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the veterinary
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Main topic : Feedback on the role of veterinary laboratories in the COVID-19 crisis

Development of a cytopathic effect-based micro-neutralization assay to evaluate the neutralizing activity of antibodies against SARS-CoV-2 in hyperimmune plasma donors

PACE L.¹, CIPOLLETTA D.¹, RONDINONE V.¹, MANZULLI V.¹, VITULANO L.¹, LOPOLITO A.¹, VETRITTO V.¹, CAVALIERE N.¹, GALANTE D.¹

¹ Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 is an enveloped, positive-sense single-stranded genomic RNA virus (+ssRNA), is the cause of the coronavirus disease 2019 (COVID-19). SARS-CoV-2, registered in the Wuhan City of China for the first time, is contagious in humans, and it has rapidly spread worldwide through close human interactions or the spilled respirational material of the infected people. The Director-General of WHO declared the COVID-19 outbreak as "a pandemic" on March 12th, 2020, as a result of the increased infection rate out of China (1).

The Experimental Zooprofilattico Institute of Puglia and Basilicata, a veterinary health institution of Italy, was selected by Italian Ministry of Health among the reference laboratories in the national project "TSUNAMI" (Transfusion of convalescent plasma for the treatment of severe pneumonia due to SARS-CoV-2), based on the study of the efficacy and therapeutic role of hyperimmune plasma in patients who developed COVID-19 disease. In this scenario, the use of serological diagnostic tests, capable of detecting any antibodies against SARS-CoV-2, assumes particular importance.

METHODS

Serum samples collected from 303 patients who acquired the Covid-19 infection between May 2020 and February 2021, were recruited as hyperimmune plasma donors for the Italian project TSUNAMI and used in the present study.

A cytopathic effect-based micro-neutralization assay was conducted in 96-well plates (2). Eight-fold dilutions (from 1:20 to 1:2560) of serum samples were tested in triplicate wells for the presence of antibodies that neutralized the infectivity of B.1 lineage of SARS-CoV-2 in Vero E6 cell monolayers. In addition, 100 TCID₅₀ of the virus in 25 µL/well were incubated with 25 µL of each dilution of serum in EMEM with 6% FBS for 1 h at 37 °C. After incubation, 2x10⁴ Vero E6 cells were added to each well. Neutralizing antibody titre was defined as the last serum dilution at which no cytopathic effect breakthrough was observed (3).

RESULTS

In all 303 serum samples analyzed, we observed a neutralizing antibody effect, ranging from a titer of 1:20 up to 1:2560 (Fig.1). Seventy-six percent of patients showed a 1:160 antibody titer, established as the threshold value for using hyperimmune plasma as a treatment for patients with COVID-19.

The remaining 24% of the analyzed sera showed an antibody titer between 1:20 and 1:80, deemed ineligible for donation by the project guidelines. The highest antibody titer was quantified as 1:2560 in 6.3% of donors (19/303).

CONCLUSIONS

The data obtained, demonstrate how the test developed by our laboratory is a valuable tool for assessing the presence of neutralizing antibodies in the sera of people cured of Covid-19. In addition, it has been shown that the antibody response toward SARS-CoV-2 infection can vary greatly among donors. Unfortunately, the TSUNAMI project did not show a benefit of plasma in terms of reducing the risk of respiratory deterioration or death in the first 30 days.

In conclusion, cooperation between human and veterinary medicine has proven effective in the fight against the Covid-19 pandemic, confirming the reliability and importance of the One Health approach.