 742				NBBS-2016 N = 421				NBBS-2012 vs. NBBS-2016
	Prop %	SE	CI95% LI	CI95% UI	Prop %	SE	CI95% LI	CI95% UI	P-value
Services received									
HIV testing and counselling	82.5%	0.048	0.650	0.922	61.1%	0.009	0.587	0.634	p < 0.001
Needle and syringes exchange	95.7%	0.009	0.925	0.976	84.4%	0.007	0.826	0.861	p < 0.001
Free condom distribution	80.5%	0.049	0.633	0.908	76.7%	0.007	0.747	0.785	p > 0.05
HBV,HCV and syphilis testing	73.4%	0.054	0.561	0.857	46.3%	0.009	0.440	0.486	p < 0.001
Information for HIV	59.5%	0.043	0.472	0.707	51.1%	0.006	0.495	0.527	p > 0.05
Knowledge of HIV transmission					66.6%				
Can a person reduce the risk of getting HIV by using a condom every time they have sex	88.4%	0.018	0.825	0.926	70.2%	0.004	0.863	0.882	p > 0.05
AIDS cannot be transmitted by mosquito bites	81.5%	0.044	0.661	0.909	65.3%	0.004	0.664	0.683	p < 0.05
HIV transmission can be reduced by having sex with only one uninfected partner who has no other partners	67.4%	0.042	0.550	0.777	87.6%	0.012	0.635	0.695	p > 0.05
Cannot become infected by sharing food with someone who has AIDS	89.3%	0.014	0.848	0.926	61.1%	0.004	0.693	0.712	p < 0.001
Healthy-looking person can have the AIDS virus	85.2%	0.013	0.812	0.885	84.4%	0.008	0.632	0.673	p < 0.001
Can a person reduce the risk of getting HIV by using clean injecting materials every time	85.1%	0.022	0.780	0.902	87.6%	0.004	0.867	0.886	p > 0.05

Drug Injection Behaviors

The durability of drug injection confirms the aging of the target population by both age and length of drug injection. In the present study, 85.6% of responders reported injecting drugs of five years and over, while respondents with an injecting experience of up to 12 months are only 1.0%.

Regarding the injected drug used in the last month the most commonly used illicit drugs were heroin (75.9%) and amphetamines (51.5%), followed by methadone hydrochloride (46.8%) and heroin&amphetamines (39.8%). PWID were engaged in various high-risk injection practices in the last month including injection with used needle/syringe (50.9%), selling used needle (57.4%), using pre-filled syringes (34.0%) or common equipment for sharing/preparation (46.3%).

TABLE 3. Injection-related characteristics and injection risk behavior among PWIDs by year 2012 and 2016

Variables	NBBS-2012 N = 742				NBBS-2016 N = 421				NBBS-2012 vs. NBBS-2016
	Prop %	SE	CI95% LI	CI95% UI	Prop %	SE	CI95% LI	CI95% UI	P-value
Duration of injection in years									
< 1 year	1.4%	0.005	0.005	0.040	1.0%	0.01	0.005	0.043	p > 0.05
1 -5 years	14.5%	0.020	0.101	0.216	13.4%	0.02	0.101	0.221	p > 0.05
> 5 years	83.3%	0.022	0.762	0.885	85.6%	0.02	0.771	0.824	p > 0.05
Drugs injected in the last 30 days									
Heroin	98.2%	0.006	0.956	0.993	75.9%	0.009	0.734	0.782	p < 0.001
Amphetamines	65.9%	0.058	0.487	0.798	51.5%	0.009	0.492	0.538	p < 0.001
Heroin and amphetamines	46.0%	0.025	0.392	0.529	39.8%	0.007	0.381	0.415	p < 0.05
Methadone	76.4%	0.038	0.645	0.852	46.8%	0.009	0.444	0.492	p < 0.001
Substinol	28.2%	0.047	0.170	0.429	1.4%	0.001	0.012	0.016	p < 0.001
Other	27.9%	0.059	0.145	0.468	6.4%	0.004	0.055	0.074	p < 0.001
Injected with used needle/syringe during the last injection	39.9%	0.105	0.164	0.691	28.4%	0.011	0.256	0.003	p > 0.05
Frequency of injection with used needle and / or syringe during the last month									
Every time/Most time	14.2%	4%	6%	31%	3.8%	0%	3%	4%	p < 0.05
Every time	1.1%	0.003	0.006	0.021	0.2%	0.000	0.002	0.003	p < 0.05
Most time	13.1%	0.040	0.053	0.286	3.6%	0.001	0.033	0.040	p < 0.05
In at least half the cases	36.8%	0.064	0.214	0.555	11.0%	0.006	0.097	0.126	p < 0.001
Occasionally	48.4%	0.105	0.226	0.751	36.1%	0.008	0.342	0.381	p < 0.05
Never	0.5%	0.001	0.003	0.008	45.2%	0.013	0.419	0.485	p < 0.001
Do not know	0.1%	0.000	0.000	0.003	3.8%	0.002	0.033	0.043	p < 0.001
Frequency of giving or selling your used needle or syringe to someone else in the last month									
Every time	0.2%	0.001	0.000	0.007	0.2%	0.000	0.002	0.003	p > 0.05
Almost every time	13.5%	0.041	0.056	0.291	4.3%	0.002	0.039	0.047	p < 0.05
Occasionally	46.4%	0.071	0.281	0.657	52.9%	0.014	0.493	0.565	p > 0.05
Never	39.5%	0.108	0.158	0.695	40.3%	0.015	0.366	0.441	p > 0.05

Variables	NBBS-2012 N = 742				NBBS-2016 N = 421				NBBS-2012 vs. NBBS-2016
	Prop %	SE	CI95% LI	CI95% UI	Prop %	SE	CI95% LI	CI95% UI	P-value
Used common instruments for sharing (preparation) in the last 30 days									
Every time	0.4%	0.002	0.001	0.011	0.9%	0.001	0.007	0.010	p < 0.05
Almost every time	5.4%	0.013	0.028	0.101	5.7%	0.002	0.052	0.063	p > 0.05
Occasionally	42.4%	0.086	0.216	0.663	39.7%	0.007	0.379	0.414	p > 0.05
Never	49.7%	0.092	0.262	0.733	48.1%	0.004	0.472	0.490	p > 0.05
Received drug dependence treatment/support	0.0%				5.6%				
Yes - treatment		0.065	0.123	0.474		0.008	0.179	0.218	p > 0.05
Yes - support	26.2%	0.089	0.118	0.585	19.8%	0.010	0.225	0.278	p > 0.05
Yes - treatment and support	30.3%	0.029	0.164	0.323	25.0%	0.002	0.345	0.354	p < 0.001
No	23.4%	0.042	0.107	0.344	35.0%	0.005	0.186	0.213	p > 0.05

Sexual Risk Behavior

The majority of participants (85.8%) reported having sex in past 1 months and 35.0% had used condoms during last intercourse. One out of ten participants (10.9%) had anal sex with male partner and 74.0% used condoms during last intercourse. Vaginal or anal sex with female partner reported 92.4% and but only 26.4% of them used condoms. More than half of the participants (64.0%) had sex with a casual partner, with a half of them (57.6%) using condoms. Selling of sex was reported by 17.5% of responders and 90.3% of them using condoms in that case.

TABLE 4. Sexual-related characteristics and sexual risk behavior among PWIDs by year 2012 and 2016.

Variables	*NBBS-2012 N = 742*				*NBBS-2016 N = 421*				*NBBS-2012 vs. NBBS-2016*
	Prop %	*SE*	*CI95% LI*	*CI95% UI*	*Prop %*	*SE*	*CI95% LI*	*CI95% UI*	*P-value*
Had sex in past 12 months	*91.9%*	*0.025*	*0.815*	*0.967*	*86.8%*	*0.005*	*0.854*	*0.881*	*p > 0.05*
Had sex in past 1 months	*83.7%*	*0.044*	*0.678*	*0.926*	*85.8%*	*0.005*	*0.843*	*0.871*	*p > 0.05*
Condom used the last time you had sex	*42.6%*	*0.011*	*0.395*	*0.457*	*35.0%*	*0.006*	*0.335*	*0.365*	*p > 0.05*
Had sex in exchange for money or gift in past 12 months	*24.1%*	*0.017*	*0.197*	*0.291*	*17.5%*	*0.008*	*0.156*	*0.196*	*p < 0.001*
Condom use with sexual partner in exchange for money or gift	*NA*	*NA*	*NA*	*NA*	*90.3%*	*0.005*	*0.891*	*0.914*	
Sex with a casual partner in last 12 months	*65.3%*	*0.049*	*0.508*	*0.775*	*64.0%*	*0.004*	*0.629*	*0.650*	*p > 0.05*
Condom used the last time you had sex with a casual partner	*57.6%*	*0.005*	*0.562*	*0.590*	*57.6%*	*0.007*	*0.559*	*0.593*	*p > 0.05*
Had anal sex with men	*11.7%*	*0.021*	*0.071*	*0.188*	*10.9%*	*0.007*	*0.093*	*0.127*	*p > 0.05*
Had anal sex with men in last 6 months	*21.5%*	*0.035*	*0.133*	*0.328*	*64.9%*	*0.018*	*0.590*	*0.703*	*p < 0.001*
Condom used the last time you had anal sex with men	*48.0%*	*0.044*	*0.362*	*0.600*	*74.0%*	*0.013*	*0.696*	*0.781*	*p < 0.001*
Condom used the last time you had anal sex with men casual men partner	*52.2%*	*0.070*	*0.309*	*0.727*	*46.9%*	*0.006*	*0.449*	*0.488*	*p > 0.05*
Had anal sex (vaginal or anal) with female partner in last 12 months	*99.1%*	*0.001*	*0.986*	*0.994*	*92.4%*	*0.005*	*0.909*	*0.937*	*p < 0.001*
Condom used the last time you had sex with a female partner	*34.1%*	*0.014*	*0.303*	*0.381*	*26.4%*	*0.003*	*0.257*	*0.272*	*p < 0.001*

HIV Testing and HIV and STI Prevalence

Most of responders (80.4%) declared to have an opportunity for a confidential HIV testing and more than half (52.4%) have been tested for HIV in last 12 months. Very few reported HIV positive status (0.6%).

Prevalence rate for HIV, HVB, HCV and syphilis was respectively 1.6%, 9.1%, 65.5% and 0.8%.

TABLE 5. Awareness of HIV status and prevalence rates of HIV, HBV, HCV and syphilis among PWID in NBBS-2012 and NBBS-2016.

Variables	NBBS-2012 N = 742				NBBS-2016 N = 421				NBBS-2012 vs. NBBS-2016
	Prop %	SE	CI95% LI	CI95% UI	Prop %	SE	CI95% LI	CI95% UI	P-value
Awareness of HIV status									
Having an opportunity for a confidential HIV testing	91.0%	0.029	0.792	0.964	80.4%	0.003	0.795	0.812	p < 0.001
Ever been tested for HIV	97.9%	0.006	0.954	0.990	95.8%	0.002	0.952	0.963	p < 0.05
HIV testing in the last 12 months	31.2%	0.052	0.188	0.470	52.4%	0.011	0.495	0.553	p < 0.001
HIV testing in the > 12 months	65.6%	0.087	0.442	0.918	45.0%	0.012	0.420	0.481	p < 0.001
Self-reported HIV status									
Positive	2.9%	0.011	0.010	0.085	0.6%	0.000	0.005	0.007	p > 0.05
Negative	97.9%	0.006	0.954	0.990	85.4%	0.007	0.836	0.871	p < 0.001
HIV and STI prevalence rate									
Anti-HIVab positive	4.0%	0.015	0.014	0.109	1.6%	0.000	0.015	0.017	p > 0.05
HbSAg positive	10.4%	0.012	0.075	0.143	9.1%	0.002	0.086	0.096	p > 0.05
Anti-HCVab positive	74.4%	0.023	0.674	0.803	65.5%	0.004	0.645	0.664	p < 0.001
Anti-Syph Ab positive	4.3%	0.005	0.031	0.058	0.8%	0.001	0.006	0.009	p < 0.05

DISCUSSION

In this study, we present data from bio-behavioral survey conducted in PWIDs in Bulgaria in 2016 to assess trends in injection risk behavior, as this is a major risk factor for HIV transmission among PWIDs.

PWID represent an estimated 10% of all HIV infections globally (22) and are 22 times more likely to be HIV-infected compared to the general population (23). The HIV prevalence in PWID population in Bulgaria is reported higher (7%) (12) than the average HIV prevalence among PWID in Europe (5.36%) (11). Overall, our findings indicate a decrease in HIV prevalence in 2016 (1.6%) among current IDUs compared with 4.0 % reported by a study conducted in 2012 (12). Nevertheless, HIV prevalence is about 23 times higher than the prevalence of the low-risk population (prevalence of 0.069%, data from Ministry of Health). The lower prevalence in 2016 is in line with the trend of decrease of new diagnoses over the past 7 years. In 2016, the number of new diagnoses was 24 vs 40 in 2012. This is also in line with the modeled prevalence of 2.2% of HIV among PWID in Bulgaria in 2016 [paper in preparation]. However, this cannot explain satisfactorily the observed low HIV prevalence in this study. One possible explanation could be a difference in the respondent's profile compared to NBBS -12. This also is supported by lower prevalence in 2016 versus 2012 for HBV (9.1% vs 10.4%), HCV (65.5% vs. 74.4%) and syphilis (0.8% vs 4.7%)

Heroin is the drug of choice among PWID in Europe, although there are sub-regional differences (24). In Central Europe, heroin is reported as the main drug injected, although studies also report between 30% and 51% injecting amphetamines as main drug (25-27). In our study the most commonly, used illicit drugs were heroin (75.9%) and amphetamines (51.5%), followed by methadone hydrochloride (46.8%) and heroin&amphetamines (39.8%).

As shown by the individual risk factor, many studies investigated the link between HIV and injecting with used or shared needle (24). The rate of receptive needle-syringe sharing in Bulgaria is reported the highest in the EU countries (47%) (24). The rate of recent needle/syringe sharing in current study is 13.8%, which is three times

lower than in prior study (51%) (12). Inversely the injecting rate from pre-filled syringe in the last 30 days in 2016 was 34.0% versus 16.9% in 2012 ($p < 0.001$).

Some of these differences may be attributed to harm reduction services have started to show its positive effects on preventing HIV infection by reducing needle and syringe sharing in this group. However, it is also possible that needle/syringe sharing behavior is under-reported because of stigmatization (28). Despite the above positive findings, injection risk behaviors in PWID is still high and remain a serious concern.

Apart from risky injection behaviors, sexual risk behaviors, including having multiple sex partners and unprotected sex were associated with HIV infection (29). The result of this study are similar to the results of NBBS-12. PWID are sexually active and most of them (85.8 %) had sexual intercourse in last one month. The extent of engagement in sexual risk behaviors varied widely among samples of PWID across countries. The proportion reporting of recent sexual risk (i.e., unprotected sex with casual partner) in European countries is between 8% in Spain and 81.6% in Lithuania (24). In our study, about two third of participants (64.0%) had sex with casual partner in last 12 months, but only half of them (57.6%) had protected sex in this case.

Commercial sex for drugs or money was reported by 17.5% of respondents. This figure is lower than in previous survey in 2012 (24.1%, $p < 0.001$) and much lower than 47% reported for Bulgaria by Degenhardt et al. (24). A positive finding is that 90.3% of responders reported condom use with sexual partner in exchange for money or gift.

Sexual intercourse of PWID with women is an important factor in passing HIV infection from the PWID population to the general population (30, 31). In our study, 92.4% of respondents reported sex (vaginal or anal) with female partner in the last 12 months. In this case, it is worrying that only 26.4% had used condoms. The reasons for inconsistent condom use is reported in other studies included feeling uncomfortable during sexual intercourse, believing that their partners were not infected with HIV and other STIs, or perceiving condom use as a mistrustful behavior within an established relationship (32). These reasons may also be applied to our study and may be due to a lack of effective behavioral intervention, especially designed for changes of sexual risk behaviors related to HIV infection in the current policies.

CONCLUSION

Our results confirm the observed trend of reduction of new HIV diagnoses within PWID in Bulgaria started by 2012. Prevention services reached the majority of the PWID. They are well informed about HIV transmission pathways and ways of prevention. Compared to the study in 2012, the rate of sharing needles and syringes and the use of pre-filled syringes decreased significantly. These results can be linked to the long-standing active interventions for HIV prevention in PWID in Bulgaria. However, the sexual risk of PWID is still high. The rate of condoms use very low by sex with commercial, casual and female partners. This is a serious prerequisite for the spread of HIV infection in general population. In this regard, prevention should be intensified in the next programming period.

With this study, we hope to provide conclusive evidence for health officers and policy makers for planning programmatic and policy responses, guiding allocation of resources for prevention and intervention, as well as monitoring and evaluating current implementation of these strategies. In summary, the results of this study suggest that programs for HIV prevention and control in the PWID (33) have been successful and have led to a significant reduction in the prevalence of HIV among them.

LIMITATIONS

Some study limitations should be highlighted. First, the survey explored a precarious population and did not reach PWID from higher social classes where cocaine use seems to be more frequently reported. Our results may not be considered as fully representative of those two groups. Second, a time location sampling was used, which bias the responder's recruitment. Second, we do not know how many PWID refused to join this study. Finally, the study was performed in the biggest cities in Bulgaria, where PWID are concentrated. This can lead to overestimation of surveyed biological and behavior indicators. The results should be considered rather as order of magnitude of the phenomenon and interpreted with caution.

Despite these limitations, this study provides valuable insights into the current PWID population four years after last survey in 2012. It will be useful for public health planning and evaluation efforts.

CONFLICT OF INTERESTS

No conflict of interests is declared.

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SEXUAL RISK BEHAVIORS AND HIV/ STI PREVALENCE IN MEN WHO HAVE SEX WITH MEN IN THE BULGARIA IN 2016: DATA FROM A CROSS-SECTIONAL BIO-BEHAVIOR STUDY

T. Varleva[1], P. Tsintsarski[2], E. Naseva[3], V. Georgieva[1], H. Taskov[4] and B. Petrunov[4]

1 – Ministry of Health, Sofia
2 – Consultant at Ministry of Health
3 – Medical University of Sofia, Faculty of Public Health
4 – National Center Infectious and Parasitic Diseases, Sofia

ABSTRACT

Introduction: The aim of this study was to assess sexual behavior and measure HIV and STI prevalence among men who have sex with men in Bulgaria in 2016.

Methods: We conducted a cross-sectional study from June to September 2016 in five major cities of Bulgaria. The time location sampling method was used to recruit MSM. Behavioral data were collected by interviewer-administered questionnaires. The blood tests were then carried out among MSM to assess their HIV and STIs status.

Results: A total 437 MSM were enrolled in this study, 44.4% of them in the capital, Sofia. Over the past month, 85.8% of MSM had had sexual intercourse with men and 31.9% of them had had sex with female partner. A sexual intercourse with a casual partner in last 12 months was reported by 85.8% of MSM and 75.2% of them had used condoms the last sexual intercourse. Having sex in exchange for money or gift in past 12 months was declared by 18.3% and buying of sex – by 6.3% of responders. An opportunity for a confidential HIV testing was reported by 95.8% of MSM. In the last 12 months, 52.4% of responders have been tested for HIV. The prevalence rate of HIV among MSM was 3.2% The prevalence rate of other STIs was as follows: 3.1% for HBV, 4.2% for HCV and 6.3% for syphilis.

Conclusion: This study confirms that HIV prevalence among MSM is 46 times higher than the low risk general population (3.1%. vs 0.069%). Though our results reflect a relative good knowledge about HIV/AIDS infection, there is an increase of new HIV-positive cases and high-risk behavior, suggesting the need for more effective HIV prevention among MSM population in Bulgaria. Thus, there is a need to rethink HIV sensitization and prevention strategies targeting hidden and stigmatized populations such as MSM.

KEYWORDS:

HBV, HCV, syphilis, surveillance

ADDRESS FOR CORRESPONDENCE:

Prof. Dr. Hristo Taskov MD, DSc,
National Center Infectious and Parasitic Diseases,
Sofia 1504, Bul. Yanko Sakazov 26,
E-mail: hristotaskov@gmail.com

INTRODUCTION

Men who have sex with men (MSM) have been a large high-risk population for human immunodeficiency virus (HIV) infection and transmission because of their more hidden and stigmatized nature (1, 2), more diverse sexual networks (3), and tendency toward risky sexual behaviors such as multiple sexual partners and condomless anal intercourse (4, 5). A priority action in Europe is to reduce new HIV infections among MSM by improving HIV combination prevention programs. (6).

Bulgaria is a country with a HIV epidemic concentrated mainly among people who inject drugs (PWIDs)(7) and (MSM) (8). There have been significant falls in HIV prevalence among IDU from 29.35 % in 2002 to 22 % in 2013 and among FSW from 5.9 % in 2002 to 5.3 % in 2013 [8, 11, 12]. However, there are signs of an increase in HIV prevalence among MSM. HIV infection among MSM was projected to increase from 1.7 % in 2005 to 2.4 % in 2013, but a recent HIV case surveillance data showed a significant increase from 2.26 % in 2012 to 3.69 % 2013 [8]. Since 2005, the annual number of new HIV diagnoses among MSM has increased from six cases to 111 cases in 2015. This is in line with the increased male-to-female ratio of HIV-positive persons from 2:1 in 2005 to 5:1 in 2016. In 2015, about 49.6% of newly diagnosed HIV cases came from MSM population. (9) Thus MSM

remain a primary group of interest for prevention and monitoring.

During the last decade, Bulgaria has implemented a number of HIV prevention programs for MSM using an evidence-based intervention approach (10). Establishment of low threshold HIV/STI testing and counselling services (LTHTS) (10), distribution of safe sex packages and health education material are among the programs implemented in Bulgaria . However the results from previous six rounds of the National Biological and Behaviour Survey in the period 2006-2012 (NBBS-06-12) indicate that the HIV incidence and prevalence has been steadily increasing among MSM in Bulgaria (8). In NBBS-06-12 among MSM carried out in 2006-2012 a number of demographic, social, and other factors that have been consistently associated with sexual risk behavior of MSM. Two European surveys among MSM, (EMIS in 2010 (11) and SIALON II in 2013 (12)) have provided an additional characteristics of MSM in Bulgaria and the capital Sofia. This series of MSM surveys was extended with Round 7 of NBBS in 2016 (NBBS-16). The purpose of this study was to perform cross-sectional bio-behavioral survey conducted among MSM to examine trends of sexual risk behavior.

Materials and Methods

Study design

NBBS-16 was conducted between June and September 2016. The study was cross-sectional, with two-stage clusters and probability time-location sampling (13, 14) which is described elsewhere in details (15). Five cities were selected as primary sampling units namely Sofia, Plovdiv, Varna, Burgas and Blagoevgrad. They were selected based on probability to size of MSM, on the rate of newly diagnosed MSM between 2010 and 2015 and where the previous rounds of NBBS-04-12 were conducted (16). The study combined blood test and behavioral face-to-face interview. Outreach workers from local non-governmental organizations (NGOs,) working with MSM in these cities were hired to reach the target population. Trained and experienced interviewers conducted face-to-face interviews. The questionnaire was an adapted version of the questionnaire previously used among MSM in NBBS-12 (8) and was based on GARPR indicators (17). Experienced medical workers provided blood collection for biological markers after the interview for all participants.

Recruitment and enrollment procedures

The most-at-risk groups are difficult to reach through classical methods thus, time location sampling was used for recruitment of participants (18). NGOs working with MSM population identify venues visiting by MSM (e.g., bars, clubs, organizations, and street locations) as well as days/time intervals when those venues are frequented. At that time, the research teams visited these places with mobile medical cabinets (MMC) for interview and testing (19). Under the second approach, the participants were recruited during their visits at LTHTS for MSM. Recruitment and interviewing then continue until the target sample size was reached.

Participants were enrolled in NBBS-16 following preliminary screening based on the following criteria: at least 16 years old and having had anal sex with a male partner within the last 6 months. Prior to enrollment into the study, the respondents were provided with comprehensive information about the study and signed a consent form. NBBS participants received compensation for their participation in form of a package with condoms and lubricants.

HIV and STIs testing procedures

All participants were tested for anti-HIV, anti-HCV and anti-Syphilis antibodies and HBsAg as well. Qualified well-trained counsellors from voluntary and confidential counselling and testing (VCT) sites provided pre-test counselling. A venous blood sample (3 mL) was obtained from each participant by a trained laboratory technician. Plasma HIV antibodies were tested by ELISA, and positive results were verified by Western blotting assay. The presence of antibodies against syphilis and HCV as well HbsAg were screened by ELISA. Treponema pallidum hemagglutination assay (TPHA) was used for confirmation of syphilis.

The same counsellor who conducted the pre-test counselling provided post-test counselling for each participant regardless of his or her HIV test result. Participants who were HIV reactive and did not know their HIV status were referred for confirmatory testing. The quality of HIV tests was monitored using quality control samples.

Data entry and analysis

The completed paper-based questionnaires were scanned by FlexiCapture-8 (ABBYY Europe Global) and then transferred to SPSS version 9.0 for pre-analysis data processing. Final database was transferred to STATA 14.2 and data analyzed with "Survey analysis" module. Sample weights and finite population coefficients (FPC) were calculated as described (14). Qualitative variables of NBBS-12 and NBBS-16 were compared using the equation (20):

$$z_1 = \frac{p_1 - p_2}{\sqrt{\left(\frac{p_1(100-p_1)}{n_1} + \frac{p_2(100-p_2)}{n_2}\right)}}$$

Quantitative variables of NBBS-12 and NBBS-16 were compared by Mann-Whitney U test.

For all analyses, we set the significance level for P values at 0.05.

Ethical statement

The ethical issues of NBBS-04-16 are described elsewhere in details (21). In brief, the study documents were approved by the Committee on Medical Ethics at the National Center of Infectious and Parasitic Diseases, Sofia (19, 22). Participation in this study was voluntary, and a written informed consent was obtained from each study participant after a detailed description of the study objective and procedures was explained to them. Participants were informed that they could stop responding to questions and discontinue their participation at any time. Interviews were conducted at a private place, and confidentiality was enhanced by assigning a unique and anonymous code to each participant (19, 22).

RESULTS

Socio-demographic characteristics

A total number of 635 participants aged 18 to 54 years were enrolled in NBBS_2012 (n=198) and NBBS_16 (n=437). Table 1 presents the general characteristics of the study population. The median age of the participants was 29 years, interquartile range 23-31 years. In the age group 20-29 years were 59.6% of them and 29.8% were in the age group 30-39 years. Regarding the educational level, 36.8% completed elementary education, 39.5% completed secondary education, and 3.2%- higher education. Most participants in 2016 study declared Bulgarian ethnicity (67.1 %) and 25.4% were from Roma origin.

TABLE 1. Demographic characteristics of MSM by year 2012 and 2016.

Variable	NBBS-2012 N = 198				NBBS-2016 N = 437				
	Median	**SE**	**Q_{25}**	**Q_{75}**	**Median**	**SE**	**Q_{25}**	**Q_{75}**	**P value**
Age	28	5.635	24	32	29	5.57	23	31	p > 0.05
Variable	**%**	**SE**	**CI95% LI**	**CI95% UI**	**%**	**SE**	**CI95% LI**	**CI95% UI**	**P value**
Education									
primary education	16.3%	0.004	0.145	0.184	14.7%	0.003	0.140	0.154	p > 0.05
elementary education	57.9%	0.004	0.560	0.597	36.8%	0.006	0.353	0.383	p < 0.001
secondary education	22.9%	0.008	0.197	0.263	39.5%	0.010	0.371	0.420	p < 0.001
higher education	1.3%	0.001	0.009	0.019	3.2%	0.002	0.028	0.037	p > 0.05
Incomplete primary education	1.6%	0.000	0.014	0.018	3.1%	0.002	0.027	0.036	p > 0.05
Ethnicity									
Mixed Origin	0.6%	0.000	0.005	0.007	1.3%	0.001	0.012	0.015	p < 0.001
Bulgarian	58.5%	0.010	0.542	0.626	67.1%	0.006	0.656	0.686	p < 0.001
Roma	38.4%	0.010	0.340	0.429	25.4%	0.004	0.244	0.265	p < 0.001
Turkish	2.6%	0.000	0.024	0.028	3.4%	0.001	0.030	0.038	p > 0.05

Services received and Knowledge of HIV transmission

Most of participants had received prevention services. Overall 80.7% reported receiving free confidential HIV testing and cancelling, free condom distribution (88.3%) and information materials about HIV infection (52.5%). Details are shown in Table 2.

Regarding the transmission routes of HIV the majority of participants gave the correct answers. Participants were significantly more likely to disprove (83.0%) the misconception that HIV could be transmitted by dining together with HIV carriers or with patients with AIDS. When asked if HIV can be transmitted via a mosquito bite, 79.8% of the participants chose the correct transmission route. Almost four fifths of participant approve the statements that the risk of getting HIV can be reduced by: "Using clean injecting materials every time" (85.1%), "Using a condom every time having sex" (85.1%) and that "Healthy-looking person can have the AIDS virus" (72.0%).

TABLE 2. Services received and knowledge of HIV transmission

Variable	NBBS-2012 N = 198				NBBS- 2016 N = 437				
	%	SE	CI95% LI	CI95% UI	%	SE	CI95% LI	CI95% UI	P value
Services received									
HIV testing and counselling	49.4%	0.012	0.442	0.547	61.1%	0.009	0.587	0.634	p < 0.001
HBV, HCV and syphilis testing	29.5%	0.005	0.276	0.315	53.7%	0.009	0.514	0.560	p < 0.001
Information for HIV	46.5%	0.005	0.443	0.488	48.9%	0.006	0.473	0.505	p > 0.05
Knowledge of HIV transmission									
Can a person reduce the risk of getting HIV by using a condom every time they have sex	97.3%	0.001	0.970	0.976	87.3%	0.004	0.863	0.882	p < 0.001
AIDS cannot be transmitted by mosquito bites	80.1%	0.002	0.791	0.811	67.3%	0.004	0.664	0.683	p < 0.05
HIV transmission can be reduced by having sex with only one uninfected partner who has no other partners	85.0%	0.005	0.828	0.870	66.6%	0.012	0.635	0.695	p < 0.001
Cannot become infected by sharing food with someone who has AIDS	87.2%	0.001	0.867	0.876	70.2%	0.004	0.693	0.712	p < 0.001
Healthy-looking person can have the AIDS virus	94.1%	0.001	0.936	0.946	65.3%	0.008	0.632	0.673	p < 0.001
Can a person reduce the risk of getting HIV by using clean injecting materials every time	95.1%	0.001	0.947	0.954	87.6%	0.004	0.867	0.886	p < 0.05

Sexual risk behavior

Almost all participants (85.8%) reported sex in the last month and 75.2% of participants used condom during their last sexual intercourse. All responders reported having anal sex in last 6 months. In that case, three-quarters (77.2%) of them have used condoms during the last sexual intercourse. The proportion of respondents, which had intercourse with a casual partner in last 12 months, is high (89.9%) and 84.3% of them reported using condoms in that case. Sex with a steady partner in last 12 month had 60.8% of participants and more than half of them (55.5%) had used condoms in that case. Every fifth (18.3%) of the respondents had sex in exchange for money or gift in past 12 months and in that case, 81.6% of them had used condom consistently. A small proportion of responders reported to pay for sex (6.3%) and 76.3% of them had used condom in last sexual intercourse. One third of respondents (31.9%) had sex with a female partner in past 12 months and 52.1% of them had used condoms. The majority of the participants (86.8%) reported anal sex with casual man partner in past 6 months and 84.3% of them used condoms in last intersexual intercourse.

TABLE 3. Sexual risk behaviors of MSM in NBBS-2012 and NBBS-2016.

Variable	NBBS-2012 N = 198				NBBS-2016 N = 437				P value 2012 vs. 2016
	%	SE	CI95% LI	CI95% UI	%	SE	CI95% LI	CI95% UI	
Had sex in last month	80.4%	0.003	0.795	0.812	85.8%	0.005	0.843	0.871	p > 0.05
Condom used the last time they had sex	62.3%	0.001	0.619	0.627	75.2%	0.007	0.732	0.771	p < 0.05
Had sex in exchange for money or gift in past 12 months	62.6%	0.002	0.617	0.636	18.3%	0.005	0.169	0.198	p < 0.001
Condom use with sexual partner in exchange for money or gift	71.4%	0.002	0.704	0.723	81.6%	0.005	0.800	0.830	p < 0.05
Sex with a casual partner in last 12 months	86.1%	0.001	0.857	0.865	89.9%	0.004	0.887	0.909	p > 0.05
Condom used the last time they had sex with a casual partner	77.1%	0.004	0.753	0.789	84.2%	0.005	0.828	0.856	p < 0.05
Sex with a casual partner in last 6 months	82.2%	0.002	0.815	0.828	86.8%	0.003	0.860	0.875	p > 0.05
Condom used the last time they had sex with a casual partner	66.2%	0.002	0.655	0.669	84.3%	0.006	0.826	0.858	p < 0.001
Had anal sex with men in last 6 months	100.0%	0.000	0.000	0.000	64.9%	0.018	0.590	0.703	p < 0.001
Condom used the last time they had anal sex with men	66.7%	0.002	0.658	0.676	77.2%	0.006	0.755	0.788	p > 0.05
Sex with a steady partner in last 12 months	64.6%	0.007	0.614	0.676	60.8%	0.002	0.602	0.613	p > 0.05
Condom used the last time they had sex with a steady partner	31.3%	0.004	0.298	0.329	55.5%	0.011	0.523	0.586	p < 0.001
Pay for sex in past 12 months	0.6%	0.000	0.005	0.007	6.3%	0.004	0.054	0.073	p < 0.001
Condom used the last time they pay for sex	100.0%	0.000	0.000	0.000	76.3%	0.007	0.743	0.782	p < 0.001
Had vaginal or anal sex with female partner in past 12 months	10.9%	0.001	0.104	0.115	31.9%	0.011	0.289	0.352	p < 0.001
Condom used the last time having vaginal or anal sex with female partner	28.9%	0.001	0.271	0.308	52.1%	0.004	0.511	0.531	p < 0.001

Awareness of HIV status and HIV/STI test results

In order to monitor HIV testing uptake, UNGASS indicator number 8 was used, which comprises the percentage of MSM tested for HIV over the last 12 months. Most of the responders (95.8%) declared to have an opportunity for a confidential HIV testing and more than half (52.4%) have been tested for HIV in last 12 months. Very few (less than 1%) of responders reported HIV positive status.

HIV Prevalence rate for HIV, HVB, HCV and syphilis was respectively 3.2%, 3.1%, 4.2% and 6.3%.

TABLE 4. Awareness of HIV status and prevalence rates of HIV, HBV, HCV and syphilis among MSM in NBBS-2012 and NBBS-2016

Variable	NBBS-2012 N = 198				NBBS-2016 N = 437				P value 2012 vs. 2016
	%	SE	CI95% LI	CI95% UI	%	SE	CI95% LI	CI95% UI	
Awareness of HIV status									
Having an opportunity for a confidential HIV testing	93.0%	0.000	0.929	0.932	95.8%	0.002	0.952	0.963	p > 0.05
HIV testing in the last 12 months	28.5%	0.011	0.242	0.333	52.4%	0.011	0.495	0.553	p < 0.001
HIV testing in the > 12 months	62.1%	0.008	0.586	0.656	47.4%	0.014	0.439	0.510	p < 0.05
Self-reported HIV status									
Posit	0.0%	0.000	0.000	0.000	0.6%	0.000	0.005	0.007	p > 0.05
Negative	100.0%	0.000	0.000	0.000	85.4%	0.007	0.836	0.871	p < 0.001
HIV and STI prevalence rate									
Anti-HIVab positive	0.0%	0.000	0.000	0.000	3.2%	0.002	0.027	0.037	p < 0.05
HbSAg positive	3.5%	0.002	0.028	0.044	3.1%	0.001	0.029	0.034	p > 0.05
Anti-HCVab positive	6.4%	0.001	0.061	0.067	4.2%	0.001	0.039	0.045	p > 0.05
Anti-Syph Ab positive	3.3%	0.001	0.029	0.037	6.3%	0.001	0.059	0.067	p < 0.05

DISCUSSION

This study was conducted as part of NBBS-06-16 in Bulgaria (23) to assess HIV prevalence and associated risk factors among MSM. The median age and the level of education of the participants are comparable to the data of the survey in 2012 (NBBS-12). They also do not differ from the data for Southeastern Europe from 2013 (24, 25).

Compared to the figures observed in NBBS-12, there is no change in knowledge about HIV infection. Participants show good knowledge, with about 85% of them responding correctly to the ways of HIV transmission and its prevention. These proportions are comparable to the data reported for South-Eastern Europe (26) and SIALON II (27).

According to our findings, MSM in Bulgaria receives good services for HIV prevention in the period 2012-2016. In this respect, the figures of all indicators concerning free distribution of condoms, information on HIV prevention, HIV testing and counseling have increased between 2012 and 2016. This increase is most significant for the access to free testing and counseling services (61.1% in 2016 vs. 49.4% in 2012). Accordingly, almost all participants (97%) declare that they have been tested for HIV. Moreover, in 2016, the proportion of MSM tested for HIV in the last 12 months increased by 20% (from 28.5% in 2012 to 52.4% in 2016). This can be attributed to the intensive HIV testing campaigns conducted by the National AIDS program 2008 – 2015(28).

In the current study the HIV prevalence rate is 3.2% which is more than 10 times higher in comparison of the rate of 0.30% observed in 2010. This is in line with prevalence rate of 3.05% observed in SIALON II survey in Sofia in 2013 (12). In this regard, it should be noted that in NBBS-12, no HIV positive case were was found, but this zero result should be interpreted with caution because of limited number of participants. The case surveillance data show a significant increase in the rate of new diagnosed HIV cases among MSM from 59 in 2012 and 72 in 2014 to 111 in 2015 (29). This is also in line with the modeled prevalence of 2.2% of HIV among MSM in Bulgaria in 2016 (unpublished data). The HIV prevalence in MSM population in Bulgaria is lower than the average HIV prevalence among MSM in Europe (11). The global prevalence of HIV among MSM is reported 19 times higher than in the general population (3, 30). According our data HIV prevalence in Bulgaria is about 46 times higher than the prevalence of the low-risk population (prevalence of 0.069 %, data from Ministry of Health). These data indicate a growing prevalence of HIV among MSM in Bulgaria.

In the current study, respondents were further tested for HBV, HCV and syphilis infections. According to our findings, the prevalence rate of these infections remains relatively constant in last 5 years. It is slightly higher compared to the figures observed in a study performed in European countries (26) (27)

In relation with the higher rate of HIV and STIs in MSM population, it is important to know the specificity of their sexual behavior.

The results of this study show an increase in 2016 in the proportion of participants using condoms the last time having sex from 62.3% in 2012 to 75.2% in 2016 as well having anal sex with man partner from 66.3% in 2012 to 72.2% in 2016. Having a steady partner in Southeast European countries is lower than in Western Europe (26)(27). Although there is no difference in the proportion of the participants having sex with steady partner (64.6% in 2012 vs. 60.8% in 2016) we observed significant increase of condom use (31.3% in 2012 vs. 55.5%in 2016) in such a case. This agrees with other observations in the region [18, 21].

There is no difference in the proportion of participants having sexual intercourse with a casual partner (82.2 in 2012 vs. 86.8%in 2016) but is still higher than the average for Europe (24, 26). In 2016, 84.3% of participants declare using condoms by anal sex with casual partner, which is with 18 % higher than in 2012 (66.2%, $p < 0.001$). The remaining quarter, however, is a high-risk group that needs more intensive and targeted interventions.

In 2016, we observed threefold decrease in the proportion of MSM having sex for money or gifts (from 62.6% in 2012 to 18.3% in 2016). It is worried that this figure is still about three times higher than the European average (5.1%) (26). A positive sign is the observation that proportion of participants using condoms with sexual partners in exchange for money or gift increased from 71.4% in 2012 to 81.6% in 2016. It is very worrying the significant increase of paying for sex (from 0.6% in 2012 to 6.3% in 2016)

Sexual intercourse of MSM with women is an important factor in passing HIV infection from the MSM population to the general population (31). In our study, 31.9% of respondents reported vaginal sex in the last 12 months, which is three times higher than reported in 2012 (10.9%) and is also higher than the average for the European countries (7.1%) (26). In addition, it should be noted that there is significant increase of condom use in that cases (from 28.9% in 2012 to 52.1% in 2016).

CONCLUSIONS

In summary, despite the good knowledge of HIV transmission, the prevalence rate and the number of new diagnoses among MSM in Bulgaria have risen significantly in the past five years. This can be related to the increased proportion of MSM with risky sexual practices. An alarming increase is the proportion of MSMs that make sex for money or sex buyers, together with low condom use and widespread unprotected intercourse with male and female partners. This apparent increase in HIV prevalence among MSM may be explained by the relatively late start of extensive preventive interventions at MSM (in 2008) in Bulgaria compared to other key populations (in 2004) (32). Another possible explanation is the significant increase of traveling for sex tourism in Western Europe. All this indicates the need to intensify prevention interventions among MSM population for safe sexual behavior, and

to implement WHO guidelines for pre- and post- exposure prophylaxis (33).

STRENGTHS AND LIMITATIONS OF THIS STUDY

NBBS-2016 is a regular bio-behaviour survey among MSM—one of the most vulnerable and understudied populations in Bulgaria and Europe. This study covered HIV high-burden sites including the capital city and four other bigger cities, thus providing results for most at risk places. This study identified important risk factors for HIV infection among MSM, which require being addressed in order to reduce HIV infection among this key population.

Limitation of the study

Our study covered only the capital city and four cities, which have the highest numbers MSM. Since cities with fewer MSM were left out, these results may not be generalized to all MSM nationally. Second participants were identified and recruited by outreach workers of community-based organizations, which could introduce bias towards MSM under their programs, leading to a recruitment bias. Finally, as this study was cross-sectional, it reports associations at a given time and may not be construed to be reporting causal relationships.

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USING CASE SURVEILLANCE DATA FOR ESTIMATION AND PROJECTION OF HIV INFECTION IN PWID AND MSM POPULATION BY COMBINING ECDC-HIV MODELING TOOL AND SPECTRUM-ESTIMATION AND PROJECTION PACKAGE

H. Taskov[1] and T. Varleva[2]

1 – National Center Infectious and Parasitic Diseases, Sofia
2 – Ministry of Health, Sofia

ABSTRACT

Background

Bulgaria is low HIV prevalence country with concentrated epidemic. The National HIV surveillance system collect case-based data for MSM and PWID. The main objective of this study was to explore an appropriate method for estimation and projection of MSM and PWID case surveillance data.

Methods

We have used a combination of ECDC-HIV Modeling Tool with Spectrum-EPP to calculate the total number of persons infected with HIV, number of new HIV infections, HIV incidence and prevalence. For computing bio-behaviour surveys data for MSM and PWID Spectrum-EPP model was used.

Result

The present results show that both models led to similar results regarding the study parameters. Although the values obtained by the studied models differ, the estimations and projection curves show similar patterns.

Conclusions

We have demonstrated that the study approach is appropriate for analysis of MSM and PWID case-based data. It has several advantages. It applies a combination of established models that are widely used. Secondly, it allows parallel analyzes of data obtained from both case surveillance and other surveys e.g. population, sentinel, BBCs etc. Therefore, a more complete and accurate picture of the dynamics of the HIV epidemic can be obtained. Finally, the described approach uses models, which are constantly developing, free and accessible.

KEYWORDS:

Bulgaria, bio-behaviour surveillance, HIV modeling, EPP.

ADDRESS FOR CORRESPONDENCE:

Prof. Dr. Hristo Taskov DSc,
National Center Infectious and Parasitic Diseases,
Sofia 1504, Bul. Yanko Sakazov 26,
Tel.: +359887304381;
E-mail: hristotaskov@gmail.com

INTRODUCTION

Mathematical modeling and computer simulation are powerful tools for assessing the impact of intervention programs. Based on the results, the health policy decisions and the direction of action can be formulated (1). The modeling of HIV infection in populations is of particular interest in understanding the dynamics of the HIV epidemic, making prognosis, and monitoring the effect of different programs. For this purpose, various models were used to evaluate the epidemic and its progression (2, 3). Currently, the SPECTUM package is the most widely used for UNAIDS annually assessing the HIV epidemic and the world (4). It includes the modules EPP, AIM, CSVAR and ECDC for estimation and projection of an epidemic. The European Center for Disease Control (ECDC) has developed the HIV Modeling tool (HMT) (5), which can be used alone or as a module in SPECTRUM. The EPP module is used by 2001 with sentinel surveillance data or surveys such as biobehaviour survey (BBS). The CSVAR and HMT require case-surveillance data.

The National HIV Surveillance System of Bulgaria consists of case surveillance, BBS, and HIV incidence (6). Individual case-based data, including the number of CD4 counts, have been collected in Bulgaria since 1997. BBS started in 2004 among the key HIV populations and nine rounds have been conducted so far (7). The estimations and projection of the epidemic in Bulgaria started in 2007 with BBS data using the Spectrum-EPP (8). The modeling was not only for the general population, but also for the

key subpopulations men who have sex with men (MSM), people who inject (drugs PWID) and sex workers. Since 2014, modeling with case-surveillance data has started with ECDC-HMT, Spectrum-CSVAR and Spectrum-ECDC. With ECDC-HMT, multiple parameters can be assessed for both general populations and subpopulations, such as PWID and MSM, but projections cannot be made. With Spectrum-CSVAR and Spectrum-ECDC projections can be made, but only for the whole population.

Bulgaria is a low prevalence country with an HIV epidemic concentrated mainly among PWID and MSM populations (9, 10). In 2009, HIV transmission due to drug injection was responsible for 43.3% of all newly diagnosed HIV cases. Although drug injection is no longer the primary mode of HIV transmission, PWIDs remain a group at high risk (10). There are signs of an increase in HIV prevalence among MSM. A recent BBS showed a significant increase of prevalence rate from 2.26 % in 2012 to 3.69 % 2013 (9). In 2015, about 49.6% of newly diagnosed HIV cases came from MSM population (11). These data show that it is of key importance for the country to estimate and project HIV infection in MSM and PWID populations.

UNAIDS recommends Spectrum-CSVAR and Spectrum- ECDC to be used for case surveillance data. As, those modules do not produce sub-population data, we investigate the possibility to combine ECDC-HMT and Spectrum-EPP models (Spectrum-EPP/HMT) to generate estimations and projections for MSM and PWID. In this study we describe the methodology of analysis by Spectrum-EPP/HMT and the comparison of results with those obtained by Spectrum-EPP and Spectrum- ECDC.

MATERIALS AND METHODS

Data on PWID and MSM from BBC during 2004-2016 (BBS-04-16) were used for modeling by Spectrum-EPP (12-15). For modeling with ECDC-HMT, we used case-surveillance data for the period 2000 -2017 uploaded in ECDC-TESSy (16). In The HMT the epidemic was defined as MSM, IDU and Remaining population (OTHER + HETERO_NONSSA + HETERO_SSA). After analysis, separate output files for MSM, IDU and Remaining Population were generated.

The following procedure was used to combine HMT output data with EPP.

1. The prevalence of each subpopulation was calculated by the formula:

$$Prevalence\ (\%) = \frac{N\_alive\_HMT}{N\_subpopulation}$$

where

N_alive_HMT - is number of alive HIV persons. Data are from output file column “N_alive”.

N_subpopulation – is the number of the respective subpopulation in the country. The number of IDUs was calculated as 0.27% from total population and the number of MSM – as 3% of adult man population (17) . The number of remaining population was calculated by subtraction the figures of MSM and IDU from total population according National Statistical Institute.

2. In Spectrum_EPP structure of national epidemic was defined as four subpopulations (MSM, IDUs, Hetero (OTHER+HETERO_NONSSA+HETERO_SSA) and Total population. Calculated prevalence and subpopulations size were entered in “Surveillance data” window of EPP. For Total population the prevalence data from, Remaining population were used. The figure 1000 was entered as number of observations.
3. Spectrum files were analyzed without calibrations. Output files from Spectrum-EPP, Spectrum-EPP/HMT and ECDC-HMT were generated. The data were entered in Excel spreadsheet for comparisons and graphics construction.

In this study, the following epidemiological parameters were selected for each subpopulation: A) Number of new HIV infections; B) Number of HIV people alive (HIV population); C) HIV incidence by +15 years old (per 1000) and D) HIV prevalence by +15 years old (percentage).

RESULTS

The results of total populations are presented on Fig. 1. For *“Number of new HIV infections”* and *“HIV incidence by +15”* Spectrum_EPP shows different pattern of curves compared to other models. For the estimation it shows highest pick value and for projection – lowest values compared to other models. For *“HIV population”* and *“Prevalence”* Spectrum_EPP shows similar pattern of curves with the other models, but the values are still lowest. This can be expected since Spectrum_EPP uses input data from BBS,

while Spectrum-ECDC, Spectrum-EPP/HMT and ECDC-HMT are using case surveillance data.

Spectrum-ECDC, Spectrum-EPP/HMT and ECDC-HMT show similar patterns of curves with lowest values of Spectrum-EPP/HMT for projections.

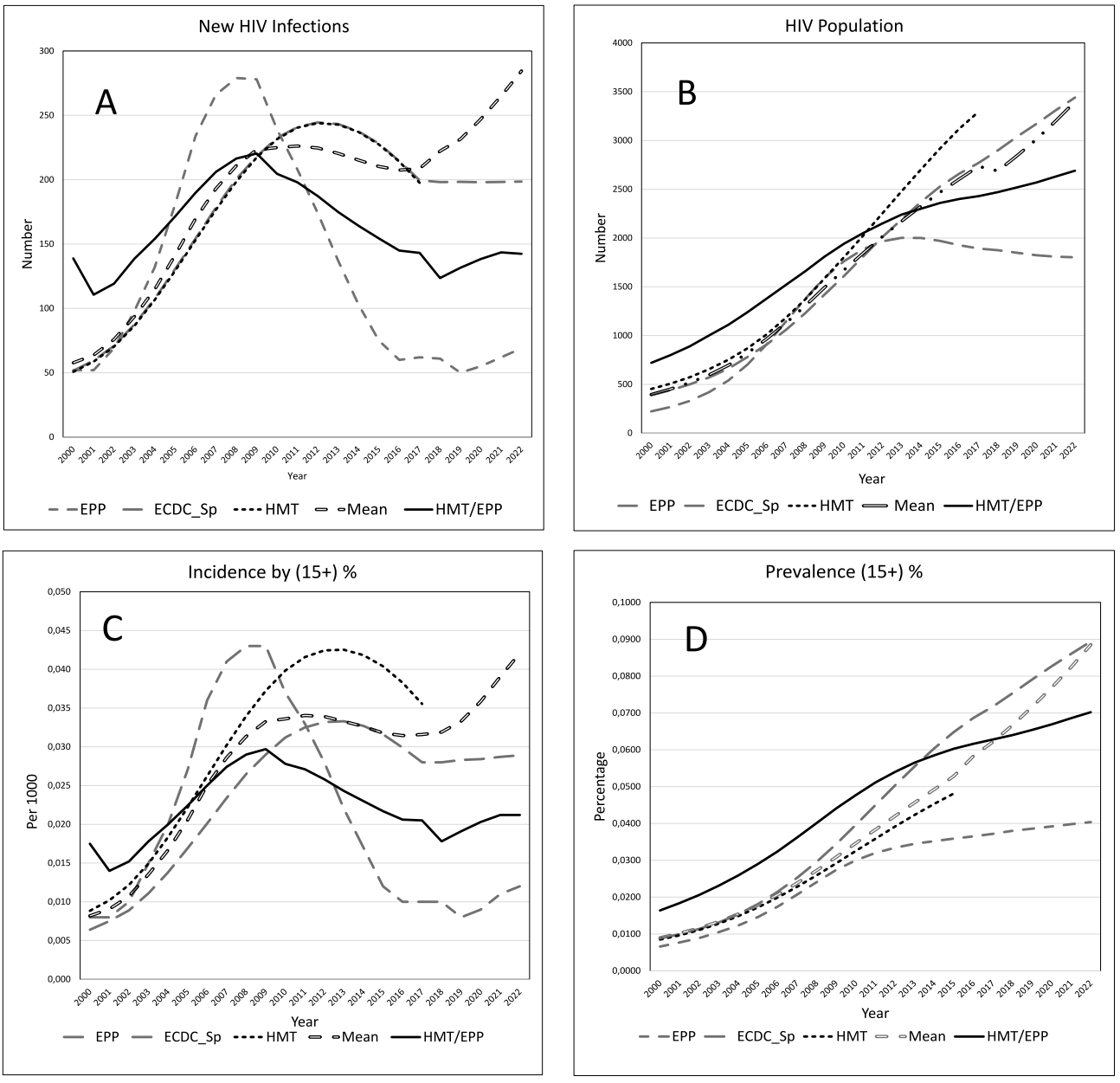

Figure 1. Results for total HIV population: A) Number of new HIV infections; B) Number of HIV people alive (HIV population); C) HIV incidence by +15 years old (per 1000) and D) HIV prevalence by +15 years old (percentage). EPP - stands for Spectrum_EPP, ECDC_Sp – stands for Spectrum-ECDC; HMT stands for ECDC-HMT and HMT-EPP – stands for combined model ECDC-HMT plus Spectrum-EPP.

MSM and PWID data were analyzed by Spectrum_EPP, Spectrum-EPP/HMT and ECDC-HMT models. Spectrum-ECDC was not used because it do not generate outputs for the subpopulations.

The results for MSM are presented on Fig. 2. All model give similar patterns of the curves. The figures obtained by ECDC-HMT are about two times lower than values derived from Spectrum_EPP and Spectrum-EPP/HMT. Spectrum-EPP/HMT generates the highest values for all parameters. One possible explanation is that the analysis by Spectrum was without calibrations. Obviously, a calibration should be performed to produce better-fitted results.

The results for PWID are presented on Fig. 3. As

with MSM and in this case all models give similar patterns of curves. With the exception of HIV population, for other parameters, the **ЛИПСВА НЯКАКЪВ ТЕКСТ!!!**

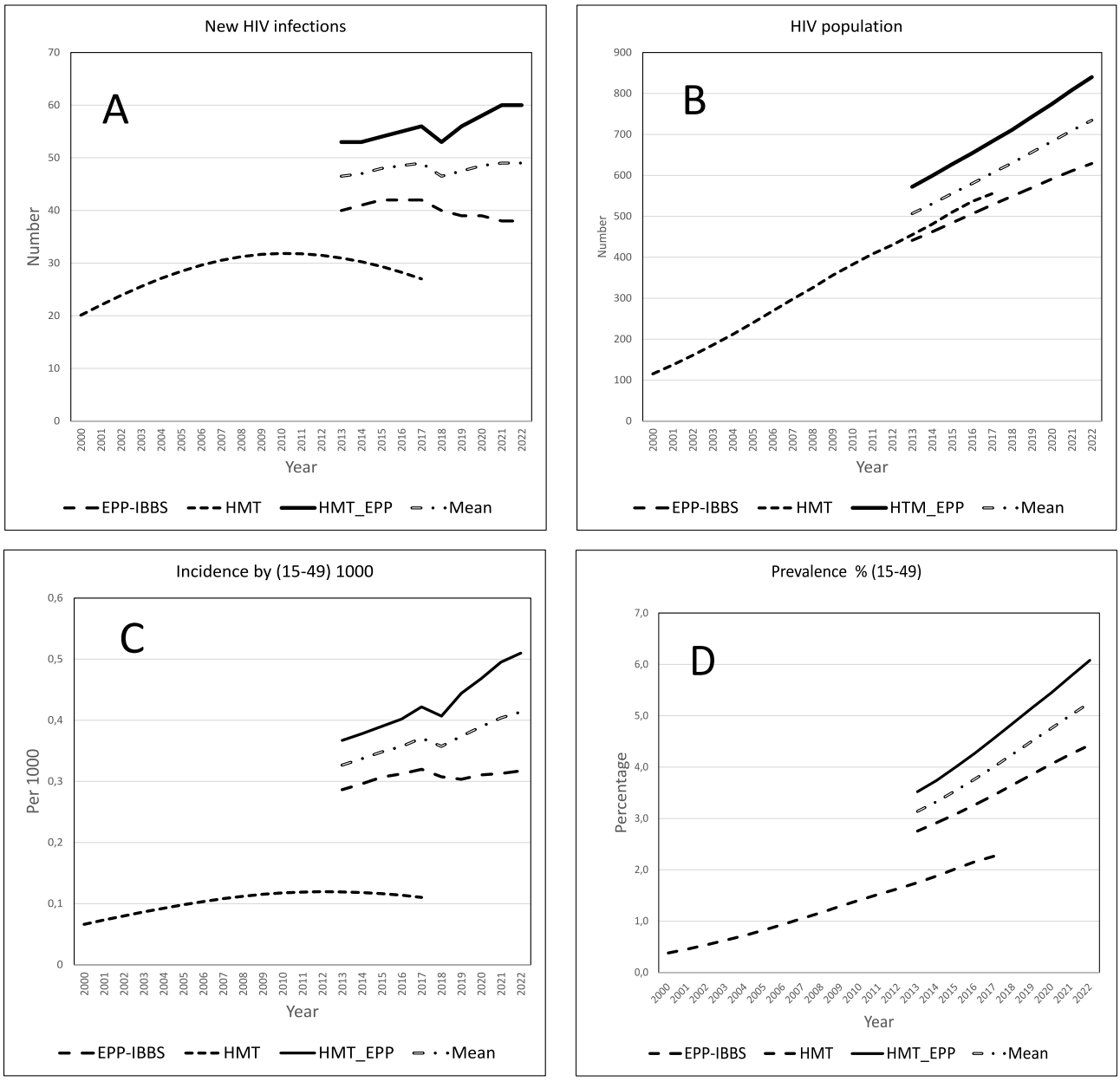

Figure 2. Results for MSM population: A) Number of new HIV infections; B) Number of HIV people alive (HIV population); C) HIV incidence by +15 years old (per 1000) and D) HIV prevalence by +15 years old (percentage). EPP - stands for Spectrum_EPP, ECDC_Sp – stands for Spectrum-ECDC; HMT stands for ECDC-HMT and HMT-EPP – stands for combined model ECDC-HMT plus Spectrum-EPP.

ECDC-HMT values are the lowest. Spectrum-EPP/HMT give the highest values. This also applies to the fact that no calibration was used in Spectrum analysis.

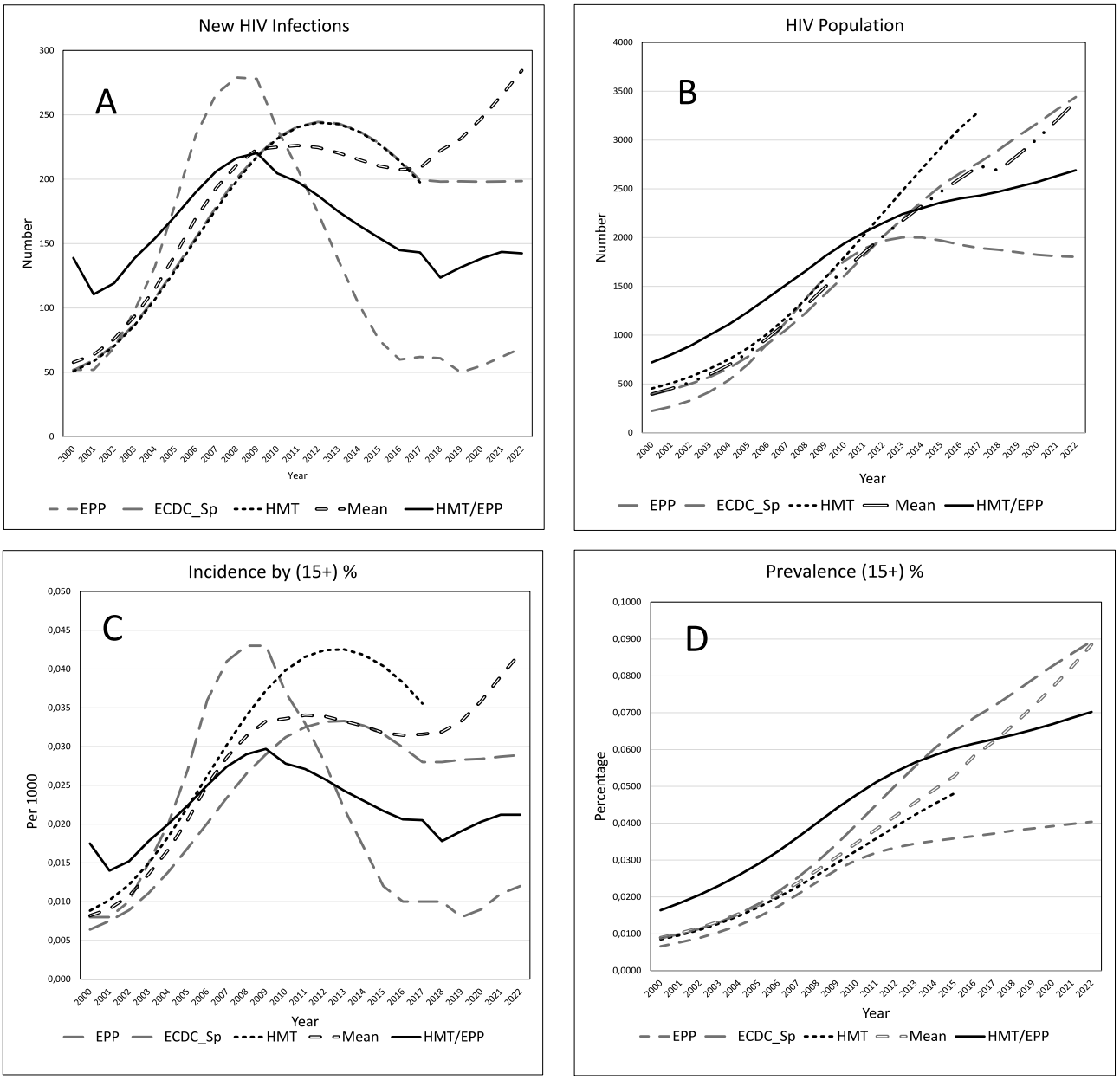

Figure 3. Results for PWID population: A) Number of new HIV infections; B) Number of HIV people alive (HIV population); C) HIV incidence by +15 years old (per 1000) and D) HIV prevalence by +15 years old (percentage). EPP - stands for Spectrum_EPP, ECDC_Sp – stands for Spectrum-ECDC; HMT stands for ECDC-HMT and HMT-EPP – stands for combined model ECDC-HMT plus Spectrum-EPP.

DISCUSSION

The estimations and projections of HIV dynamics in countries with low prevalence and concentrated epidemics is a challenge. There is no commonly accepted model for these cases. UNAIDS use Spectrum/EPP to produce official estimates for most countries in the world. Its limitation is the utilization of survey's data. Bulgaria has quality case based data since 1987 and they should be used in a proper way. There are several models for this, but they do not give either results or projections for key subpopulations. To overcome this, we applied a new approach, combining ECDC-HTM with the Spectrum-EPP. The approach was used to analyze case-based data on MSM and PWID in Bulgaria. At the same time, we analyzed the available BBC data for the same groups with the classic Spectrum-EPP model. Although the values obtained by the studied models differ, the estimations and projection curves show similar patterns. Probably additional calibration is required in the analysis

by Spectrum. Further research is needed in this direction.

In conclusion, we have demonstrated that this approach is appropriate for analysis of case-based data from subpopulations as MSM and PWID. Currently we are in process of testing this method for sex workers and Roma population. The described method has several advantages. It applies a combination of established models that are widely used. Secondly, it allows parallel analyzes of data obtained from both case surveillance and other surveys e.g. population, sentinel, BBCs etc. In this way, a more complete and accurate picture of the dynamics of HIV epidemic can be obtained. Finally, the described approach uses models, which are constantly developing, free and accessible.

For the analysis, we have used data from Bulgaria. We hope our approach is also applicable to other countries with a concentrated HIV epidemic.

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