

Short communication

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SEROPREVALENCE OF PORCINE RESPIRATORY CORONAVIRUS AND TRANSMISSIBLE GASTROENTERITIS VIRUS INFECTIONS ON COMMERCIAL PIG FARMS IN CENTRAL SERBIA

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Abstract

Porcine respiratory coronavirus is an enzootic, viral, respiratory disease of pigs, which manifests with mild clinical signs, but it takes part in the etiopathogenesis of the porcine respiratory disease complex. The virus was first discovered in Belgium in 1984 as a deletion mutant of the transmissible gastroenteritis virus. The two viruses are strongly antigenically related which is why they cross-react in serological tests. In this study, we tested 276 serum samples from different categories of pigs using ELISA test, which allows differentiation between the porcine respiratory coronavirus infection and transmissible gastroenteritis. The seroconversion for coronavirus infection was determined in 80.4% of tested samples. Out of 222 positive samples, 219 samples (98.6%) were positive for porcine respiratory coronavirus antibodies, while 3 (1.01%) samples were positive for transmissible gastroenteritis virus antibodies. Depending on the production category, 97.7% of piglets, 83% of sows, and 35% of gilts tested positive for porcine respiratory coronavirus antibodies. In total, 2.3% of piglets tested positive for transmissible gastroenteritis virus antibodies. Taking into account the characteristics of the ELISA test, its sensitivity and specificity, this result can be considered a false positive, because of a cross-reaction between the porcine respiratory coronavirus antibodies and the transmissible gastroenteritis virus. Specific antibodies in other swine production categories against the transmissible gastroenteritis virus were not determined.

Key words: Porcine respiratory coronavirus, transmissible gastroenteritis virus, seroprevalence, Serbia

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SEROPREVALENCIJA INFEKCIJE SVINJA RESPIRATORNIM KORONA VIRUSOM I VIRUSOM TRANSMISIVNOG GASTROENTERITISA NA KOMERCIJALNIM FARMAMA SVINJA U CENTRALNOJ SRBIJI

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Kratak sadržaj

Respiratorni korona virus svinja je uzročnik enzootskog respiratornog oboljenja svinja koje se manifestuje blagim kliničkim simptomima, ali učestvuje i u etiopatogenezi kompleksa respiratornog oboljenja svinja. Prvi put je otkriven u Belgiji 1984. kao delecioni-mutant virusa transmisivnog gastroenteritisa. Virusi su antigenski vrlo slični, usled čega unakrsno reaguju u serološkim reakcijama. U ovoj studiji, testirana su 276 uzorka krvnih seruma poreklom od svinja različitih proizvodnih kategorija komercijalnim ELISA testom koji omogućava razlikovanje infekcije respiratornim korona virusom od infekcije virusom transmisivnog gastroenteritisa. Kod 80,4% uzoraka, utvrđena je serokonverzija na korona virusnu infekciju. Od 222 pozitivna uzorka, 219 uzoraka (98,6%) je bilo pozitivno na specifična antitela protiv respiratornog korona virusa, dok su 3 (1,01%) uzorka bila pozitivna na antitela protiv virusa TGE. U zavisnosti od proizvodne kategorije, 97,7% prasadi, 83% krmača i 35% nazimica je bilo pozitivno na antitela protiv respiratornog korona virusa svinja. Kod 2,3% prasadi utvrđena su antitela protiv virusa transmisivnog gastroenteritisa. Uzimajući u obzir karakteristike ELISA testa, osetljivost i specifičnost, ovaj rezultat se može smatrati lažno pozitivnim, najverovatnije usled unakrsne reakcije antitela protiv respiratornog korona virusa i virusa transmisivnog gastroenteritisa. Pored toga, specifična antitela protiv virusa transmisivnog gastroenteritisa kod drugih kategorija svinja nisu ustanovljena.

Ključne reči: respiratorni korona virus svinja, virus transmisivnog gastroenteritisa svinja, seroprevalencija, Srbija

INTRODUCTION

Porcine respiratory coronavirus (PRCV) causes an enzootic, mild, respiratory disease in pigs. The first report of the virus was recorded after a prevalence study for transmissible gastroenteritis virus (TGEV) which revealed that over 60% of tested pigs had neutralising antibodies against TGEV without being vaccinated or developing the symptoms (Pensaert et al., 1986). It was reported that the virus that caused the infection was a mutant of TGEV, with a deletion (170-190 kDa) in the gene for spike (S) protein (Lin et al., 2015). Both PRCV and TGEV belong to the *Coronaviridae* family, and the *Alphacoronavirus* genus (International Committee on the Taxonomy of Viruses, ICTV.). Soon after the discovery of PRCV, it spread throughout the world suppressing TGEV in many regions (Whittaker, 2017). The TGEV causes gastrointestinal disease with a high affinity for the intestinal tract, and a high mortality rate among piglets. The deletion in the S gene resulted in a change in the spike protein of PRCV, thus inhibiting binding to the sialic acid and entering enterocytes (Turlewicz-Podbielska and Pomorska-Mól, 2021). The PRCV replicates in the lungs and causes a subclinical to mild infection of the respiratory tract, but induces a strong immunological response to the infection raising the levels of interferons and interleukins (Turlewicz-Podbielska and Pomorska-Mól, 2021; Keep et al., 2022). Since TGEV and PRCV are closely antigenically related, the antibodies against PRCV cross-react with the TGEV antigen thus protecting pigs against TGEV infection (Whittaker, 2017). Since its initial spread, PRCV has been reported as the dominant strain, with only a few sporadic outbreaks of TGE in Europe (Lőrincz et al., 2014) China (Zhenhui et al., 2015) and the USA (Chen et al., 2019). Coronaviruses have a high recombination rate (Whittaker, 2017), and there is a potential for a new mutant TGEV to arise, which could potentially break through the partial immunity acquired after PRCV infection. Recently there have been reports across Europe and Asia of novel swine enteric alphacoronavirus (SeACoV) (Pan et al., 2017) which represents a chimeric strain of TGEV genomic backbone and porcine epidemic diarrhoea virus (PDEV) spike protein (Akimkin et al., 2016; Boniotti et al., 2016).

There have been reports of difficulties in differentiating TGEV antibodies from PRCV antibodies, caused by the similarity in the antibody response to the viruses (Valkó et al., 2019). To circumvent this, various ELISA tests were created that would allow a quick assessment of serological status of the herd.

This study aimed to determine the seroprevalence of PRCV and TGEV infection in domestic swine on commercial farms in Central Serbia by testing different production categories of pigs for the presence of antibodies against PRCV and TGEV.

MATERIAL AND METHODS

Serum samples used in this study originated from Central Serbia, from commercial units, and were kept in the serum bank at the Serbian Institute of Veterinary Science. The total number of samples tested was 276. The samples were tested by blocking commercial ELISA test (INgezim Corona Diferencial 2.0, Ingenasa, Madrid, Spain), according to the manufacturer's instructions, and the optical density (OD) was measured at 450 nm with an ELISA reader (Multiscan, LabSystems). Based on the recommendations of the cut-off values by the manufacturer, the samples were considered positive or negative to PRCV or TGEV. The diagnostic sensitivity and specificity, according to the manufacturer, are 94% and 98.2% respectively (INgezim Corona Diferencial 2.0, 2022).

RESULTS AND DISCUSSION

Out of 276 tested samples, 222 (80.4%) samples were positive for coronavirus antibodies. For PRCV antibodies, 219 samples tested positive, which makes up 79.3% of the total samples and 98.6% of all positive samples. For TGEV antibodies, 3 samples were positive, which makes up 1.09% of the overall sample or 1.4% of all positive samples. Depending on the production category, 97.7% of piglets, 83% of sows, and 35% of gilts tested positive for porcine respiratory coronavirus antibodies (Table 1.).

Table 1. Table showing the number and percentage of positive and negative samples according to the production category.

Production category of pigs	Number of tested samples	Number of positive samples (%) for PRCV antibodies	Number of positive samples (%) for TGEV antibodies
Sow	88	73 (83%)	0
Piglet	128	125 (97.7%)	3 (2.3%)
Gilt	60	21 (35%)	0

Porcine respiratory coronavirus causes a subclinical disease in pigs, which in some cases results in fever, sneezing or mild coughing, and combined with other pathogens such as porcine respiratory and reproductive syndrome (PRRS) and swine influenza virus (SIV) can be a part of a post-weaning porcine

respiratory disease complex (PRDC) that causes a significant economic loss in swine industry (Brockmeier et al., 2002). However, strains 135 and 137 of PRCV are capable of producing similar pulmonary lesions to that of the swine influenza virus (Keep et al., 2022). Furthermore, a co-infection of PRCV and PRRS induces a disease with severe respiratory signs (Jung et al., 2009). Ever since the emergence of PRCV, the incidence of TGEV has decreased significantly through partial cross-protection by anti-PRCV antibodies. Nonetheless, there still might be a low TGEV circulation without a clinical manifestation usually explained by a high enough titre of antibodies against PRCV (Kim et al., 2000). Namely, only continuous reinfection allows for a high enough titre of antibodies for adequate protection against TGEV (Kim et al., 2000). Low seroprevalence of PRCV infection was recorded in wild boars in the region: 0.7% in Croatia (Roic et al., 2012), and 3% in Slovenia (Vengust et al., 2006). However, PRCV infection was not detected in wild boars in Serbia (Milicevic et al., 2016). On the contrary, a high seroprevalence of PRCV infection had been recorded in domestic swine in Slovenia at 65% (Vengust et al., 2006), while, in a study by Lőrincz et al. (2014) over 70% of tested gilts and 100% of tested sows seroconverted against PRCV in Hungary. These findings are in accordance with the results in this study where the overall seropositivity to PRCV infection was 79.3%, suggesting that there isn't a circulation of PRCV between the population of domestic swine and wild boars in Central Serbia. The reports regarding seroprevalence of TGE in neighbouring countries have been similar. Seroprevalence of TGE was 0.4% in Croatia (Roic et al., 2012; Brnić et al., 2020), and in wild boars in Slovenia TGE was not detected (Vengust et al., 2006). The results were similar in Serbia where TGEV was not detected either (Milicevic et al., 2010). In domestic swine, there was a single outbreak of TGE in the previous decade in Hungary (Lőrincz et al., 2014). In Serbia, there were reports of porcine epidemic diarrhoea virus (Prodanov-Radulović et al., 2017), but there were no reported outbreaks of TGE in domestic swine. In this study, 2.3% of piglets tested positive for TGE antibodies, which is similar to a study by Valkó et al., (2019) where a single serum positive for TGE antibodies was detected. The high-level serological cross-reactivity represents a hindrance in diagnosis since it allows for a false-positive result, especially at the individual pig level, which lowers the accuracy of blocking ELISA tests (Magtoto et al., 2019). In this study, coronavirus antibodies were detected in all tested production categories, with the highest seroprevalence in piglets, where all of the piglets tested positive for coronavirus antibodies, and 97.7% for antibodies against PRCV. Taking into account the characteristics of the ELISA test, its sensitivity and specificity, it can be concluded that 3 piglets that tested positive

for TGE antibodies were false-positive result, probably because of the cross-reaction between the PRCV antibodies and the TGEV. Following this, none of the tested sows or gilts had antibodies against TGEV, which would be expected if there were positive piglets. On the contrary, a high percentage of PRCV seropositive piglets is in accordance with the high percentage of seropositive sows, probably caused by their continuous reinfection. The other 65% of gilts were negative for antibodies against TGEV and PRCV coronavirus-antibodies, which could be connected to the waning of antibodies under the detection level of the ELISA kit. This could represent a risk since there is a report from 2014 by Lőrincz et al. (2014), which describes a re-emergence event of TGEV in piglets from primiparous gilts which had low levels of PRCV antibodies.

CONCLUSION

The seroprevalence of PRCV infection in domestic swine on commercial farms in Central Serbia is high (79.3%). Antibodies against PRCV were detected in all tested production categories of pigs, with the highest seroprevalence being in piglets (97.7%). The 2.3% of piglets tested positive for TGEV antibodies. Antibodies against TGEV were not found in either sows or gilts. Considering the characteristics of the ELISA test, coupled with a high degree of cross-reactivity between PRCV antibodies and TGEV and the lack of antibodies against TGEV in other tested production categories, this result can be considered a false positive.

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Author's Contribution:

DG and VLJ designed the study. JMZ carried out the serological tests, MN and BM contributed to the interpretation of the results and BK and VM DLJ supervised the findings of this work

Competing interest

The authors declare that they have no competing interests.

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