04768 Detailed epitope mapping of SARS-CoV-2 nucleoprotein reveals specific immunoresponse in cats and dogs housed with COVID-19 patients

12. COVID-19

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Background

Since the initial emergence in December 2019, the Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been reported in over 200 countries, representing an unprecedented challenge related to disease control worldwide.
Several animal species have been found to be susceptible to SARS-CoV-2 after experimental and natural infections. In this context, cases of human to animal transmission have been reported, raising concern about the potential role of companion animals in the pandemic and stressing the need for reliable animal testing. SARS-CoV-2 nucleoprotein (Np) is highly used in immunoassays since it is overexpressed during infection and highly immunogenic in infected patients thus representing an ideal antigen to develop a COVID-19 antibody test.

Methods

A panel of pandemic (23 COVID19 human sera and 15 sera of animals housed with COVID19 patients) and pre-pandemic sera (3 human and 3 animal sera) collected between March and November 2020 were subjected to epitope mapping study. Rabbit and guinea pig immune sera produced against three Beta coronaviruses

(Bovine Beta-CoV, Porcine hemagglutinating encephalomyelitis virus and Human CoV OC43), 3 Feline Coronavirus positive sera, 270 pre-pandemic and 15 pandemic animal sera were used to validate a double antigen ELISA test.
For the epitope mapping study, four recombinant Np subunits were PCR amplified, cloned in pGEX-2T vector, purified and used as antigen in an indirect ELISA test. The Np C-terminal subunit was subsequently used to generate a double-antigen ELISA test for multi-species immunoglobulins detection.
Plaque reduction neutralization test (PRNT) was used to confirm a specific immunoresponse in animal sera.

Results

The detailed epitope mapping of SARS-CoV-2 Np allowed the identification of the most antigenic region in the C-terminus domain of the protein, which was used to develop a sensitive and specific double antigen-based ELISA. The panel of pre-pandemic sera and sera of animals immunized against related coronaviruses assessed the assay specificity at 99.5%. Positive sera belonging from animals housed with COVID-19 patients were confirmed with the newly developed double-antigen ELISA and PRNT test.

Conclusions

The serological assay, targeting a highly specific viral antigen, developed in the present study represents a valuable tool for multispecies monitoring of COVID-19 infection in susceptible animals.