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Challenges in Implementing DVI Best Practice Guidelines Due to the Brumadinho Disaster

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Abstract

The broke of a tailings dam from Vale S.A. destroyed a gigantic area killing 270 persons in Brumadinho/Brazil. Organizing activities after a mass disaster is a complex process that requires the involvement of many people and resources in the laboratory. To DNA identification of the victims, a daunting work had to be made by DNA laboratory staff in a collaborative effort with many partners. Efforts to implement good practice guidelines in Disaster Victim Identification (DVI) have revealed several important aspects that need to change in the forensic DNA laboratory. This article highlights the challenges of implementing DVI best practice guidelines in resource-poor settings, but with professionals from different sectors engaged in the same goal of briefly identifying victims and helping families and society.

Keywords

Disaster, Victim, Identification, DNA, Brumadinho

1. Introduction

Mineral resource exploration activities generate waste that, in Brazil, is stored in dams. A succession of errors since the implementation of the dams and their inspection led to rupture and release of waste in the cities of Mariana and Brumadinho leading to a huge environmental and mass disaster [1]. The identification

of victims of mass disasters is often challenging due to the scale and nature of the disasters. A mass disaster is caused by an unexpected event, occurred by natural, or accidental and until intentional acts, resulting in a large number of victims that need to be identified [2]. They can be natural disasters, terrorist attacks, mass car accidents, plane crashes and explosions, among others [3] [4]. International organizations such as Interpol, the World Health Organization (WHO) and the International Committee of the Red Cross (ICRC) have developed and revised their guidelines for these situations [4] [5] [6]. Adaptations in the methodology of these guidelines were also necessary in mass disasters such as those that occurred in Brazilian towns, Mariana (2017) and Brumadinho (2019).

2. Methodology

This paper is the report of the experience of the Forensic Biology Laboratory Expert Group from Civil Police of Minas Gerais in Brazil and all data presented were obtained from its records. The forensic DNA laboratory is located in the city of Belo Horizonte, in Minas Gerais State. Forensic experts are responsible for carrying out all DNA analyzes of all biological samples found and collected at the crime scene in Minas Gerais State. The efficiency combined with the qualification of the group of experts produces reports that make it possible to contribute to the criminal investigation of various crimes, including paternity, missing persons, and sexual assaults. In recognition of the role forensic DNA laboratories play during the DVI response, the DNA Commission of the International Society for Forensic Genetics (ISFG) published several recommendations to provide guidance to DNA laboratories covering all aspects of DNA [6]. The discussion below focuses on the Disaster Victim Identification (DVI) process and the challenge our group faced in making changes and adaptations to the DNA analysis methodology.

3. Brumadinho Tailings Dam Failure

Brumadinho is located about 50 km from Belo Horizone, the capital of the state of Minas Gerais, Brazil. On January 25, 2019, during lunchtime, a dam belonging to the mining company Vale S/A, ruptured in the town of Brumadinho. Immediately the waste cascade hit a huge area including the mining company's structure, the administration offices, the staff canteen, maintenance workshops, loading terminal and railway lines; it also reached a village and a nearby inn. Over the course of the next few days, the mud destroyed houses, plantations, businesses, Rio Doce (a river near the dam) and killed hundreds of humans and animal. As result, a large number of people became homeless and state of Minas Gerais reached a record number of deaths by dam failure in Brazil, killing 270 people. Rescue teams are still looking for the missing victims.

Brumadinho dam failure is a reminder that, in November 2015, the worst environmental disaster in Brazil's history occurred when a dam owned by Samarco

(a joint venture between Vale S.A and BHP Billition) also failed in the state of Minas Gerais, where the toxic mud that reached the Rio Doce was taken to the Atlantic Ocean, leaving 19 fatal victims.

However, due to the damage it caused, the Brumadinho disaster is one of the biggest environmental disasters in history [7]. The mining waste destroyed a perimeter of 32 km, including the Paraopeba River, located 10 km from the dam. The ecosystem in the area was completely destroyed, lots of animals and plants were immersed in the mud. Until November 2023, 3 victims were still missing from a total number of 270 human fatalities from the dam break. A difficult job had to be done by the DNA laboratory staff from the Civil Police of Brazil located in Minas Gerais, in a collaborative effort with many partners (forensic anthropology teams from the Medical Examiner's Office André Roquette (IMLARBH) Civil Police of Federal District and the Federal Police of Brazil. Thus, the objective of this work is to present some adaptations in the routine of the DNA laboratory that had to be made to implement international guidelines for the identification of victims of mass disasters (DVI).

4. Laboratory Team Coordination

Each mass disaster has its own characteristics and involve different approaches in victim identification. Even though forensic geneticists are often not included as first responders, DNA sample collection and strategy for DNA based victim identification need to be part of the community's preparedness plan [6]. The victim identification process was carried out by the State Secretary for Justice and Public Security of the State of Minas Gerais, with support from the Civil Police of Minas Gerais, the Federal District and the Federal Police of Brazil.

In DVI, DNA profiling is considered to be one of the most reliable and efficient means to identify bodies or separated body parts. It requires a post mortem DNA sample, as well as an antemortem DNA sample of the presumed victim or their biological relative(s) for comparison. Due to the nature of this event, it is expected huge numbers of victims and body fragments. The forensic DNA laboratory team set aside its regular activities to dedicate mainly on the DVI process. The initial team was made up of experts from the DNA laboratory, later joined by experts from Minas Gerais with expertise in genetics, by laboratory technicians hired by the company Vale S.A, by some retired specialists from the DNA laboratory, in addition to experts the police force from other states and from the Federal Police.

Following Prinz [6] recommendations, all the new members, even those with previous experience in DNA analysis, received a proper training and were supervised to follow the standard operating procedures. To optimize the workflow, the staff was divided in groups, which would be responsible for each part of the process, from training and the coordination of sampling process to statistical analysis of DNA profiling data. Throughout all of this process, regular meetings were necessary to stablish new approaches.

5. Post Mortem Samples

Forensic anthropology teams from the Medical Examiner's Office André Roquette (IMLAR) conducted procedures related to the primary identification, including sampling for DNA analysis. Simultaneously, other steps had to be taken in the criminal investigation: such as confirmation of death; determination of the cause of death; and determination of aggravating or qualifying circumstances that played a part in the victim's death [8].

6. Identification of Victims

Araujo *et al.*, 2022, concluded that 95.9% of the victims were identified during the 1st year after the disaster; 78.9% were identified in the first 10 weeks. In addition, polytraumatic injury, accounted for 86.2% of deaths, was observed more frequently among mine employees than among community residents. Since it was the biggest humanitarian disaster and work accident in the country's history and posed challenges in the management and identification of multiple victims, all efforts were made to efficiently identify the victims and minimize the mourning of families and friends. In the first weeks, friction ridge analysis was mostly used for identification [3]. Fingerprints were subsequently collected using the Alethia System.

Due to the amount of mud, the rescue process was complicated. A careful search method was firstly implemented, to find living victims and not produce fragmentation of the bodies found. In some rare instances, well-preserved bodies were found, which allowed the collection of multiple samples from less affected areas. Different from recommendation [6], no criteria of sampling accounting for the size of fragmented remains were established. Nevertheless**,** fragments were individualized without the presumption of belonging to the same individual. The first approach for the post-mortem (PM) sampling was to use the previously established protocol, in which soft tissues (skeletal muscle, organ tissues and skin) or blood are preferable sources, and hard tissues (teeth and bones) are collected from bodies in putrefaction [9]. In addition to this approach, the collection of cartilaginous tissue was responsible for better DNA profiling. However, the mud flood was responsible for a gigantic degree of body fragmentation. Consequently, many soft tissue fragments were found in the field that made a triage approach for sampling impossible. Establishing and maintaining chain of custody was essential for the sample's reliability. The first documentation of the samples, with clear chain of custody details, was made by a field team that was accompanied by the Coroner's Office until the arrival of the samples at the DNA laboratory. Each collected fragment was classified and described in a specific form, receiving a standardized number that identified the sample in the whole process. These numbers were used to label the samples from Coroner's Office to the DNA laboratory and were described in the DNA reports returned to the Coroner's Office which then proceed with reconciliation methods. The Coroner's Office developed a database that was shared with the DNA laboratory, which

enabled a strict control of the flow of 469 Post-mortem (PM) samples from one laboratory to the other. To minimize the decomposition process, PM samples of different kinds of tissues were stored in −80˚C after arriving at the DNA laboratory.

7. Ante-Mortem (AM) Sampling

Another point in evidence is obtaining AM samples. This material collected by the AM teams that searched for the fingerprint files of individuals declared missing by their relatives.

For the identification carried out by DNA analysis, Ante-mortem (AM) samples were collected from the victims' relatives in the DNA laboratory by duly trained volunteers and health professionals from Minas Gerais State, with previous experience in sampling, following international standard procedures [10]. Training covered all aspects of the process, from the quality of the sampling methodology to the evaluation of kinship sampling, with the intention of drawing a family tree with the most appropriate relatives to avoid the necessity of reanalysis or even resampling as recommended by Prinz [6]. Every step of the sampling protocol was trained and simulated before calling the families for collection. A forensic geneticist was in attendance at the collection center for consultations during the course of AM sampling. Before sampling the families, the collection team had already provided elimination samples (voluntarily collected DNA samples from individuals not involved in the alleged crime), which were input in the CODIS (Combined DNA Index System database from FBI) elimination category [8]. *Whatman*^{TM} *EasiCollect,* devices from *GE Healthcare* which were previously assigned to collect samples of convicted offenders to input in CODIS database were redirected to the sampling of DVI AM samples. These collection kits had achieved two important criteria: long-term archival storage, and the use of robotics for processing, which increased the speed and reliability of the processing analysis [11]. Throughout the sampling process, copies of identification documents of each relative and collection forms were filled with all data needed to evaluate kinship and establish a complete chain of custody. The same procedure was performed for direct references provided by each family. Standardized collection forms and sample collection kits were used to improve the reliability, accuracy and efficiency of the collection process.

8. Samples to DNA

First part—Preparation and extraction

In this DVI event, human remains or its parts were found in different stages of decomposition, under the ground or immersed in the mud, and then Forensic Laboratory had to process different kinds of tissues according to theirs different stages of decomposition in order to identify the DVI victims. Degradation of soft tissues is particularly evident after a short period of time, which is a consequence of the rapid natural bacterial increase in decomposing bodies, especially those exposed to high temperatures in tropical countries such as Brazil [12]. There-

fore, bone samples are often the only, and usually the best, biological material available for DNA typing. However, DNA recovery from degraded specimens is a significant challenge due to the environmental, bacterial, and postmortem DNA damage. Nowadays there are many different protocols for the efficient isolation of highly purified DNA from bone cells. Therefore, our methods for preparing samples are based on instructions recommended by the FBI and the implementation of international guidelines for the identification of victims of disasters (DVI).

PM samples were submitted to a cleaning procedure before DNA extraction to avoid contamination, cross contamination, PCR inhibition and degradation. Soft tissues were cleaned with distilled water many times to remove the mud. Long bone fragments were sent by IMLAR, where all the bones had been cut into 0.5 cm long fragments, according to previously standardized procedures. DNA extraction from hard tissues offers results that are more satisfactory since it constitutes a natural deposit of nucleic acids protected from the environment.

After the first cleaning step, bones fragments were then scraped with a scalpel to remove the soft tissues and subjected to another cleaning step with 1% sodium hypochlorite, avoiding damage to the samples, but ensuring that the impregnated slurry was removed and could not interfere with DNA processing. Afterwards, bone fragments were washed with distilled water and immersed in EDTA 0.5 M (molecular biology grade) solution from 1 to 5 days. Then, these fragments were sliced with a scalpel to increase surface area of the bone with the reagents and enzymes coming from the extraction buffer. DNA extraction protocols from bones generally have two phases: in the first, the lysis phases, in which are used detergents and Proteinase K to break down the cells and solubilize the DNA, disrupting nucleus and cells membranes. In the second phase, the DNA purification, to eliminate remaining cellular debris and unwanted material like proteins, RNA and other macromolecules using enzymatic and/or chemical methods.

PM samples received a DNA purification step, using QIAquick® PCR purification kit (QIAGEN) to improve results. The most challenging samples were submitted to Phenol/Chloroform DNA extraction. Two different extractions from the same PM sample were performed to fulfill the duplication policy [9]. Direct references samples were submitted to DNA IQ™Casework for Maxwell® (Promega Corporation) previously established in the DNA laboratory. Following Montelius and Lindblom [10] recommendations, in quantification analysis using *Quantifiler™ Trio DNA Quantification kit* on a *QuantStudio™* 5 *Real-Time PCR System* (*Applied Biosystems*), to improve analytical results, the degree of degradation of PM samples was evaluated. Samples, which presented a high degree of degradation, were submitted to a lower dilution than the quantification suggested by the software DNA IQ™Casework for Maxwell® (Promega Corporation) to compensate for the lack of quality in the sample. Besides DNA concentration estimation, *Quantifiler™ Trio DNA Quantification kit* verified possible presence of inhibitors in DNA extract.

Well-performed DNA analysis is essential for the accurate identification of remains from mass disasters. Alternatives in the process of obtaining DNA can increase the success in obtaining genetic typing data from highly degraded samples. Laboratories involved in mass disaster analysis also need to process the daily load of other cases received for analysis. Therefore, additional analytical tools could be purchased to achieve the goal: fast identification of the samples with high accuracy methods. Robotics systems were implemented to reduce human error, minimize events of contamination, and increase traceability and sample processing speed [11]. FTA 1.2 mm diameter samples were obtained from relatives' samples using CPA200TM (Applied Biosystems) and PM samples DNA extraction using DNA IQ™ Casework for Maxwell® (Promega Corporation).

9. DNA Genotyping

According to Alonso [9], nuclear STR markers exhibit the most discriminative power to analyze family references. The following order to choose from which family members to collect samples was used: 1) mother or father of the victim, or even both, 2) biological mate of the missing person and their offspring, and 3) full biological siblings.

Powerplex® Fusion 6C System, of *Promega Corporation* is a set standardized in the DNA laboratory and presents a great number of independent loci, as indicated by Lee *et al.* [12], and from which some short amplicons may be obtained, increasing the success rate with degraded DNA as indicated by Alonso [9]. The amplicons were separated and detected on ABI 3500 of *Applied Biosystems* and allele designation performed on *Genemapper™ ID-X Forensic Data Analysis and Expert System software, Software Version* 1.6*,* of *Applied Biosystems.* Standard autosomal STR typing was the main technique applied. As supporting tools, Y chromosome was performed using PowerPlex® Y23 System (Promega Corporation), and Massive Parallel Sequencing was performed using ForenSeq (Verogen) analysis.

Genotyping analysis followed four possible approaches:

1) DNA profile achieved with high quality interpretable genotyping and can be input in CODIS for match research;

2) DNA profile achieved with low peak heights, peak imbalance at heterozygous loci, and allele dropout due to stochastic effects. DNA extract was submitted to a new PCR reaction with more DNA input;

3) DNA profile achieved with off-scale peak heights, stutter peaks, pull-up peaks, and incomplete adenylation (-A peaks) due to high input quantity of DNA. Amplicons were submitted to dilution and another capillary electrophoresis.

4) DNA profile achieved with features of low copy number and degradation were submitted to a new sampling and DNA extraction.

Only partial profiles were obtained from some samples; therefore, retesting was necessary in these cases. If another partial profile from the same sample emerges, both were then combined in a composite profile following DNA pro-

files interpretation criteria [6].

10. DNA Matching and Statistical Analysis

In order to verify matches between remains and kinship, or direct references, CODIS 7.0 and 8.0 softwares (Federal Bureau of Investigation, FBI) were used as a single database. DNA profiles of FTA cards from relatives were inserted in CODIS. For each family a pedigree tree was established. After a screening process of DNA profile quality from PM samples, they were uploaded from GeneMapper to CODIS to avoid transcription errors [10]. Firstly, each PM sample DNA profile was submitted to a search for matching to verify the presence of other samples with the same DNA profile in order to associate matching body parts. After this process, the DNA profile was submitted to a search looking for matches with pedigrees. Nevertheless, computer processing was very slow and we decided to start searching for a "shared allele" which provided faster matches. Therefore, the pedigree which the PM sample belonged to was established. The next step was a reverse search using the pedigree against PM samples to verify the match.

We used the software *Familias* version 3.2.5 to achieve statistical confirmation of the matches and to calculate likelihood ratio (LR) to indicate the strength of evidence, using a compiled database [8] [13] for allele frequency. The DNA laboratory team chose not to use the prior probability since part of this event could be categorized as an open event and then it was impossible to estimate the number of victims.

11. Results and Discussion

This work presented the modifications and adaptations, in the forensic DNA laboratory, with the objective of carrying out the identification of the victims of the disaster that occurred in Brumadinho/MG. A series of methodological and organizational possibilities have been presented so that other Forensic Laboratories, linked to the network of DNA laboratories maintained by SENASP (National Secretariat for Public Security) could inform themselves about the methodology and about the standard procedures already stipulated in the forensic literature in addition to the DVI procedures. It also highlights the importance of working in team, both within the laboratory and in cooperation and integration with other expert areas such as those developed by forensic anthropology as well as all those involved in the identification of victims based on DVI protocols.

The Brumadinho case brings some specificities that complicated the identification of victims by DNA profiling, such as the large number of victims, fragmentation of the bodies and DNA degradation caused by the toxic mud. Some of these conditions, like high levels of body fragmentation, sample disintegration, and a partially open DVI event, make Brumadinho's case analogous to the World Trade Center (WTC) tragedy. In these kinds of DVI, the goal of DNA identification is to determine the DNA profile of each remaining bodily part [14]. Two

factors that likely differentiate both DVI events are [15] the population density in the WTC area, culminating in thousands of victims, and [5], in some cases the mud was responsible for the conservation of samples due to its capacity of creating a completely toxic environment which can avoid the biological degradation by microorganisms. Working hard and successfully with DNA analysis since 1998, the DNA laboratory had never come across such high number of victims, at the same time, so the Vale S.A. dam rupture became a challenge unlike all the ones previously encountered. All adaptations in laboratory structure, the number of professionals involved, and the massive quantity of samples contributed to a most challenging scenario for the team. Moreover, the unpredictability of mass disasters always brings a stressful environment, involving families of victims, government agencies, and the press. The DNA laboratory team had to face a long-lasting renovation of the laboratory structure to settle new equipment and the temporary increase of personnel. Due to the international impact of this tragedy, at multiple times the law enforcement was pressured to share the work with private laboratories. However, following the ISFG recommendation [16], the work could not be shared, because the DNA laboratory is the only laboratory in Minas Gerais State with the capacity and continuous experience with these specified sample types. The DNA laboratory and Coroner's Office established a collaboration method which was fundamental to achieve the quickness and accuracy of the identification process. Even though both are not co-located as recommended by Hartman [5], the flow of samples and information between the DNA laboratory and the Coroner's Office followed a remarkable chain of custody, which allowed a fast and exact identification of the victims. ISFG recommends that, if possible, the DNA-based identification has to be anchored by anthropological data. Nonetheless, Interpol recognizes DNA as a primary identifier method, and considering the degree of fragmentation of the bodies, just DNA could be used as an identification method for some samples [16]. As the samples arrived, the DNA laboratory staff acknowledged the degree of fragmentation of the victims' bodies and concluded that each fragment should be analyzed, because each one could identify a different person and provide proper closure to a family. Before collection, besides the contact with the toxic mud, many fragments were submitted to humidity, high temperatures and UV-radiation, factors that can directly account for DNA degradation and provide new challenges for identification by DNA. Due to the number of samples arriving in the laboratory and the new approaches to maximize efficiency, the probability of errors increased a lot. To avoid the possibility of a mismatch caused by accidental mix-ups of samples, as part of quality assurance protocols, all PM samples were tested in duplicate at different moments. Some overlapping DNA profiles from duplicate or even triplicate analysis were combined to achieve a suitable profile to statistical analysis. Nevertheless, many samples were not amplified by STR markers even when submitted to a second purification phase after extraction. Three possible hypotheses were delineated for the unsuccessful results: 1) Pres-

ence of PCR inhibitors which were impossible to remove 2) high DNA degradation or 3) tissues of nonhuman remain which were not screened properly at the IML because of the degree of fragmentation. Quantifiler™ Trio DNA Quantification kit, used as DNA quantification method, showed that many samples had the presence of PCR inhibitors and high DNA degradation. Purification methods used to remove PCR inhibitors were inefficient in favor to find analyzable DNA profiles and even increasing DNA input in PCR to compensate for the lack of DNA quality, because of DNA degradation, was not enough to produce good DNA profiles. In association with laboratories from Universidade Federal de Minas Gerais, we could test those challenging samples that were not amplified by STR markers using DNA barcoding techniques to verify if some of them did not amplify because they were not human remains and verify which other species were victims of the Brumadinho dam failure.

At the moment, November 2023, 592 PM samples were conducted for DNA analysis and individuals were identified exclusively by DNA, and 7 others by DNA and other primary methods (Forensic Dentistry and Forensic Papilloscopy). Comparing the collaboration of DNA analysis as a tool for victim's identification with other disasters in Brazil [17], such as the mudslides in the mountainous region of the State of Rio de Janeiro in January 2011 (5.2% of individuals were identified by DNA) and a plane crash in the Brazilian Amazon rainforest in September 2006 (8.4% individuals were identified by DNA), the identification of victims of the Brumadinho dam collapse was higher (17.7% individuals were identified by DNA) due to the daunting degree of fragmentation of the bodies, making DNA the unique method capable of identifying these fragments. It can be considered a work in progress, because from 270 initial missing victims, 267 were identified by different primary methods, therefore we have yet to identify 3 missing individuals. Searching restarted after a break due to the COVID-19 pandemic, and there is still hope that other bodies or even fragments could be found to identify these missing victims. It is completely unpleasant to think about more dam breaks in Brazil. However, the Dam Safety Report shows that many of them are at great risk of collapse. Therefore, the development of a Dam Safety Control Program and maintenance of these dams is imperative, so that the Brazilian government would be obligated to design a national response plan for any future dam breaks.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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