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2 MACAQUES

3 **Supplementary materials:**

4 **Supplementary Material 1. RNA extraction**

5 For RNA extraction, 250µL of blood or 250mg of the supernatant obtained from
6 manually homogenized tissue (achieved by homogenizing the tissue with a micro pastel) was
7 combined with 750µL of Trizol LS reagent (TRIzol™ Reagent, Invitrogen™). The mix was
8 vortexed for 1 minute and incubated at room temperature for 5 minutes. The mix was added
9 with 200 µL of chloroform, vortexed for 15 seconds, and incubated for 15 minutes before
10 centrifugation at 12,000 x g for 15 minutes. The aqueous phase was transferred to a 1.5 ml
11 microtube and added with 500 µL isopropanol. The solution was incubated at room
12 temperature for 10 minutes and centrifuged at 12,000 x g for 10 minutes. The supernatant was
13 discarded, and 1000 µL of 70% ethanol was added to the tube. The solution was then
14 centrifuged at 7,500 x g for 5 minutes. The supernatant was discarded and air-dried for 10
15 minutes. The RNA pellet was resuspended in 20µL of sterile distilled water and stored in the -
16 20°C freezer until use.

17

18 **Supplementary Material 2. β-actin qPCR**

19 Genomic β-actin was amplified from DNA samples under the following conditions:
20 the reaction mixture contained a final concentration of 10 µM of forward
21 (GTSTGGATYGGHGGHTCBATC) and reverse (GAYTCRTCRTAYTCCTSCTTG)
22 primers, and the HEX-labeled probe (ACCTTCCAGCAGATGTGGATC) (Piorkowski et al.
23 2014) prepared by mixing 20 µL of 10 µM forward primer, 20 µL of 10 µM reverse primer,
24 and 3.75 µL of 10 µM probe. The qPCR cycling protocol for β-actin consisted of an initial
25 denaturation at 95 °C for 5 min, and 45 cycles of 95 °C for 10 s, 50 °C for 20 s, and 72 °C for
26 20 s.

27

28 **Supplementary Material 3. Metabarcoding 16S rRNA gene analysis**

29 A metabarcoding 16S analysis was performed to explore the microbial composition
30 present in the samples. This advanced analysis can detect the presence of other bacteria
31 potentially involved in the epizootic, offering a more comprehensive view of the
32 microbiological situation (Loman and al, 2013). High-throughput sequencing of the 16S
33 rRNA gene (variable regions V3-V4) was performed by Novogene (Novogene 16S Amplicon
34 QIIME2) on extracted DNA from blood and lung tissue samples (N=4), using specific primers
35 (341F: CCTAYGGGRBGCASCAG, 806R: GGACTACNNGGGTATCTAAT). Briefly, PCR
36 reactions were carried out with 15 µL of Phusion® High-Fidelity PCR Master Mix (New
37 England Biolabs), 0.2 µM of forward and reverse primers, and 10 ng of template DNA.
38 Thermal cycling consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of
39 denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s, and
40 a final elongation at 72°C for 5 min. The PCR products of proper size were selected through
41 2% agarose gel electrophoresis.
42 The same amounts of PCR products from each sample were pooled, end-repaired, A-tailed,
43 and ligated with Illumina adapters using the TruSeq® DNA PCR-Free Sample Preparation
44 Kit (Illumina, USA), following the manufacturer's recommendations. The library quality was
45 checked with Qubit@ 2.0 Fluorometer (Thermo Scientific) and real-time PCR for
46 quantification, while a bioanalyzer (Agilent Bioanalyzer 2100) was used for size distribution
47 detection. Quantified libraries were pooled and sequenced on an Illumina nextseq 2000
48 platform to generate 250bp paired-end raw reads. Paired-end reads were assigned to samples
49 based on their unique barcode and truncated by cutting off the barcode and primer sequence.
50 Paired-end reads were merged using FLASH (V1.2. 1 1). To make the results of bioinformatic
51 analysis more accurate and reliable, splicing and filtering of raw data were done to obtain

52 clean data using Fastp (Version 0.23.1) software. Based on clean data, DADA2 of QIIME2
53 software (Version QIIME2-202202) was used for noise reduction and to obtain initial
54 Amplicon Sequence Variants (ASVs). Representative sequences of each ASV were annotated
55 to obtain the corresponding species information (QIIME2 software), and multiple sequence
56 alignment was performed to study phylogenetic relationships and the differences in dominant
57 species among samples. The top 10 taxa of each sample at each taxonomic rank (Phylum,
58 Class, Order, Family, Genus, Species) were selected to plot the distribution histogram of
59 relative abundance.

60 **References:**

61 Loman NJ, Constantinidou C, Christner M, Rohde H, Chan JZM, Quick J, Weir JC, Quince
62 C, Smith GP et al. 2013. A culture-independent sequence-based metagenomics
63 approach to the investigation of an outbreak of Shiga-toxigenic *Escherichia coli* O104:
64 H4. *Jama* 309 :1502-1510.

65 Piorkowski G, Baronti C, de Lamballerie X, de Fabritus L, Bichaud L, Pastorino BA, Bessaud
66 M. 2014. Development of generic Taqman PCR and RT-PCR assays for the detection
67 of DNA and mRNA of β-actin-encoding sequences in a wide range of animal species.
68 *J Virol Methods* 202:101-105.

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