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Main topic : One Health

Development of one-step multiplex qPCR/RT-qPCR assays for simultaneous detection of SARS-CoV-2 and other viral and bacterial agents associated with canine and feline respiratory disease

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Introduction
Canine infectious respiratory disease complex (CIRDC) and feline upper respiratory tract disease (URTD) are the primary causes of respiratory disease in companion animals and are associated with a wide array of viruses and bacteria acting as either individual etiologic agents or in combination, making etiologic diagnosis challenging. Additionally, SARS-CoV-2 has been reported to infect both dogs and cats. Therefore, the rapid detection and differentiation of SARS-CoV-2 from other common viral and bacterial agents in a single specimen is critical.

Methods
Here, two panels of one-step TaqMan® multiplex qPCR/RT-qPCR were developed and validated for the identification of CIRDC and feline URTD-associated agents along with SARS-CoV-2. The canine respiratory panel included the detection of 8 viruses and 4 bacteria. The feline respiratory panel included the detection of 4 viruses and 3 bacteria. The analytical performance of each assay was evaluated using reference strains of each pathogen and plasmid DNA or *in vitro* transcribed RNA containing the target sequences. The clinical performance was evaluated using 76 and 63 nasal/nasopharyngeal swabs obtained from CIRDC-suspected dogs and URTD-suspected cats, respectively.

Results
All the multiplex assays demonstrated high specificity, analytical sensitivity, efficiency, and linearity. Among the clinical samples derived from dogs, *Mycoplasma canis*, *M. cynos* and canine respiratory coronavirus were the most prevalent pathogens. The emerging canine pneumovirus was detected in four samples. Among the clinical samples from cats, *M. felis* was the most prevalent pathogen, followed by feline herpesvirus type-1, *Chlamydia felis* and feline calicivirus. SARS-CoV-2 was detected in four canine and two feline samples. A high prevalence of co-infection was detected, with a rate of 29% and 59% in the canine and feline respiratory samples, respectively.

Conclusions
These two newly panels of one-step TaqMan® multiplex qPCR/RT-qPCR are useful and reliable for the rapid detection and identification of canine and feline respiratory pathogens, along with SARS-CoV-2. The high prevalence of co-infection highlights the need for the simultaneous detection of multiple pathogens using such panels in order to implement proper treatment and/or prevention plans.