Development of a plate reduction neutralization test and microneutralization assay for a SARS-CoV-2 seroepidemiologic survey in company animals in Mexico

Mario Solís-Hernández[1], * Dehesa-Freddy[1], Pastrana-Unzueta Roxana[1], Carillo-Guzmán Nadia[1], Francisco Liljehult-Fuentes[1], Villarreal-Silva Marcela[1], Pérez-De la Rosa Juan Diego[1], Humberto Ramirez-Mendoza[2], José Guillermo Estrada Franco[3], Ninnet Evelyn Gómez Romero[1], Roberto Navarro-López[1], Fabiola Carolina Espinosa-Gómez[4], and Elizabeth Bautista[4]

[1]Servicio Nacional de Sanidad Inocuidad y Calidad Agroalimentaria
[2]Universidad Nacional Autonoma de Mexico Facultad de Medicina Veterinaria y Zootecnia
[3]Instituto Politecnico Nacional
[4]Universidad Popular Autonoma del Estado de Puebla

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Abstract

SARS-CoV-2 is a virus that originated in Wuhan, China, in 2019 and has since spread worldwide, infecting people and animals. The objective of this study was to implement the plaque reduction neutralization test (PRNT90) and microneutralization (MN) to evaluate the presence of antibodies against SARS-CoV-2 in companion animals. First, a virus was isolated and sequenced, which showed a similarity of 99.9% with the original Wuhan strain. This virus had a titer of 3.55 DICC $_{50}$; for the PRNT90, it was adjusted to 30-40 plaque-forming units (PFU) and for the MN, at 100 DICC $_{50}$. For both assays, serial double dilutions of the sera were performed from 1:10 to 1:5120. Serum samples from people with negative and positive status were used as controls, as well as samples from companion animals (dogs and cats). In the case of human samples, the titers were from 1:10 to 1:5120 with PRNT and from 1:10 to 1:1280 with MN ($r^2 = 0.957$). Analysis of animal samples (dogs n = 521 and cats n = 29) revealed 10 positive samples, six from dogs and four from cats. Cats had higher titers than dogs. Titers ranged from 1:80 to 1:320 by MN and from 1:160 to 1:640 by PRNT90 for cats and from 1:10 to 1:40 by MN and from 1:10 to 1:80 PRNT90 for dogs ($r^2 = 0.621$). In both human and animal samples, the highest titers were observed with the PRNT90 test; however, the MN test is easier to perform than the PRNT90 test, so it is suggested as the test of choice for animal seroprevalence studies.

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