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# **PROBLEMS**

of Infectious and Parasitic Diseases

### ISSN 0204-9155

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# PROBLEMS OF INFECTIOUS AND PARASITIC DISEASES VOLUME 43, SUPPLEMENT/2015

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Manuscripts must be written in English, using British spelling. All manuscripts should be single-spaced, with wide margins and numbered pages. MS Word should be used for word processing, 12-point Times New Roman font.

Named authors must fit the following three criteria:

- 1. Substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data;
- 2. Drafting the article or revising it critically for important intellectual content; and
- 3. Final approval of the version to be published.

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#### **ABSTRACT**

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#### **ACKNOWLEDGEMENTS**

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#### Journal articles:

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Rosa PA, Hogan D, Margolis N. *Molecular analysis of the major outer surface protein locus from a divergent Borrelia burgdorferi isolate from Europe*. In: Schutzer SE. Lyme borreliosis: Molecular and immunologic aspects. Cold Spring Harbor Laboratory Press, 1992, 95-110.

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Identifying details of patients should be omitted. Identifying information, including patients' names, initials, or hospital numbers, should not be published unless the patient (or parent or guardian) gives written informed consent for publication. When informed consent has been obtained it should be indicated in the published article.

# Dear readers,

I am pleased to present to you this special issue – supplement of Problems of Infectious and Parasitic Diseases, issued by the National Center of Infectious and Parasitic Diseases, which includes five summaries of reports for the long work done by the program "Prevention and Control of HIV/AIDS" at the Ministry of Health, funded by the Global Fund to fight AIDS, tuberculosis and malaria. Anyone interested could find the full text of the reports in Bulgarian language, which soon will be published. They reflect the efforts of hundreds of medical professionals, social workers, members of NGOs, municipal officials, ministries and many other public and business organizations united in their struggle for improved control and prevention of one of the most important diseases of the present time targeting key risk groups in this respect: prisoners, injecting drug users, young Roma men, female and male sex workers and men who have sex with men.

The editor-in-chief prof. T. Kantardzhiev kindly gave us the opportunity to present the summaries of those reports in English which is of great importance as it will enable the results of the enormous volume and of great medical and social importance of work done within the program to become available to the wider international audience, that will undoubtedly contribute to the prestige of our country, which organizes and conducts the fight against HIV/AIDS according to the latest requirements.

With a wish to continue the efforts of all governmental and non-governmental institutions in the fight against this serious and socially significant disease associated with great financial burden, I would like to express my most sincere gratitude to all Program participants for their persistent, longlasting labor, which allows to introduce ourselves worthy to the Bulgarian public based on the activities carried out and the results obtained under the Program.

Prof. Hristo Taskov, MD, DSc

# REPORT OF INTEGRATED BIO-BIHEVIORAL SURVEILLANCE OF HIV AMONG PRISONERS, 2006-2011, BULGARIA. ABSTRACT

Tonka Varleva, Vyara Georgieva, Emilia Naseva, Tsvetana Yakimova, Mariya Zamfirova, Hristo Taskov, Bogdan Petrunov

Program "Prevention and control of HIV/AIDS", Ministry of Health, Bulgaria

The program "Prevention and Control of HIV/AIDS" was launched in 2004 according to the agreement between the Global Fund to Fight AIDS, Tuberculosis and Malaria and the Ministry of Health. Thanks to financial support from the Global Fund a national system of second generation epidemiological surveillance was established ensuring the implementation of integrated biological and behavioural surveillance (IBBS), including those specified in the two national programs for prevention and control of HIV/AIDS (with a period of duration of 2004-2007 and following a period of operation from 2008 to 2015) for the most vulnerable groups of the population, namely:

- ➤ Injecting Drug Users (IDU);
- Men who have sex with men (MSM);
- ➤ Young Roma people exposed to the greatest risk (injecting drug users, men who have sex with men, sex workers, persons who have served a sentence of imprisonment and

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mobile people);

- > Imprisoned persons;
- > Sex workers;
- Young people at risk;
- ➤ People living with HIV/AIDS;
- Migrants and refugees seeking asylum in the country;

With the launch of the program in 2004 several important new prerequisites were created for improving the control of the distribution and the dynamics of the infection among the group of persons serving sentence of imprisonment:

- After 2007, when the first agreement for cooperation was signed between the Minister of Health and Minister of Justice, which enabled external to the system of justice medical professionals to provide services in voluntary counselling and testing for HIV, hepatitis B and C and syphilis, the activities on prevention of HIV, the sexually transmitted infections and blood-transmissible hepatitis B and C were extended by the holding of regular lectures on health education on the aforementioned topics.
- The healthcare professionals providing medical services for inmates, as well as inspectors carrying out social and educational activities in prisons have contributed to actively motivating and encouraging imprisoned persons to use the services for voluntary counselling and testing (VCT) for HIV and participate in ongoing health education lectures.

These efforts made it possible to increase the detection of infected persons, especially through services for voluntary counselling and testing. A really indic-

ative illustration is the fact that for the period since 1986 and after, when the practice of carrying out screening and confirmatory tests for HIV in Bulgaria was introduced, by 2005, the number of detected HIV positive persons in the places of imprisonment (PoI) was a total of 3. After expanding the access to VCT on site in the places of imprisonment for medical specialists outside the judicial system from the Consulting rooms for anonymous and free counselling and testing for HIV/AIDS (CRAFC-TA), the number of annually registered HIV positive persons has multiplied, with a peak in 2008 and 2009 when 28 new HIV-positive persons were discovered in 2008 and respectively 30 HIV-positive persons in 2009.

# I. Demographic characteristics

Number of tested prisoners in the Pol arranged by towns and years as follows: in 2006 – 600 persons, 2007 – 754, 2008 – 1050, 2009 – 1151 and 2011 – 702 persons. In 2006, the testing included four prisons – in Sofia, Burgas, Pleven and Stara Zagora. In 2007, two more prisons, in Plovdiv and Varna, were added. In the following years 2008, 2009, 2011, the research included the prison in Pazardzhik, by which a total of 7 prisons with the largest number of prisoners and data on available risk behaviours of prisoners were covered.

In the years, the majority of respondents fall within the age group 20-29 years, followed by the group within 30-39, within 40-49 and followed by 50+. The smallest is percentage of persons under 20. The table shows the distribution of respondents by age group in the years in which the research was conducted. The educational degree of half of the persons involved in the research

was lower than secondary. More than half of all respondents identify themselves as belonging to the Bulgarian ethnic group, and also they profess Orthodox Christianity.

# II. Illegal drugs

Every fourth respondent has used illegal drugs in their lifetime, and every tenth has injected illegal drugs. The percentage of the drug users, those with a history of use before arrest, increases in the years. This can be explained on the one hand with the higher confidence of the study participants in the people who carried out the research and on the other hand with the effect of article 354 A of the Penal Code, due to the application of which, more people arrested with small doses of drugs for personal use fall in jail. With the growing penetration of oral drugs, the trend is that the number of IDUs among people of young age, up to 24, declines.

# III. Injecting behaviour in Pol and outside

Every 10th prisoner in 2006 announced that they have injected illegal drugs. Over the years this percentage grows and in the research in 2011 every fifth reported experience in injecting drugs. Every fifth prisoner reported that during their running stay in the Pol they had been injecting drugs. The difference in rates is twice in the years 2008 and 2009. In 2008, 13.33 percent reported they had injected illegal drugs in their current stay in prison, and in the next year 2009 their number doubled – 26.98%.

# IV. Practice of tattooing

The results of the research revealed that at least 50% of the prisoners undergo tat-

toos in prison. In almost every second one of them last tattoo was made more than 12 months ago. Also every second one reported that used, but disinfected needles were used the last time when they had a tattoo, and every sixth respondent acknowledges that their latest tattoo had been made with needles already used by others. The greatest percentage of tattooed prisoners was tattooed with used, but disinfected needles.

### V. Sexual behaviour

Over 90% of respondents reported of having sex in the last year. The age for starting sexual life is about 16 years of age on average. Most of the respondents know that condoms protect, but are not in the habit of using it. For the majority of respondents the certainty that their partner is not infected comes from the appearance of the person – how they look, tidy, healthy. Over 80% of prisoners reported that they had not used a condom during their last sexual intercourse. Relatively high is the percentage, 15% on average of the respondents, whose last sexual contact was with a man. The percentage of men who had sex with another man in prison in the past six months and have used a condom during their last sexual contact was the highest in 2007 - 46%, followed by the present in 2006 - 30%. The number of prisoners using condoms fall dramatically in 2008 to only 7.75% and remained at approximately the same levels of 20.41% in 2009 and 22.80% in 2011.

# VI. Knowledge, perceptions and attitudes about HIV/AIDS

Over 90% of all respondents are aware of the fact that the risk of HIV can be reduced by the use of condoms. With regard to the issues related to knowledge about the ways of transmission of HIV/AIDS an increasing awareness is definitely observed among the respondents, particularly in matters of transmission of HIV/AIDS through mosquito bites, where the number of false positive answers decreased from 48.9% to 21.3% in 2011 as well as on question "Can a person be infected with HIV/AIDS, if they eat from the food of someone infected," where the number of false positive responses decreased from 24.5% in 2006 to 13.2% in 2011. The responses to other questions showed a steady increase in the knowledge and awareness of the group. Over 90% of the respondents gave the correct answer that a person can prevent HIV infection by using new needles, syringes and other injecting equipment. Within 70%-80% of the respondents believe that a person can be protected from HIV infection if they switch from injection to non-injection way of using drugs. Based on the presented results it can be alleged that the respondents' knowledge about HIV infection and the ways of its transmission were significantly increased during the period of operation of the program.

# VII. Results from testing on HIV, viral hepatitis B, viral hepatitis C and syphilis of blood samples from participants in the research

In 2006, neither of the participants in the research was diagnosed HIV positive. In the following years 2007, 2008 and 2011 there is 1%, and in 2009 2% of the respondents had a HIV positive result. The highest is the percentage of positive test results for hepatitis C antibodies: from 14% in 2006 to 26% in 2011, followed by hepatitis B screening test: between 10% and 14% in different years. Positive for syphilis are 6% on average. The results show that the prevalence of the

aforementioned infections is much higher among prisoners than among the general population.

# VIII. Conclusions and recommendations

The results show that regularly ongoing HIV-prevention activities among the group of prisoners since 2006 is related primarily to increasing the knowledge and attitudes of people in prisons on HIV infection, thanks to regular health education lectures held in the Pol by the teams of CRAFCTA.

With regard to the other indicators – use of illegal drugs in prisons, illegal practice

of injection, tattooing, sexual behaviour and condom use no attitudes and behaviours for reducing the risk of infection are observed. This suggests that for the prevention of HIV and hepatitis B and C in prisons it is necessary that the following additional measures are taken:

- ➤ To create conditions for access of addicted persons to individual sterile injection kits.
- ➤ To ensure access of prisoners to safer tattooing – the options are different: from an external service provider to supplying the tattooists in prisons with the necessary supplies for safe tattooing.

REPORT OF INTEGRATED BIO-BIHEVIORAL SURVEILLANCE OF HIV AMONG PEOPLE WHO INJECT DRUGS, 2004-2012, BULGARIA. ABSTRACT

Tonka Varleva, Tsveta Raicheva, Emilia Naseva, Tsvetana Yakimova, Vyara Georgieva, Mariya Zamfirova, Hristo Taskov, Bogdan Petrunov

Program "Prevention and control of HIV/AIDS", Ministry of Health, Bulgaria

The program "Prevention and Control of HIV/AIDS" is financed by the Global Fund to Fight AIDS, Tuberculosis and Malaria and is aimed at providing adequate and quality health and social services for the most vulnerable groups at different levels of intervention, raising the expert knowledge and skills and the institutional commitment, as well as implementation of significant positive changes in the community rules, risky sexual behavior and behavior associated with drug use in the prevention and control of HIV/AIDS in Bulgaria.

The main purpose in the implementation of the Program "Prevention and Control of HIV/AIDS" is to contribute to reducing new cases of infection with HIV. The activities are aimed at:

 Expanding the availability, scope and quality of services for voluntary counseling and testing as a basis

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- for prevention, treatment and support with a special focus on groups at greatest risk;
- Reducing the specific vulnerability in the groups at greatest risk (injecting drug users – IDUs, Roma communities, prostitutes, young people at risk, people living with HIV, men who have sex with men – MSM), increasing coverage and ensuring access to comprehensive, high quality programs and services addressing the specific needs and priorities of these groups;

Under an agreement between the Global Fund to Fight AIDS, Tuberculosis and Malaria and the Ministry of Health, Bulgaria receives grant for the implementation of activities under the program "Prevention and Control of HIV/AIDS". With the launch of the program in 2004 several important new prerequisites were created for improving the supervision of the stage, type and dynamics of HIV infection.

These efforts made it possible to increase the detection of infected persons, especially through the services for voluntary counseling and testing.

The main task of Objective 4 "Reducing vulnerability to HIV among injecting drug users (IDUs) by increasing the scope of the group with a full package of preventive interventions" is to keep low HIV prevalence among IDUs by providing various preventive interventions: effective interventions in training and promoting the practice of safe injection and safe sexual behavior; activities of voluntary, free, accessible and confidential counseling, testing and referral of persons with high-risk behavior to treatment. An important part of the work is to provide activities for comprehensive support for IDUs that are carried out in drop-in

centers and through the service Case management, as well as providing treatment to people with high-risk behavior in treatment programs with methadone hydrochloride.

# Data on injecting drug users and their connection with HIV

In the recent years, according to the National Focal Point on Drugs and Drug Addiction certain dynamics in the main indicators of drug use in Bulgaria is observed, which corresponds to general trends in the European Union. Overall, cannabis (particularly marijuana) is the most widely used drug. Following synthetic drugs – amphetamines and substances such as ecstasy, while the use of cocaine is a trend of slight increase.

Heroin is the drug most associated with the problematic drug use in Bulgaria. Around 21,000 of problematic drug users apply the injection form of intake.

The pooled estimate of the number of problematic opioid users in Bulgaria is about 26,000. There is a reason to believe that the number of heroin users in the recent years has remained relatively constant and stable, with indications for starting the reduction.

The trend in Bulgaria to discover new HIV-infected persons whose infection occurred by injection drug use remains still valid. This fact is particularly noticeable after 2004, when thanks to the Program "Prevention and Control of HIV/AIDS" of the Ministry of Health, funded by the Global Fund, many NGOs were invited to work with this vulnerable group and low-threshold centres for anonymous and free counseling and testing for HIV/AIDS were started which increased the availabilities and the provision of necessary services for IDUs as well as the testing opportunities of

intravenous drug users for other blood transmitted diseases.

The risky sexual behavior is a substantial and independent factor for HIV transmission among IDUs in Bulgaria.

Drug users are one of the most vulnerable groups to HIV infection in the country and the world. They are often a marginalized and hard to reach group with a variety of health, social and legal problems.

# Methodology of IBBS

The study is cross sectional in its design. The sample has been made among the voluntary responding IDUs. Respondents were recruited at the service delivery points, served by teams of NGOs working with HIV vulnerable groups. To participate in the survey each respondent was granted a small material incentive. The study added for the collection of biological and behavioral data.

To participate in the survey, persons who meet the following criteria were selected:

- Persons, IDUs, aged over 18 years, regardless of their sex;
- Persons, willing to participate in the study.

The study involved injecting drugs users for the period 2004-2012 as follows: 2004 – 626 participants, 2005 – 1019 participants, 2006 – 1042 participants, 2007 – 1015 participants, 2008 – 1421 participant 2009 – 1374 participants, 2011 – 999 participants and in 2012 – 742 participants.

# **Demographic characteristics**

The largest part of the respondents fall within the age group of 21-35 years, i.e. young people. The share of the youngest respondents, up to 20 years of age, declined over the years, while those over 36 years of age increase, which is associat-

ed with the aging of the main part of the group of IDUs.

The predominant ethnic group among the respondents is Bulgarian, but in the last two years the share of Roma originated ones increases, which is a typical phenomenon for the entire group of IDUs in the country.

The majority of the surveyed IDUs have secondary or primary education or even lower. From the responses received it is evident that the marital/partner status of the respondents remains relatively unchanged over the years. The majority of respondents live alone – within 57%-70% in the various years, followed by the group of unmarried/singles, living with a partner.

#### Sexual behavior

IDU group is sexually active – between 80% and 90% of interviewees reported having had sex in the last year, and between 74% and 80% of them –in the last month as well.

The presence of a permanent partner is typical of the interviewed IDUs – over 60% reported that they had one for the past 12 months; between 50% and 65% report of having non-regular partners.

The share of people who reported that they have had sexual partners-clients is about 10% and remains constant over the years. The proportion of men who have sex with men (for the last 6 months) is relatively low.

# Injecting behavior

The division of the respondents in terms of the durability of injecting confirms the target population aging: on the one hand by age itself, and on the other hand by "length of experience in injecting drugs". In 2004, the main group of respondents were in the range of duration of injecting up to 5 years (47.1%). In 2012, the divi-

sion of the group of respondents looks as follows: 19.8% of the respondents are in the group of injecting up to 5 years and 78% inject drugs for a period of more than 5 years. The issue of drug use in the last month is part of the questionnaire over the 2005-2008 period. For respondents the drug of choice throughout all the years is heroin, followed by marijuana and amphetamines, but there is a slight increase in the proportion of use of amphetamines and a slight decrease in relation to using marijuana.

The substance of choice for injecting is heroin. There is a significant increase in the number of persons injecting methadone hydrochloride: from 5.3% in 2005 to 25.5% in 2009. These data show a trend of increasing misuse of prescribed medications in the recent years.

Important and interesting are the following responses which demonstrate an alarming trend of combined use of different groups of substances: for example heroinand amphetamine use in 2009 was 24.7% compared to 2005, when this proportion was 7.3%.

The usage of heroin and cocaine in 2009 increased to 21% compared to 2005, when this proportion was 5.4%, whereas an increase of intravenous amphetamine use from 15.3% in 2005 to 41% in 2009 has been observed. These findings warn about the necessity of introducing and approbating new interventions and services in the field of harm reduction from drug use.

Regarding the issue of substances used by the respondents in their lifetime, the answers are as follows: heroin and marijuana use reaches over 89% of respondents, followed by the use of sedatives and sleeping remedies (76% до 81%), amphetamines (71% до 75%) and methadone (66,80% до 70,40%).

In terms of the question concerning the number of people who the respondents took used needles and syringes from last month, no significant changes are shown during the period of the program.

In terms of the question concerning the number of people who the respondents gave their used needle/syringe to last month, over the years, there is a positive trend of reducing the proportion of respondents who have given their needle and syringe to more than five people.

When answering the question of using someone else's used needle/syringe while injecting the last time the percentage of persons who have not used someone else's needle/syringe in 2004 was 73.3, and in 2012 it was 75.7%.

To the question "How often have you injected with someone else's used needle/syringe last month?" the percentage of persons who did not inject themselves with someone else's used needle/syringe increased from 54.9% in 2004 to 65.1% in 2012.

An apparent improvement of the injecting behavior is demonstrated in the responses to the following question: "How often have you used a common cap, filter or water to make a drug for the period of last month?", as well as regarding the issue: "Use of a drug that someone previously

had divided and measured in the syringe used by someone else".

# Knowledge, behaviors and attitudes with regard to HIV/AIDS

In respect of matters relating to knowledge about the ways of transmission of HIV/ AIDS an increase in the knowledge of respondents can be definitely observed. Based on this it can be claimed that the respondents' knowledge about HIV infection and the ways of its spread have significantly been increased during the period of the program validity.

# Access and availability of interventions

The results received from the study showed a significant improvement in the availability of different services and an increase of their recognizability by the target group and also confidence increase towards the programs.

# **Biological results**

When the Program was launched in 2004, the percentage of participants with a positive HIV result was 0.5%, while in 2012 it was 10.6%. The proportion of positive results for hepatitis B is relatively constant over the years. Hepatitis C prevalence among the participants in the study is high.

REPORT OF INTEGRATED BIO-BIHEVIORAL SURVEILLANCE OF HIV AMONG 18-25 YEARS OLD MALES IN ROMA COMMUNITY, 2005-2012, BULGARIA. ABSTRACT

Tonka Varleva, Elena Kabakchieva, Emilia Naseva, Tsvetana Yakimova, Vyara Georgieva, Mariya Zamfirova, Hristo Taskov, Boqdan Petrunov

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The Roma community represents a significant part of the total population and is considered the most vulnerable to HIV infection among the ethnic minorities. This is the result of numerous overlaping factors associated with the traditional way of life in the community and socio-economic characteristics: rapid onset of de-socialization, social isolation, low general and health education, high unemployment rates - up to 90%, lower general and economic culture in the group, lack of social skills and motivation for socialization which facts determine the increasing rate of prostitution, drug use, crime, mobility. These problems can be added to the insufficient coverage of health services, especially the prevention program; condom use is still culturally unacceptable in many Roma communities; there are persistent taboos on sex; there is a double moral standard - strong control over the

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Tel. +359 2 946 14 82 E-mail: aidsprogram@hdp.bg sexual behavior of women and "complete freedom" with regard to men. The vulnerability to HIV / AIDS infections is further enhanced by culturally accepted anal sex as part of the repertoire of sexual practices. A great part of the Roma community is affected by the STIs, which represents an open door for HIV. The prevailing negative image created by the media defines a certain attitude of mistrust towards the messages addressed to the community. All these implies strategic planning of specific complementary activities and interventions directly targeted at Roma communities. Sustainable reduction of the vulnerability in Roma communities can only be successful by applying interdisciplinary policies for development and integration.

# Activity in Objective 5 and territorial coverage

The main aim: Limiting the spread of HIV and AIDS by reducing the vulnerability to HIV and STIs among the Roma community. The interventions include outreach and counseling for HIV and STIs, distribution of free condoms and culturally-tailored health educational materials, voluntary counseling and testing for HIV and STIs, free medical examinations for STIs, training of youth leaders in peer education, case management for those at highest risk, guidance and accompanying to other existing services, conducting AIDS campaigns within the community.

Major target groups of these interventions are the young Roma population aged 12-25 years, and specific groups at particularly high risk of spreading HIV - sex workers who have sex with men; intravenous drug users already infected with HIV and their sexual partners.

The activities under Component 5 of the Program have taken place with different

duration among the Roma communities in 13 regional cities of Burgas, Varna, Sliven, Sofia, Stara Zagora, Plovdiv and Pazardzhik (for 11 years), Haskovo, Kyustendil (9 years) Vidin and Yambol (5 years), Blagoevgrad and Montana (2 years), according to the reported current results and periodic analyzes of the situation. Since 2009, the scope of activities implemented by sub-contractors has expanded in order to provide services among the target group at regional level. Integrated biological and behavioural surveillance (IBBS) among men 15-25 years from the Roma community are held periodically in Burgas, Varna, Sofia, Plovdiv, as well as in some of the studies included Pazardzhik, Blagoevgrad and Stara Zagora.

The wide coverage of cities / areas in which the study was conducted within the period 2005 - 2012, i.e. 7 regions and the considerable number of tested persons - 5289, can allow us to make more generalized characterization with regards to the risks of HIV infection among the Roma community in the country.

# Limitations of the study

Documents of the European Commission shows that 10% of Bulgaria's population are Roma, while Roma are 23% of those entering the working age (16 years of age). In cities where the survey was conducted, there is the highest concentration of Roma population in the country. This presupposes a large number of target group and the relatively low percentage of the sample, which falls in the study. For this reason, in the different years, the scope of the survey respondents fall with various social and risk profiles, there is a high turnover and filling with new people because of the age limit of the study. Thus, the main three criteria that are constant in composition of the sample were: ethnicity, age and gender. In order to achieve more accurate and clear picture of the risk factors and sexual practices of the target group, the methodology used is adapted and enriched within the longitudinal study. This leads to a problematic situation for the comparison of the findings as change in the attitudes and in behavior throughout the whole period of time.

# Results and anlyses

• Demographic characteristics

The respondents covered by the survey are relatively evenly distributed into two age groups up to 20 years (average 47%) and between 20 and 25 years (average 46%). The biggest group of respondents who have primary education is nearly 45% of the sample. Approximately similar is the percentage of respondents with basic education - about 40%, not attending school constitute 16% of the total sample.

#### Sexual behavior

 Anal sex - Sexual contacts with women

Acording to data from the period 2005-2007 nearly 70% of respondents reported that they practiced an anal sex with women in the last six months. Half of them are doing this with more than one partner (between 2 and 5 women). The distribution of this data by age shows that the anal practice is of significantly greater proportion in case of young men up to 19 years of age (including), compared with the group of young men over 20 years. A likely explanation for this is the "preservation of virginity" of young girls, and the use of anal sex as a method to prevent unwanted pregnancy. With regard to the age of over

20 the anal sex is also a common practice, but the majority of men in this period already have a marriage partner. Hardly one in three reported that they used condom at the last anal intercourse with a woman, as most participants themselves offered to use it, and the most common reason for not using a condom is because they do not like condoms. In the period 2006 - 2008 there was an increase in the number of participants in the survey who give a positive answer to

this question with 50 %.

 Anal sex - Sexual contacts with men For the period 2005 -2008, more than 50% of respondents reported practicing anal sex with men, regardless of the different marital status of the respondents in the sample over the years. The number of partners ranges from "1-5" and between "11-90", as the first group dominates significantly. The practice of anal sex is widespread in the Roma community. The practice is more common in this community rather than among the general population and it is one of the factors, together with the low health education that put the Roma population at high risk for HIV / AIDS infection. For the period of the study the use of condom during sex with men increased from 34% to 62%.

### Sex with non-regular partners

The promiscuous sexual behavior is typical of the young men of all ethnicities. The results from the IBBS over the years show the following: three out of four respondents said they had had sex with non-regular partners in the last year. Every third of them had between 2 and 6 non-regular partner-men and half of them - between 2 and 5 female partners. One out of seven stated that he had used a condom every taim during sex with non-regular partners-man in the last

12 months. Over half of the participants in the survey did not discuss the issues of HIV and other STIs with none of their non-relular sexual partners. Less than half of the respondents reported they had used a condom during their last sexual intercourse with non-regular partners. The use of condom with occasional partner has increased from 40% to 65%.

 Sex against payment (prostitutes – respondent selling sex services)

A quarter of the respondents reported that they had had clients for sex in the last 12 months. There is a positive trend towards a progressive increase in the number of respondents who answered that they had used a condom with clients from 57% to 85%.

Sexul behaviour and religion

Within the period 2005-2009, a link is sought between the different characteristics that can describe cultural differences in behavior. A leading criterion in this regard is religion - Christian and Muslim. From the comparative analyzes made during the past four years in Roma communities in different regions of the country it can be stated that the cultural or behavioral characteristics regarding sexual behavior is not determined by their religious background. The graphs enclosed illustrate that conclusion by data.

## Injecting drug use

Within the period 2005-2008 there is data about respondents who reported this behavior. After this period IDU by the Roma are directed to participate in a study of another objective of the program. The data show that there is an extremely high percentage of respondents who use injecting drugs using shared needles and syringes (66% to 72%). This trend is relatively steady, indicating that sharing a needle and syringe is an established

norm among IDU community within the Roma community.

# **Biological results**

The highest number of HIV positive cases covered by the IBBS is in 2008 (when the IDU from the community were included in the sample) – they reached 52, which represents almost 6% of the sample. Regarding Hepatitis B, C and Syphilis the positive cases are at the highest level during the first year of the IBBC (2005). Hepatitis B results were 16.65% and Hepatitis C were 12.36% of the sample. The cases of syphilis were 44, which is 3.14% of the sample.

### **UNGASS** indicators

Indicator A: Percentage of the group at highest risk who have tested themselves for HIV in the last 12 months and are aware of the results

The data show that the majority of respondents who have been tested for HIV (90%) know the results of their testing, this percentage increases significantly in the last two years of the study.

Indicator B: Percentage of the group at highest risk who have been reached through programs for HIV prevention

 Is it possible in your community for someone to make a confidential test to find out if they are infected with HIV?

The levels of awareness of the confidential testing increased over the period of the study, in 2007, 2008 and 2011, three out of four stated that it was possible to make a confidential test in their community.

• What kind of social or health services have you received over the last 12 months?

Between half and two thirds of the participants in the study had contact with

outreach workers last year. The most frequently received services are: distribution of free condoms, getting health education materials - HEM, voluntary counseling and testing for HIV and other, and the share of person who claim that they had received these services is very high.

### Conclusion

- 1. The surveys made with regrd to the knowledge, attitudes and behavior clearly show the vulnerability of young men from the closed Roma communities to HIV and STIs (lower education, widespread practice of anal sex, sharing of needles and syringes in IDUs, paid sexual servises) and prove the need for continuous and systematic work to reduce vulnerability.
- A key conclusion from monitoring the effect of interventions is the need for enhanced professional involvement in service delivery and quality control.
- 3. Cultural norms and social behavior have similar characteristics in different communities, despite the existence of a different religion, language, ancestral history. The leading factor for successful intervention is the well-structured set of services and service quality and trust in service providers.
- 4. The nature of the work with the community sould be maintained (for example, over 50% of young men have primary education or have no education, 70% practiced anal sex, relatively high rates of participation in paid sexual services, low access to health services, increasing migration processes and other risk factors).

#### REPORT OF INTEGRATED BIO-BIHEVIORAL SURVEILLANCE OF HIV AMONG ROMA MALES...

- 5. At the same time a special focus must be placed to people with multiple risk practices MSM, sex workers, injecting drug users.
- 6. To maintain activities aimed at the social norm campaigns, trainings of youth leaders, outreach actibities.
- 7. Periodic analysis of the situation should be conducted in each area because of the great dynamics of
- risk factors for HIV: labor migration to countries with a high prevalence of infection, involvement in prostitution and others. Thus, small towns and villages may prove to be at significant risk of spreading of HIV infection.
- 8. To continue and extend networking with all local agencies and teams.

REPORT OF INTEGRATED BIO-BIHEVIORAL SURVEILLANCE OF HIV AMONG MALES AND FEMALES SEX WORKERS, 2004-2012, BULGARIA. ABSTRACT

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Since launching the Program for Preveng tion and Control of HIV/AIDS, financed by the Global Fund to Fight HIV, Tuber-culosis and Malaria in 2004, Integrated Biological and Behavioral Surveillance (IBBC) among persons of different risk groups has been carried out within the Program and outreach workers have been providing preventive and health services amongst the groups mentioned. IBBC includes testing for HIV, hepatitis B, hepatitis C and syphilis and completing of a questionnaire related to the health and social status of the respondents.

The main goal of Objective 6 under the program PCHA is to improve the general health and the social status of the target group through the following: reducing the risk of HIV infection and preventive measures to reduce infection with sexually transmitted infections and poly-drug use; increasing the number

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of the persons tested for the HIV virus; increasing the motivation and supporting clients for easier access to specialized hospitals and general medical services; covering a large proportion of women and men with risky sexual behavior (services are reachable to partners of sex workers); encouraging clients to take independent decisions; involving public institutions and stimulating the local authorities to support programs aimed at the target group and effectively reducing the adverse repercussions on society.

The activities under Objective 6 have been planned and carried out since 2004. In the beginning, teams were selected in ten cities of the country - Sofia, Varna, Burgas, Ruse, Plovdiv, Stara Zagora, Haskovo, Sandanski (region of Blagoevgrad), Pazardjik and Pleven. Nine non-governmental organizations have been working on the territory of 22 regions in Bulgaria for the period 2009-2015. Each team consists of a coordinator, outreach workers, medical professionals a doctor and a nurse, and a local expert. All sub-recipients have cars and mobile medical units, where clients of the Objective can be consulted and tested for HIV and STIs anonymously and free of charge.

When analyzing the results of the epidemiological control under Objective 6, it must be taken into consideration that data from Blagoevgrad, Pleven and Pazardjik have been collected through studies and interviews by teams of organizations working in the towns of Sandanski, Lovech and Plovdiv. The number of participants included in the study over the years is 3790. The study has been held among sex workers and carried out by specially trained outreach workers who made the trust contact with

the target group representatives. The study was conducted within the period 2004-2008 in all cities where services are provided under Objective 6, and within 2011-2012 it was carried out in the same places, but with updated questionnaires. All participants in the study were selected according to standardized criteria applicable to the whole research over the years.

# Information on the risk of spreading of HIV/AIDS in this target group:

The Republic of Bulgaria is located in a region with a HIV epidemic, the number of persons infected by blood and sexually transmitted infections is growing throughout the territory of the country. Women and men, offering sex services, are an especially risky group in terms of prevalence of HIV and STIs. The spread of HIV and STIs, the low social status, the discrimination against the vulnerable groups and the general marginalization at the everyday life level continue to be topical issues for the country. A part of the reasons for this situation among the target group is: hard to access preventive examinations and treatment: low culture of sanitation and education: low social status; practicing unsafe sex; drug use, including non-sterile needles and syringes, or use of somebody else's ones: low level of awareness about the risks of transmission of blood-transmitted infections and ways of prevention caused by the mobility of some of the clients; commitment to the criminal world.

# Brief characteristics of the target group:

The group of individuals that perform paid sexual services in Bulgaria amounts to 12 000-15 000 according to data from expert assessments and observations of

the outreach teams in the cities where activities are carried out under the Program. The ethnicity of the persons offering sexual services is: Bulgarian, Roma, Turkish, representatives originating from former Soviet republics, immigrants and refugees from Africa and the Middle East, others. Some of the clients use psycho-active substances by injection, nasally and by inhalation.

# Major sub-groups:

- Persons working outdoors those persons who provide sexual services on major roads, ring roads of major cities, close to the railway and the bus stations, in certain neighborhoods, streets, squares, etc. are included here;
- Persons working indoors those persons who offer paid sexual services in clubs, bars, apartments, massage studios, etc. are included here;
- VIP and escort persons these are highly paid and elite prostitutes and the access to them for the outreach workers is very limited;
- Persons working by advertisements

   persons who offer sexual services
   primarily through advertisements in newspapers and the internet;
- Students (university and school)

   the access to them is also very
   hindered inasmuch as they do not
   determine themselves as offering
   paid sexual services;

Each of the aforementioned subgroups of persons offering paid sexual services could be determined as a risky one, but in fact outreach workers mainly cover the first two subgroups — persons working

outdoors and indoors. They occupy about 60-70% of the whole group, i.e. a number of about 7000-9000. One third of them are of Roma origin, up to an average of about 5% use injecting drugs. Due to the high mobility driven by different factors (seasonality of the business/tourism, frequent police raids, job opportunities outside Bulgaria, the future law regulating prostitution being discussed, etc.) outreach workers reach approximately up to 60% of the actual size of the target group – i.e. from 4 200 to 5 400 clients.

A subgroup to the overall target group of the Objective are also the partners and clients of the persons offering sexual services as they are among the most vulnerable groups — with multiple sexual contacts. The number of the ones belonging to the "escort" (among them we can mention the guards, managers of clubs and bars, security and taxi drivers) is determined by the size of the group proposing sexual services — there is one person in charge of an average of 3-5 girls.

In the analysis below the developments in attitudes and behavior of the target group will be extensively reviewed and monitored, both in the cities, working under the Objective as well as in total over the years. This will show the overall picture in terms of knowledge, attitudes and morbidity. Discussed and compared are data only on a part of the questions that the target group was asked as well as those that measure the awareness and the attitudes, and monitored by UNGASS (the indicators are: Number and percentage of persons who used a condom with their last client; Distribution of participants according to whether they used a sterile needle and syringe the last time they injected themselves;

Percentage of the group at highest risk who both correctly identify the ways of preventing of HIV infection through sexual transmission and reject the major misconceptions about HIV transmission as well; Percentage of the group at highest risk who have tested themselves for HIV in the last 12 months and are aware of their results).

# Main findings and results based on the studies carried out within IBBC:

- Characteristic of the target group is the age limit of the majority who offer paid sex services to move between 20-30 years of age.
- Traditionally, women constitute the majority of the target group, the data concerning men are only for 2008.
   In the questionnaires up to 2009 men were entered in the category of the transgender, and from 2009 onwards questionnaires for men and women have been used, wherefrom information about the gender of the participants in the study has been taken.
- The majority of respondents declare themselves to be of Bulgarian origin, followed by the group of Roma ethnicity. The representatives of the Turkish ethnicity are a lower number, due to the concentration of representatives of this ethnic group in certain regions of the country as well as by reason of the cultural characteristics that prevent representatives of this ethnicity to identify themselves as such.
- A high percentage of respondents have given a positive answer to the question whether they used a condom during their last sexual contact with a client.

- In general, the percentage of persons injecting psychoactive substances among the group of those offering paid sex services, is low.
- For the period of carrying out of the studies between 70 and 90% of the interviewed representatives of the group correctly identify ways of preventing HIV infection through sexual transmission and simultaneously reject the major misconceptions about HIV transmission.
- As a result of the activities implemented by the outreach teams since the beginning of the Program in 2004, there has been a gradation in the indicator "Percentage of the group at highest risk who have tested themselves for HIV in the last 12 months and are aware of their results".
- Prevalence of HIV, syphilis, hepatitis
   B and C among the groups at high risk:
  - The data for syphilis indicate values around around 10%. The special characteristic of the testing for syphilis within IBBC is that the results are from antibody test and they show both an old and a new infection.
  - The results of hepatitis B and hepatitis C indicate infection among the target group show within 5-7%.
  - HIV prevalence among the group of persons offering paid sex services is within 1% of those surveyed in the period 2004-2012.
- The percentage of clients who received their results after HIV testing remains high over the years –

- about 97-98%, which is indicative of the confidence contact, the good knowledge of the outreach areas and the clients and the specifics of the work implemented by the outreach workers.
- There is also a high percentage regarding the issue of reaching high risk clients by HIV prevention programs, suggesting a high level of awareness on behalf of clients with regard to the services provided under the Program PCHA.

The intensive work of teams for outreach activities aims at comprising a maximum number of clients and maintaining confidence contact and is performed according to preliminary schedules in line with needs recognized. The clients' awareness of the risk of infection with sexually transmitted infections, the services they can benefit from, as well as the relatively low prevalence of STIs among the target group of sex workers, demonstrate the effectiveness of the Program PCHA in providing low-threshold services for people at greatest risk.

The termination of the implemented activities hides the risk of infecting a large number of clients. This is due to the fact that the group of the persons offering sexual services is not static. Thich is evident by the fact that the outreach teams continuously reach new clients in already developed outreach areas. The relationship of the group with various representatives of the general population poses a risk in terms of the prevalence of STIs among the general population. In this connection, it is necessary that providing low-threshold services is to be maintained even after closing the financing granted by the Global Fund to Fight HIV, Tuberculosis and Malaria.

REPORT OF INTEGRATED BIO-BIHEVIORAL SURVEILLANCE OF HIV AMONG MEN WHO HAVE SEX WITH MEN, 2006-2012, BULGARIA. ABSTRACT

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The program "Prevention and Control of HIV/AIDS" is financed by the Global Fund to Fight AIDS, Tuberculosis and Malaria and it aims at providing adequate and quality health and social services to the most vulnerable groups at different levels of intervention, improving experts' knowledge and skills and the institutional commitment and implementing significant positive changes in the community standards, the risky sexual behavior as well as behavior associated with drug use with regard to the prevention and control of HIV/AIDS in Bulgaria.

The main goal in implementing the program "Prevention and Control of HIV/AIDS" is to contribute to reducing the number of new cases of infection with HIV. The activities are aimed at:

 Expanding the access, scope and quality of services for voluntary counseling and testing as a basis for prevention, treatment and support with a special focus on groups being most at risk;

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Tel. +359 2 946 14 82 E-mail: aidsprogram@hdp.bg Reducing specific vulnerability in the groups at greatest risk (injecting drug users – IDUs, Roma communities, prostitutes, young people at risk, people living with HIV, men who have sex with men – MSM), increasing coverage and ensuring access to comprehensive, high quality programs and services addressing the specific needs and priorities of these groups;

The proportion of infected men and women compared to the total number of registered cases in the period 1986-2006 was 2:1, which suggests that a significant proportion of men might fall into the homo/bisexual transmissible category. In the recent years, the period within 2009-2014 this ratio increases to 4:1 in favor of men. In 2005, 30% of the men receiving ARV therapy are MSM. Data from the national register of HIV in 2006 show that 78% of people infected with HIV are men, suggesting of the possibility of a hidden epidemic in the MSM group.

Considering the high risk in the group of men who have sex with men, the program "Prevention and Control of HIV/AIDS" was extended in 2009 by another objective: Objective 9: "Reducing the vulnerability to HIV among men who have sex with men (MSM) by expanding the coverage of the group with a full package of preventive interventions.

Men who have sex with men are a group difficult to be reached, and data regarding it is insufficient. Based on various studies in Eastern Europe, the expert assessment of the size of the group in Bulgaria is 3% of the male population in the country (aged between 15 and 49 years), or approximately 60 000 persons.

The term men who have sex with men (MSM), is mainly used in studying the spread of HIV and refers to men who engage in sexual activity with other men, whether they identify themselves as gay, bisexual, heterosexual or do not use terms to describe their sexual orientation. MSM is

considered a category, defined according to the way of behavior, not the sexual identity. The purpose of the term is to address certain category of men who are at high risk of sexually transmitted infections, especially HIV/AIDS. So the term MSM includes the groups of men who identify themselves as homosexual (gay), but also those who are bisexual, transgender or heterosexual. It covers both men who are willing and voluntarily participate in sex with other men, and those who, due to certain circumstances, do so under pressure and/or coercion.

Objective 9 begins working initially in three areas (Sofia, Varna and Plovdiv) and later the towns of Blagoevgrad and Burgas were included.

Within 2009-2010, under Obj. 9 Operational research was conducted to determine a package of effective interventions for HIV prevention among MSM. It includes laboratory study, data collection on spot and the preparation of final analytical report with recommendations. According to some of the results of the operational research, the group of MSM is at least 4-5%, not 3%, and homosexuality is most widespread among the lowest and highest educational levels, which requires a specific focus on the policies namely among these educational and age groups. In terms of the size of the MSM community in the various cities there are significant differences between the winter and the summer seasons. The group migrates seasonally. The community itself is heterogeneous and also the unifying factor of homosexual orientation, and it seems that there is no other unifying factor that creates the sense of community. This is especially visible in the places different from Sofia. Within the community there are strong divisions and sub-groups based on socio-economic, ethnic and other grounds. Except for the negative attitudes and intolerant attitudes of most of the general public to the gay community, in the MSM group itself there are negative and discriminatory attitudes among its various strata.

Within the program "Prevention and Control of HIV/AIDS" data are published periodically on the internet site of UNAIDS on the progress of the country in terms of HIV prevention. Part of the reported indicators are calculated based on data from the IBBS implemented and the prevention activities themselves carried out by NGOs – sub-recipients under the program.

The share of MSMs that have received an HIV test in the last 12 months and know are aware of their result is 54.04% in 2012, which is more than one and a half times more compared with the output in 2006. The reported results from IBBS coverage of HIV preventive services among MSM have also significantly increased from 28.64% in 2006 to 78.79% in 2012. At the same time, the use of condom with last sexual partner in 2012 is relatively high – 66.16% of MSM involved in the survey have declared it

The existence and involvement of a network of NGOs for the implementation of information and prevention activities among MSM is an important prerequisite for the national response to HIV. The results show an increase in prevention services provided over the years.

## Methodology of IBBS

The study is cut, the cross section includes those who were willing to respond. Respondents were recruited at the places of service delivery by teams of NGOs working with HIV vulnerable groups. To participate in the survey, each respondent was granted a small financial stimulus. Biological and behavioral data were collected with the help of the study.

## **Results from IBBS**

The studies over the years cover different size groups – from the community of men who have sex with men: from 175 in 2006 in 670 (2011) to 198 (2012).

The largest part of the studies MSM are within the age group 20-29, but a decrease

of the youngest can be observed at the expense of an increase among the older.

The largest number of respondents during the years of the survey identify themselves as belonging to the Bulgarian ethnic group (between half of them and nearly all). The share of the respondents from the Roma ethnic group varies depending on the methodology for recruiting participants in the study and the involvement in this process of teams working on other objectives of the program, the dynamics in Sofia is weaker compared to the rest of the country.

Between one-third of the respondents (in 2008) to over half have completed secondary education, while approximately one fifth are graduates, which corresponds to the distribution of the educational status of the population in the country, as expected, respondents in Sofia are persons with higher educated, as compared with those in the rest of the country.

Two-thirds of the persons interviewed in 2006-2007 said they sometimes or always drink alcohol before sex.

Between 1/3 and ½ of respondents have tried marijuana, the use remained relatively stable over the years. An increase in the proportion of amphetamine users is observed in the different studies.

Injecting drug use varied over the years from 1% to 8%, and was highest in 2008 and this is mainly due to the drug using in the country as a whole, rather than in Sofia

### Regular partners

Almost half of the respondents interviewed in the period 2008-2009 said that in the last three months they had not had a regular sexual partner. The same, but for a period of 6 months, was reported by persons surveyed in 2011 and 2012

During the period 2008-2012 the reporting of condom use every time with a regular partner increased from 10% to over 37%, which applies to both for Sofia and for the rest of the country.

The sexual activity of the studied participants in the last three months with regular partners is within the norm.

### Non-regular partners

Two out of five respondents in 2008 said they had not had any non-regular sexual partner in the last three months. In 2009, their share dropped to 7.5%. In subsequent studies ½ to 1/5 of respondents reported that they had had no non-regular sexual partner in the last 6 months.

The study shows a relatively high sexual activity of men in terms of their non-regular partners. For 30 or more sexual contacts for a quarter of the year share between 1/7 and 1/3 of the participants in the study.

About half of the respondents in 2011 and 2012 reported on a monthly basis for between 6 to 30 sexual contacts.

From 2/3 in 2008 to 4/5 in 2012 from the studied persons reported of having had non-regular partners and said they had used a condom the last time they had anal sex with such a partner.

Between 1/3 and 1/2 of the respondents said they had used a condom every time during anal contacts with non-regular partners.

A problem remains the relatively high proportion of men in the study who used occasionally or do not use protective equipment during anal sex with their non-regular sexual partners.

# Sexual history: partners against payment

More than 2/3 to almost all of the respondents did not have sex with partners who pay them for sex in the last 3-6 months. At the same time a very small percentage of the respondents (under 2%) had partners who pay for sex.

And about two-thirds of the people who have sex with clients say they used a condom the last time they had anal sex with such a partner.

## Sexual history: sex with women

During the study in 2008 about a quarter of respondents say that they have done sex with a woman over the past year. In the coming years, given the more active work in the community and reaching more homosexual men, this share dropped to 1 in every 10 persons.

# Knowledge, behaviors and attitudes regarding HIV/AIDS

Generally, the knowledge about HIV among respondents was high and increased over the years, and a role in this regard is mainly of the efforts of NGO employees, sub-recipients under the program in the fieldwork.

Over three quarters of the persons surveyed in 2006 believe that it is possible to be tested for HIV confidentially. This percentage changes in a positive direction in the coming years, in 2012 almost all of the participants are of the same opinion.

Less than half of the respondents (2006 and 2007) have ever tested themselves for HIV in their lives. This share rises to almost all of the respondents in 2012.

The share of persons who received the test result for HIV remained relatively high over the years and covers almost all respondents, of which 60% were tested during the last year.

## Sexually transmitted infections (STIs)

Between 5% and 18% of those surveyed (between 2006 and 2008) men who have heard of STIs, said that they had had genital or anal discharge/inflammation during the last year. In the studies, carried out during the following years, from all participating men, between 2.5% and 5% said they had experienced symptoms of STIs – discharge/ulceration of the penis during the last month.

# Access and availability of interventions Between half and two thirds of the participants (in the studies in the period 2006-

2008) had contact with outreach workers last year.

The most common services received from clients who have had contact with outreach workers providing prevention services within the program during the last 12 months are the following: distribution of free condoms, getting visual aid materials (health education materials – HEM), voluntary counseling and testing (VCT) for HIV and other STIs, and the share of persons who declare that they have received their results is very high.

# Biological results

The proportion of participants with a positive HIV result ranges from 0 to 3.3 percent; with a positive result for hepatitis B is between 4 and 10%; diagnosed with hepatitis C are persons between 2 and 11%; the spread of syphilis is between 3 and 5%.

## **Conclusions**

The results show that in the group of men who have sex with men regular HIV prevention activities are carried out, especially in the period of implementation of Objective 9 (2009-2015 AD). The results achieved are also due to the efforts made under the program "Prevention and Control of HIV/AIDS", financially supported by the Global Fund to Fight AIDS. Tuberculosis and Malaria.

The funding from the Global Fund to Fight AIDS, Tuberculosis and Malaria ends at the end of 2015, then the country should plan its activities based on its own or other donor financial resources to maintain the HIV epidemic in this country at a low level. It is also necessary to implement programs and activities to reduce stigma and discrimination against MSM and HIV-infected persons since this would contribute both to the improvement of health services and to the demand for these services on behalf of the vulnerable groups.

### **CONFLICT OF INTEREST STATEMENT (AUTHORS)**

# CONFLICT OF INTEREST STATEMENT (AUTHORS)

I certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Author name	Date	Signature
When there is conflict of interest	t, specify the company title and the re	elationship with the Author.
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I certify that have no personal or me for review.	financial conflict of interest with au	thors of the manuscript provided
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# STATEMENT ABOUT PROTECTION OF HUMAN SUBJECTS AND ANIMALS IN RESEARCH

I certify that this study involving human subjects is in accordance with the Helsinky declaration of 1975 as revised in 2000 and that it has been approved by the relevant institutional Ethical Committee.

Author name	Date	Signature
I certify that this study involving a and use of laboratory animals.	animals followed the institution	nal and national guide for the care
Author name	Date	Signature