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INTRODUCTION

In 2019, the SARS-CoV-2 virus emerged in China and spread around the world¹. Most patients develop pneumonia with symptoms such as fever, fatigue, anosmia, hyposmia and cough². Diagnosis is made by serological and molecular tests. Molecular tests are more effective in the early detection of infection, detecting viral RNA with PCR techniques³.

Next-Generation Sequencing (NGS) technologies have rapidly become the preferred method for many applications in virology. One of the advantages of this technology is the reconstruction of whole viral genomes directly from clinical samples, even for unknown or poorly characterized viruses⁴. In the case of SARS-CoV-2, NGS technologies are being developed in variant identification and control. These variants may be more effective in their spread, due to mutations that give the virus a selective advantage, making its characterization important⁵.

MATERIAL & METHODS

Sample collection, extraction and RT-PCR

Every day, samples of corpses are received by the medico-legal services of Lisbon region for SARS-CoV-2 diagnosis. As the n in study was low, n=40, we added a group of 36 living individuals, with respiratory symptoms, whose samples were collected in the same period. RNA extraction from nasopharyngeal samples was performed with the EZ1 DSP Virus Extraction Kit (QIAGEN). An RT-PCR assay was performed to detect the N and E genes as well as human RNase P gene.

NGS Sequencing

For sequencing, we used the MinION device from Oxford Nanopore Technologies (figure 1) with the NEBNext® ARTIC SARS-CoV-2 Companion Kit. The files obtained from the samples were imported in the INSaFLU platform. Results were sent to NEXTCLADE for variant analysis. In figure 2 we can see an example of the results obtained.



Figure 1 - MinION sequencer

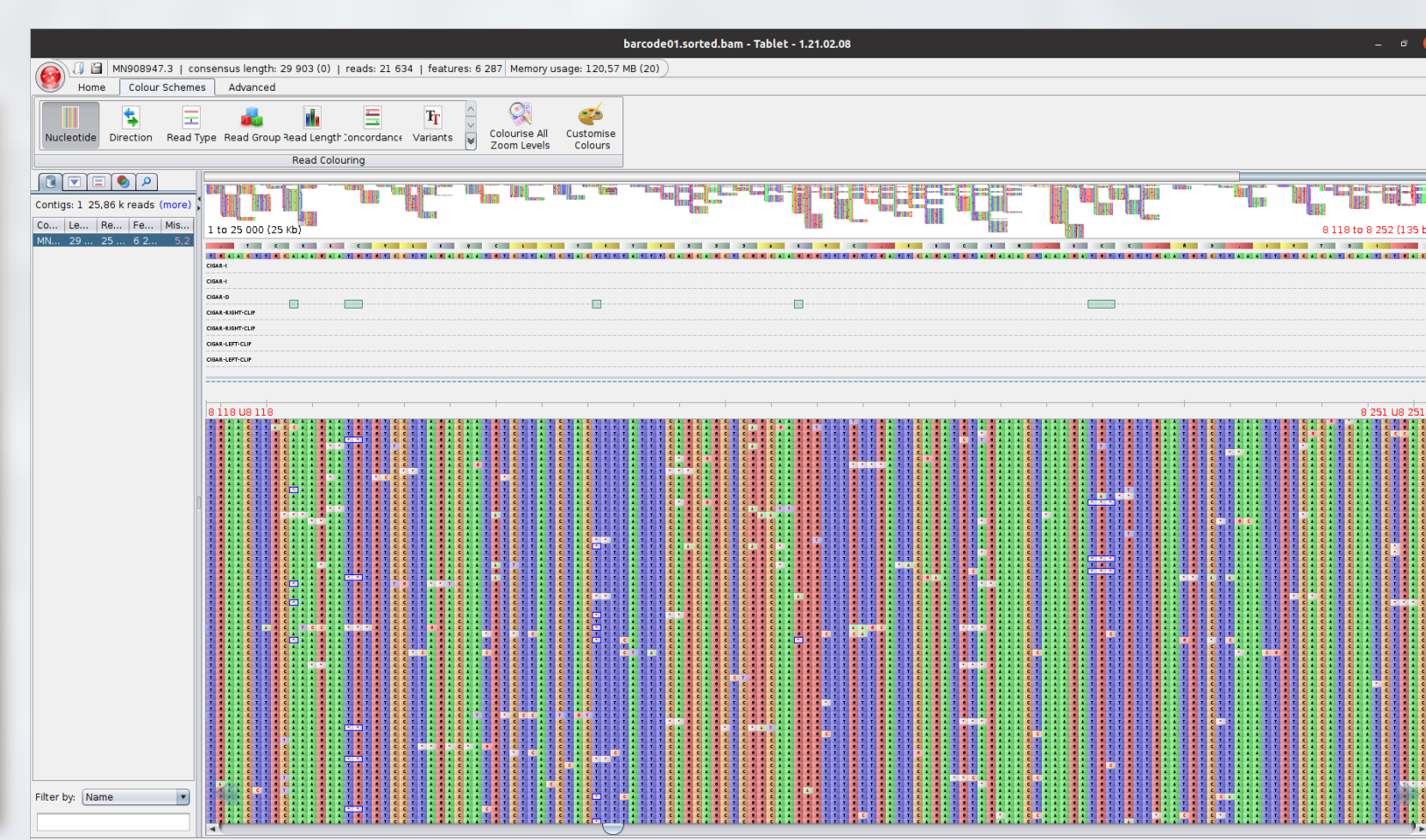


Figure 2 - Example of the results of one of the samples obtained with the MinION sequencer

After characterization of the variants present in our samples, a comparison of the results obtained by our NGS-MinION sequencing with the NGS-Sanger Based reference method (figure 3) performed in a reference laboratory was made. In figure 4 we can observe an example of results that can be obtained with this sequencing.



Figure 3 - SeqStudio Genetic Analyzer

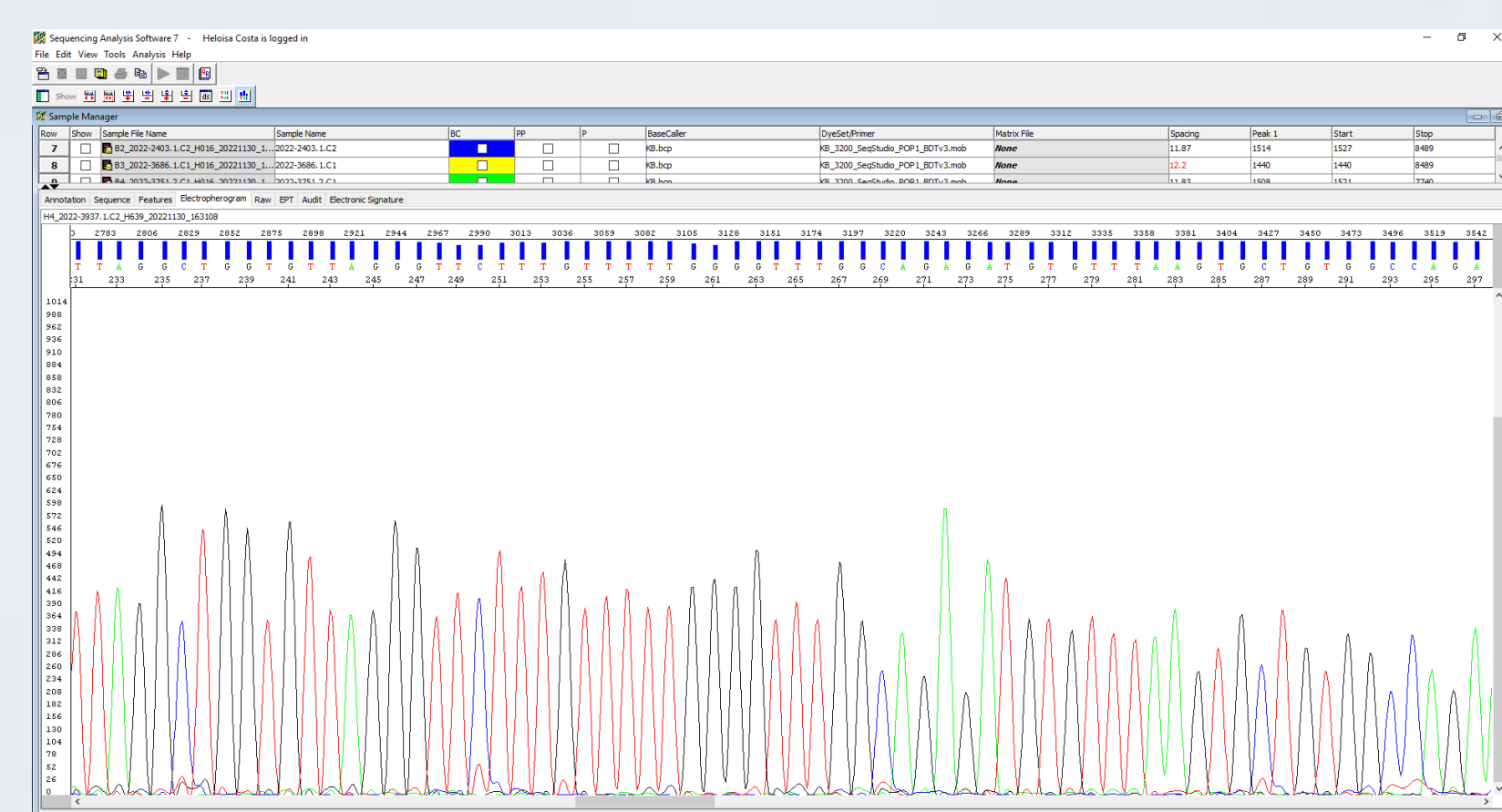


Figure 4 - Example of the results that can be obtained with the SeqStudio Genetic Analyzer

RESULTS

Four variants were detected in the group of cadaver individuals: 20A (1 sample), 20E (EU1) (16 samples), 20I (Alpha) (8 samples), and 21J (Delta) (15 samples), with their respective lineages. For the Delta variant it was also possible to discriminate sub lineages that descended from the main lineage B.1.617.2 (AY.5, AY.5.1, AY.5.1, AY.98.1, AY.124 and AY.125).

In the group of living individuals, the same 4 variants were detected, however, at different frequencies: 20A (1 sample), 20E (EU1) (10 samples), 20I (Alpha) (5 samples), and 21J (Delta) (20 samples), along with their respective lineages. For the Delta variant it was also possible to discriminate sub lineages (AY.4.7, AY.5, AY.22, AY.43, AY.122, AY.124 and AY.125).

The results obtained in each of the groups were separated by semester, in order to have a temporal perception of the evolution of the variants. In the group of cadaver individuals, represented in figure 5, we noticed that in the first semester of the year 2021 there was a prevalence of the variants 20E (EU1) and 20I (Alpha). However, in the second semester of the same year, it is possible to observe a reversal of these results, with the variant 21J (Delta) becoming dominant. The same pattern can be observed in the group of living individuals, represented in figure 6.

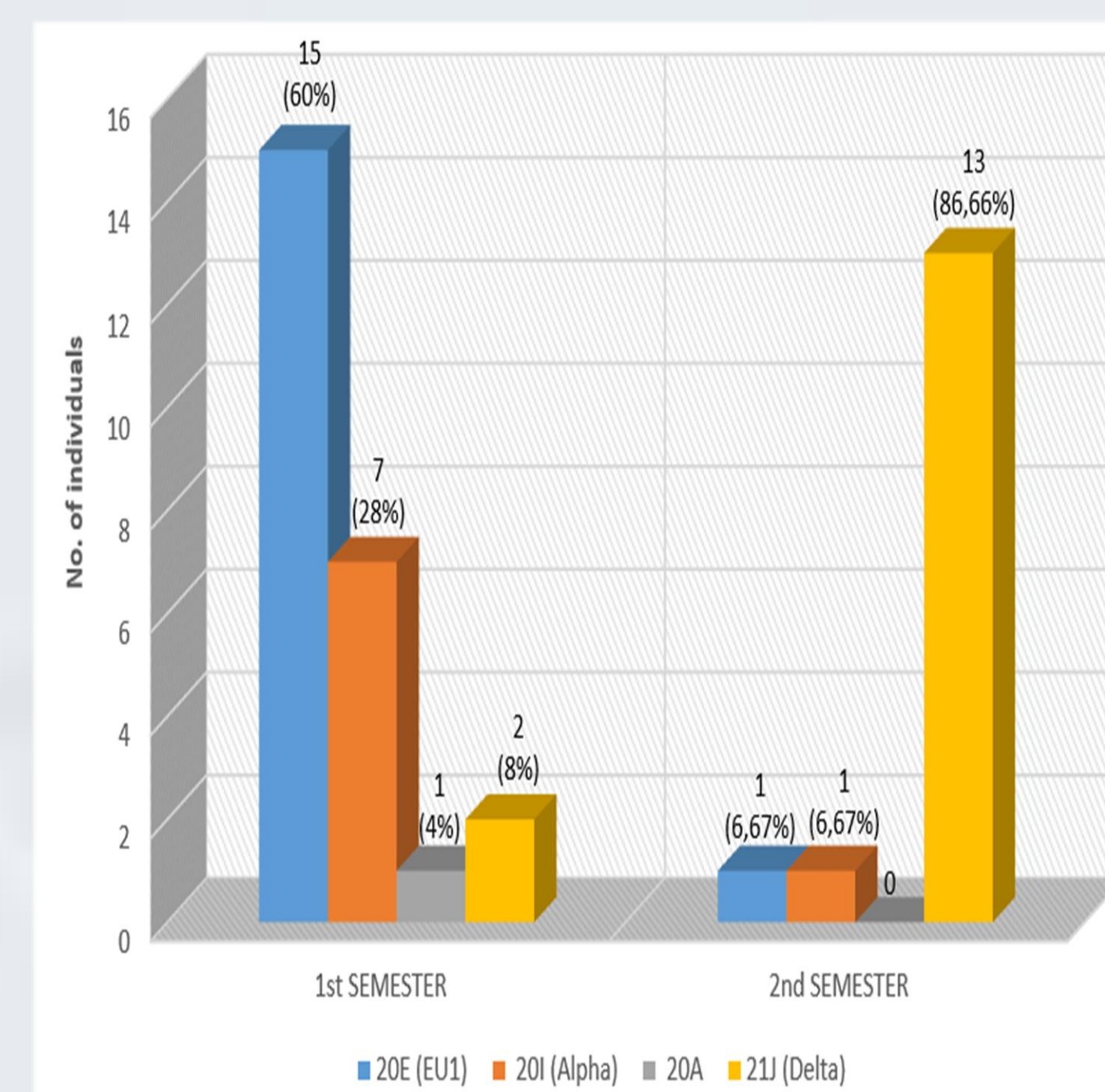


Figure 5 - Distribution per semester of the variants detected in the group of cadaver individuals (2021)

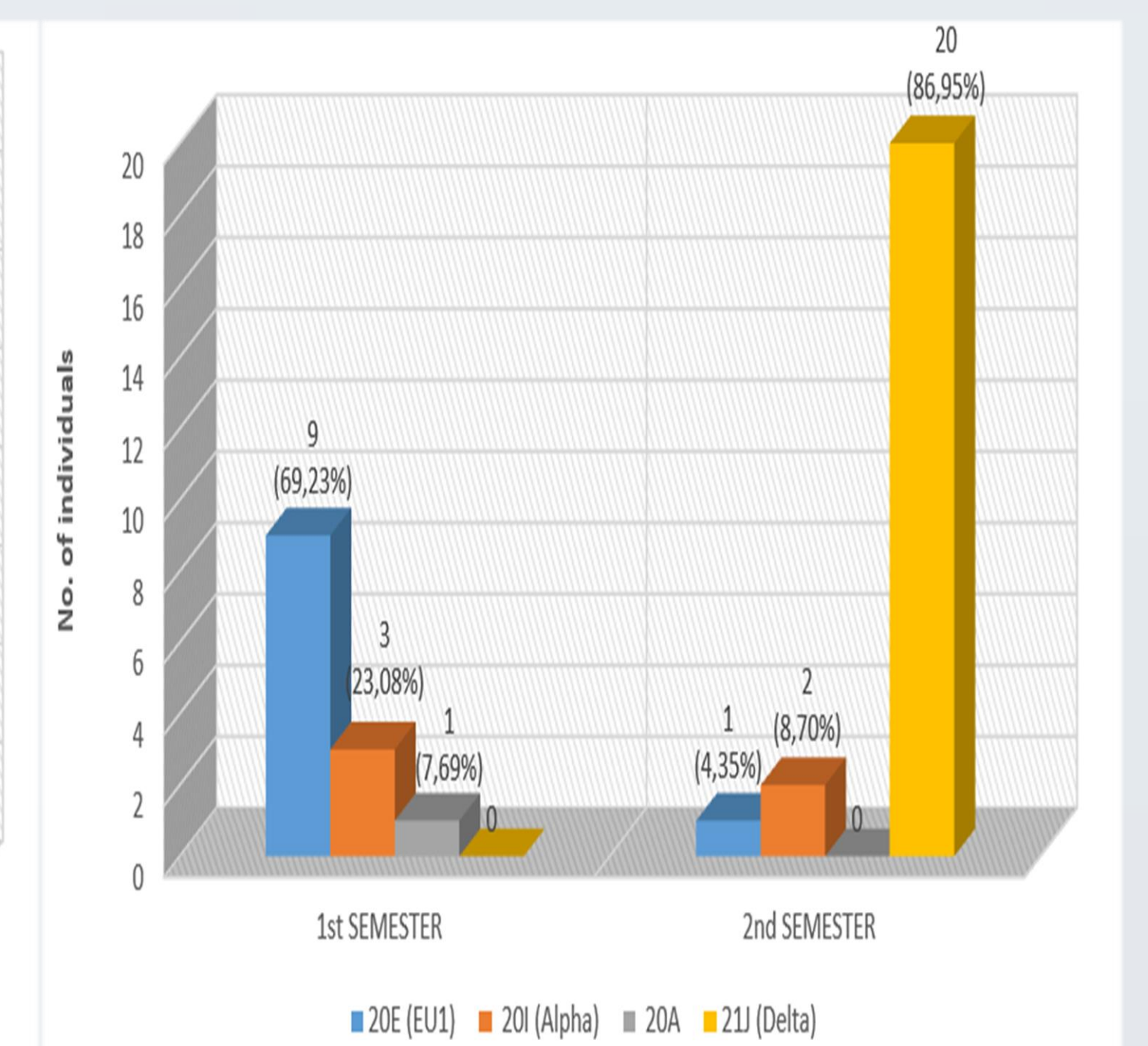


Figure 6 - Distribution per semester of the variants detected in the group of living individuals (2021)

DISCUSSION/CONCLUSION

Constant surveillance for new variants is of utmost importance, both in living individuals and in cadavers, and this study is a contribution to the epidemiological panorama in the Lisbon region. Studying corpses samples for the presence of SARS-CoV-2 virus leads to better screening for its presence in the population.

With this study, we were able to detect SARS-CoV-2 variants in both cadaver and living individuals. The variants were characterized according to their mutations to determine their respective lineages. In both groups, we were able to identify several SNVs (single nucleotide variants) characteristic of the various lineages and their amino acid substitutions, as well as some deletions.

Our results were in agreement with the results obtained by the NGS-Sanger Based reference method, and in the specific cases of the delta variant, NGS-MinION sequencing also allowed discrimination of sub lineages.

Analyzing the distribution of the variants of the samples, in the semesters of 2021, it was found that in the 1st semester there was a predominance of the variants 20E (EU1) and 20I (Alpha), being, in the 2nd semester, replaced by the variant 21J (Delta). Our data were according to the available scientific bibliography.

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