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BACKLOG PROCESSING OF TRACES OF SEXUAL CRIMES IN THE STATE OF PARANÁ

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Abstract: The Project: ``Backlog of Traces of Sexual Crimes`` was created by the Management Committee of the Integrated Network of Genetic Profile Banks, which directed investments to supply equipment and inputs to the States of the Federation that had presented targets for processing their related liabilities to sexual crimes. The Forensic Molecular Genetics Laboratory (LGMF) of the Scientific Police of the State of Paraná committed to processing two thousand traces of its liabilities, thus being awarded the automated platform: *ID STARlet Hamilton*. Traces of sexual crimes that occurred between 2010 and 2021 were processed, originating from approximately 900 victims. Differential lysis was performed manually, while purification and other steps took place on the platform: *ID STARlet Hamilton*. In a period of less than a year, after installing the equipment received by the National Public Security Secretariat, the LGMF completed the processing and analysis of the two thousand traces it initially proposed. Thus, 786 male genetic profiles were entered into the National Genetic Profiles Bank (BNPG), resulting in 350 coincidences observed, involving 196 victims. Furthermore, the intersection of these matches generated 79 Coincidence Reports, covering coincidences with: other states, convicts, identified suspects and cases with no suspects yet. The Backlog of Sexual Crime Traces Project allowed a significant increase in the Genetic Profiles Database of the Scientific Police of Paraná, which was ranked 3rd in the national ranking of absolute number of traces entered into the BNPG in 2021.

Keywords: Forensic Genetics; Rape; CODIS; RIBPG; Sexual Violence.

INTRODUCTION

Sexual violence can be defined as any sexual act or contact where the victim is used for the sexual gratification of their attacker without their consent, through the use of force, intimidation, coercion, blackmail, bribery, manipulation, threat or taking advantage of a situation. of vulnerability [1]. In 2020, 60,926 cases of sexual violence were recorded in Brazil, 86.9% of the victims being female [2]. Impunity has been considered responsible for the perpetuation of this crime, given that the conviction rate for sexual crimes is approximately 1% in Brazil [3,4].

The insertion and search of genetic profiles, originating from traces of sexual crimes, in genetic profile banks is a very useful tool for justice. This strategy makes it possible to identify perpetrators of unsolved crimes, confirm the identity of already identified criminals, exclude unjustly accused suspects, in addition to connecting different cases with the same perpetrator (serial crimes).

The Sexual Crime Trace Backlog Project was proposed and prepared between 2018 and 2019 by the Management Committee of the Integrated Genetic Profile Bank Network (RIBPG), which carried out surveys and proposed documents, procedures, acquisitions and training. The project aimed to process the backlog of more than 150,000 biological samples from sexual crimes awaiting analysis in the country's official forensic units [5]. The State of Paraná had a liability of six thousand samples of sexual crimes and committed to processing two thousand traces within a period of 1 year.

During 2020, the Forensic Molecular Genetics Laboratory of the Scientific Police of Paraná (LGMF) collected data relating to the traces to be examined. That year, the National Public Security Secretariat (SENASP) delivered equipment and inputs for the development of the Project, valued at more than 1.5 million reais. Paraná was awarded the large automation platform: *ID STARLet Hamilton*, essential equipment to process this quantity of traces within the period stipulated by the program. Until then, genetic tests were carried out at the LGMF using manual methods and semi-automated systems, with simultaneous processing of a reduced number of samples and incompatible with the project's demands.

In 2021, the LGMF carried out the complete processing of the two thousand traces it proposed, coming from around 900 victims of sexual crimes, crimes that occurred between the years 2010 and 2021. As a result, 786 male genetic profiles were inserted into the RIBPG, the which provided 350 observed coincidences, involving 196 victims.

MATERIAL AND METHODS

SAMPLE SELECTION

Two categories of samples were selected. The first was named "backlog" and included cases of sexual violence that occurred between 2010 and 2017, in which at least one trace sample presented as a preliminary result the presence of "several spermatozoa" determined after optical microscopy examination. The linking of the case to a Police Report or Police Inquiry was also considered as a criterion for inclusion, in order to allow future investigative actions by the police forces. The second category was named "routine" and in it cases of sexual violence from the LGMF case series were processed, with an official request to carry out a genetic examination, received during the year 2021, the project execution period, regardless of the preliminary result of semen research.

DNA EXTRACTION

A differential DNA extraction protocol was performed. For such issue, 400 µL of extraction buffer (10 mM Tris; 10 mM EDTA; 100 mM NaCl; 2% SDS) and 10µL of Proteinase K (20mg/mL) were added to 200µL sample (macerated swabs, clothes or other traces in PBS). The microtubes were incubated in thermoblocks at 56°C with shaking at 750 rpm for 30 minutes. After this first lysis, the samples were centrifuged for 5 minutes at 13,000 rpm. Then, 50µL of the supernatant (non-sperm fraction - FNE) was transferred to a new microtube, which was stored (2-8ºC) and the rest of the supernatant was discarded. The precipitate was subjected to a second lysis with 340 µL of extraction buffer e 10µL of Proteinase K, under the same conditions as the first lysis, for 15 minutes. After centrifugation for 5 minutes at 13,000 rpm, the supernatant was discarded and the precipitate was washed with 750µL of ultrapure water twice, resulting in the sperm fraction (FE), after removing the final supernatant [6]. 500µL of lysis buffer from the kit was added to the FNE and FE fractions.: PrepFiler™ Automated Forensic DNA Extraction (*Applied Biosystems®*) and, only in the FE fraction, 8.3µL of DTT (dithiothreitol) at 1M was also added. The samples were incubated in a thermoblock at 70°C with shaking at 750 rpm for 40 minutes [7]. After incubation, the samples were subjected to automated purification on the equipment: *ID STARlet Hamilton* according to the manufacturer's recommendations [8], with the final volume of extract 65µL. This automation platform allows the processing of up to 85 samples simultaneously.

QUANTIFICATION OF DNA (QPCR)

DNA quantification was performed using quantitative PCR on the equipment: *7500 real-time PCR System (Applied Biosystems®)*, using the kits: *Power Quant System (Promega®)* e Inve*stigator Quantiplex Pro (Qiagen®)* according to manufacturers' protocols [9,10]. The preparation of the reactions was carried out on the ID STARlet Hamilton equipment according to the manufacturer's recommendations [8].

DNA AMPLIFICATION (PCR)

DNA extracts from the FEs and FNEs fractions were amplified using the kits: *Global Filer™ PCR Amplification Kit (Applied Biosystems®), Yfiler™ Plus (Applied Biosystems®), PowerPlex Fusion® 6C (Promega®) and PowerPlex Y23® (Promega®)* in thermal cyclers: *Veriti®* (*Applied Biosystems®*), according to the manufacturers' instructions [11,12,13,14]. Amplification reactions were prepared in ID STARlet Hamilton automation.
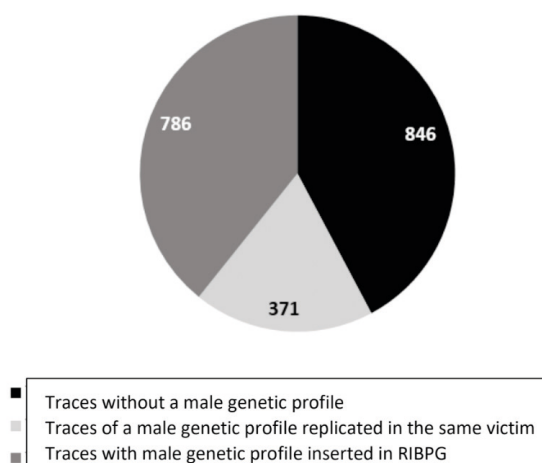
CAPILLARY ELECTROPHORESIS

The amplified samples were subjected to capillary electrophoresis in the genetic analyzer: *ABI 3500® (Applied Biosystems®)* with *ABI 3500 Series Data Collection Software 3 (Applied Biosystems®)*. Plate preparation was carried out on the equipment *QIAgility* (Quiagen®) using 1µL of the amplified PCR product, 9,6µL of formamide: *HiDi™ (Applied Biosystems®)* and 0,4µL of *Gene Scan™ 600 LIZ® dye Size Standard v2.0 (Applied Biosystems®)* in reactions with kits: *Globalfiler ™* e *Yfiler™* or 9,5µL of formamide *HiDi™ (Applied Biosystems®)* and 0,5 µL of *WEN Internal Lane Standard 500 (Promega®) in reactions with kits*: *PowerPlex Fusion® 6C* and PowerPlex Y23®).

ANALYSIS OF GENETIC PROFILES AND INCLUSION IN THE NATIONAL GENETIC PROFILES BANK (BNPG)

The genetic profiles obtained were analyzed in the *GeneMapper®ID-X Software v.1.4 (Applied Biosystems®)* and those that met the admissibility criteria set out in the RIBPG Operational Procedures Manual v.4 [15] were entered into the CODIS software of the Integrated Genetic Profile Bank Network.

RESULTS

In total, 2003 traces, coming from 913 victims of sexual crimes, were processed and analyzed in the Backlog Project for Traces of Sexual Crimes in the State of Paraná. Of the processed traces, 1157 showed viable male genetic profiles, resulting in 786 insertions in the BNPG (Fig.1).


Traces without a male genetic profile
Traces of a male genetic profile replicated in the same victim
Traces with male genetic profile inserted in RIBPG

Figure 1.: Relationship between the number of traces processed, obtaining viable male genetic profiles and inclusion in the BNPG.

The ratio between the number of traces processed and the number of viable male genetic profiles obtained is represented in Fig.2. It is possible to observe the difference in proportion between the results from the samples selected from the categories: "backlog" and "routine" (mentioned in title 2.1).

Figure 2: Proportion of male genetic profiles obtained from samples from the backlog (positive sperm) and routine (positive and negative sperm).

After inserting the 786 male genetic profiles into the BNPG, 350 coincidences were identified with the bank's data, resulting in 79 Genetic Profile Coincidence Report reports. These coincidences were classified into four categories defined for this study (Fig. 3).
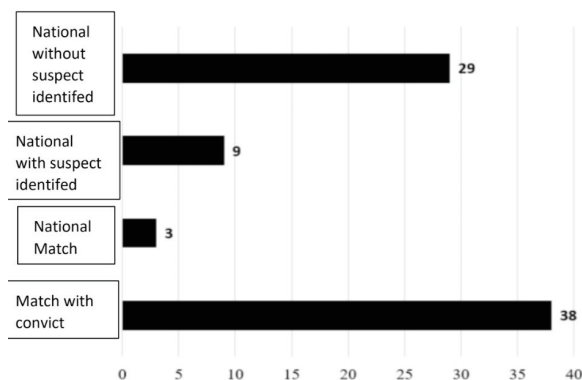

Figure 3. Number of coincidence reports subdivided into the categories defined for this study.

By crossing information in BNPG, it was possible to concatenate different cases of sexual violence related to one and the same perpetrator, characterizing serial crimes. In total, 39 serial attackers were detected, in 9 of the cases the perpetrator was identified, and

in 30 cases, the perpetrator was not identified. Fig. 4 shows the number of serial aggressors identified, for which 2, 7, 9 and 17 victims were listed. Fig. 5 shows the number of unidentified serial aggressors involved in crimes with 2, 3, 4, 5, 8 and 14 victims.
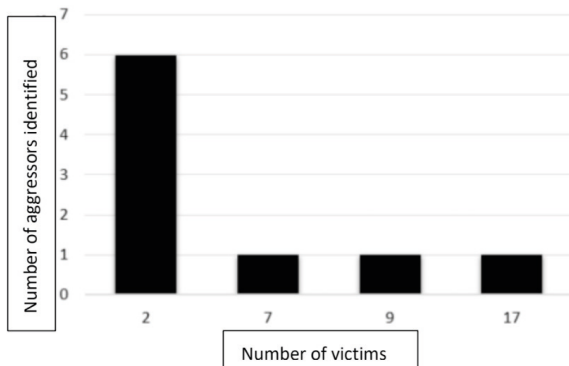

Figure 4: Number of serial offenders identified with 2, 7, 9 and 17 victims of sexual violence.
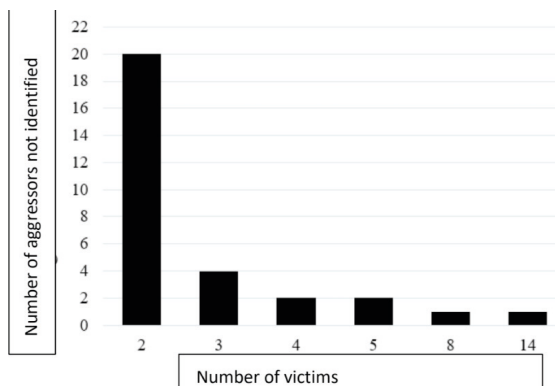
Figure 5: Number of unidentified serial offenders with 2, 3, 4, 5, 8 and 14 victims of sexual violence.

The male genetic profiles identified in traces of 786 victims of sexual violence were entered into the BNPG. Figure 6 shows the number of victims whose male profiles had some type of coincidence with data from the bank.
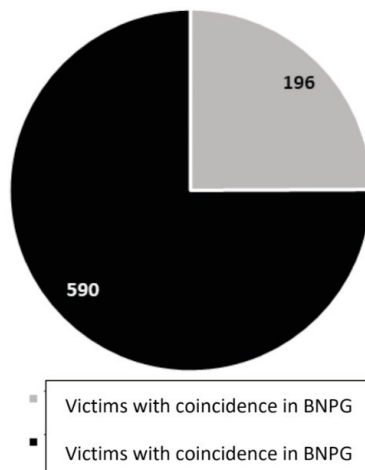
Figure 6: Relationship between the number of victims with and without coincidence in the BNPG, among those who had an aggressor profile inserted.

DISCUSSION

The National Bank of Genetic Profiles has already helped in the elucidation of more than 3,400 criminal investigations in Brazil and of these, 227 had the help of profiles mapped by the Scientific Police of Paraná. The Backlog Project on Traces of Sexual Crimes proposed and financed by SENASP allowed the State of Paraná to have a significant increase in the number of genetic profiles, originating from sexual crimes, included in the BNPG. As a result of processing the 2003 traces, 786 genetic profiles of traces were inserted into the network, an impressive increase of approximately 49% in the category of traces inserted. This number represents a significant contribution to the investigations of crimes that occurred between 2010 and 2021. In Fig. 1, it is possible to see that 371 viable profiles were not inserted because they were replicated in more than one trace from the same victim. In situations where more than one material is collected from the same victim (e.g. vaginal swab and panties), it is common for these traces to present the same male genetic profile.

Among the 2003 traces processed, only 26 came from male victims and the analysis of

these traces was conditioned on the existence of the male victim's reference profile. This way, it was possible to detect the presence of a male profile of the alleged aggressor in these samples.

The selection criteria used to list the samples in the "backlog" category allowed the obtaining of male genetic profiles in 62.5% of the processed traces. On the other hand, among samples from the "routine" category, which did not necessarily present a preliminary positive semen research result, the percentage of male genetic profiles obtained in relation to the number of traces processed was considerably lower (21.5%), as shown in Fig.2. Due to the high number of cases of sexual violence in the LGMF's liabilities, this project used the selection criteria for the backlog samples in order to apply the resources received in cases where there were indications of obtaining profiles that could be included in the BNPG. Therefore, those who had at least a trace of the presence of several spermatozoa were selected, so that after differential extraction, they had a real chance of presenting male genetic profiles. However, this is not the reality of the forensic laboratory routine. Only 20% of all traces of victims of sexual crimes processed make it possible to obtain profiles of the aggressors, as demonstrated in this study. This is due to several factors, including: the time elapsed between the attack and the collection of the trace; the victim's behavior after the attack (taking a shower, washing clothes, brushing teeth); azoospermia or oligospermia of the aggressor, among others.

Obtaining 350 coincidences in RIBPG, after inserting 786 genetic profiles, was a record for LGMF. Furthermore, the intersection of these matches resulted in 79 Coincidence Reports, which for study purposes we subdivided into national matches, matches with convicts, matches with identified suspects and matches without suspects (Fig. 3). Two of the three national matches occurred with suspects from the States of Rio Grande do Sul and São Paulo, while the third national match took place with a trace from the State of São Paulo. Obtaining 38 matches with convicts reflects the importance of inserting profiles of this category into the BNPG, work that has been developed by the State of Paraná since 2019, currently accounting for 4576 genetic profiles of convicts. Matches with identified suspects refer to coincidences with profiles from cases previously entered into the bank and which were already included with suspects selected during the police investigation. Finally, the 29 matches between traces for which it was not possible to identify, through RIBPG, any suspect, were classified as matches without a suspect.

Sexual aggressors tend to be repeat offenders, and it is not uncommon for different victims of the same criminal to be identified [16]. The RIBPG connected different cases with the same perpetrator, highlighting serial crimes. Some matches of crimes of the same authorship occurred with identified suspects (Fig. 4) while others were with cases without identified suspects (Fig. 5). In the State of Paraná there is an identified serial aggressor who has 17 victims to date and there is another aggressor with 14 victims who has not yet been identified. The analysis of these figures allows us to conclude that sexual offenders tend to be repeat offenders in committing this crime, highlighting the importance of continuously inserting trace profiles in the BNPG, to identify connections that are often not yet related in the police investigation. The Coincidence Report, sent to the competent authorities, listing the different victims of the same criminal, tends to help in elucidating these crimes. To date, through this project, BNPG has assisted in investigations involving 196 victims of sexual violence in the State of Paraná (Fig.6).

Although the number of coincidences was quite relevant, no correlation was found in the BNPG for 75% of the victims who had their respective aggressor's profile entered into the RIBPG's CODIS software (Fig.6). It is expected that police investigations will be able to identify new suspects for these crimes and the insertion of profiles of convicts will contribute to the identification of these attackers.

CONCLUSION

The Project: ``Backlog of Traces of Sexual Crimes`` in the state of Paraná was fully carried out in a period of less than one year from the date of installation of the large automation platform: *ID STARLet Hamilton*, allowing a significant increase in the Genetic Profiles Bank of the Scientific Police of Paraná. This increase can be evidenced by the 3rd place ranking of the State of Paraná in the national ranking of absolute number of traces included in the BNPG, serving as an important contribution to aiding police investigations.

THANKS

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