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Short Communication

Serological survey suggests circulation of coronavirus on wild Suina from Argentina, 2014–2017

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Abstract: Swine coronaviruses affecting pigs have been studied sporadically in wildlife. In Argentina, epidemiological surveillance of TGEV/PRCV is conducted only in domestic pigs. The aim was to assess the prevalence of TGEV/PRCV in wild Suina. Antibodies against these diseases in wild boar and captive collared peccary were surveyed by ELISA. Antibodies against TGEV were found in three collared peccaries (n = 87). No TGEV/PRCV antibodies were detected in wild boar (n = 160). Preventive measures should be conducted in contact nodes where the transmission of agents may increase. Epidemiological surveillance in wildlife populations and in captive animals before their reintroduction should be attempted.

Keywords: Collared peccaries, Epidemiological surveillance, Porcine respiratory coronavirus, Porcine transmissible gastroenteritis virus, Serology, Wild boar

Porcine transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV) are swine coronaviruses of the genus *Alphacoronavirus* (González et al. 2003; Saif et al. 2019). TGEV causes a highly contagious enteric disease in swine and it is considered notifiable by the World Organization for Animal Health (OIE 2019). PRCV is a natural deletion mutant of TGEV with an altered tissue tropism towards the respiratory system (Kim et al. 2000). This condition allows PRCV infections to cause cross-protection against TGEV, a phenomenon that has altered its global epidemiology (Kim et al. 2000; Vlasova et al. 2020).

The risk of TGEV and PRCV spread increases in areas of high swine density, as they are transmitted via faecal–oral route or aerosols, respectively (Saif et al. 2019). Serological tests are the most widely used for coronavirus diagnosis in animals, but cell culture and immunofluorescence can also be used (Lin et al. 2015; Miyazaki et al. 2010; Valkó et al. 2019). Little information is available regarding coronavirus infections in European wild boars and feral

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pigs (Moutelíková et al. 2016; Saliki et al. 1998; Vengust et al. 2006; Woods et al. 1990).

In South America, there are large populations of two species of wild Suina (Order Artiodactyla): the wild boar (*Sus scrofa*) is an invasive and rapidly expanding species in Argentina and neighbouring countries (Ballari et al. 2019), whereas the collared peccary (*Pecari tajacu*) is a native species considered "vulnerable" in Argentina (Camino et al. 2019), which underlines the relevance of research and conservation actions.

The interaction between invasive species, native wildlife and domestic animals implies an increased risk of pathogen transmission (Barrios-Garcia and Ballari 2012; La Sala et al. 2021). Moreover, disease transmission is a concern when wildlife reintroductions involve captive-bred animals as source populations, which can increase the risk of decline or extinction of wild populations (Earnhardt 2010).

In Argentina, epidemiological surveillance of TGEV and PRCV is conducted by the reference laboratory, the National Service of Agri-Food Health and Quality (SENASA) (Martinez, Buenos Aires, Argentina), and involves mainly domestic pigs, with little information on wildlife populations (Carpinetti et al. 2017) or captive animals destined for reintroduction. The presence of TGEV in the domestic pig population of Argentina seems to be relatively recent (Carné 2014; Piñeyro et al. 2018).

With this background, this study assessed the presence of antibodies against TGEV and PRCV in free ranging wild boar and captive collared peccaries from Argentina.

The studied species were sampled between 2014 and 2017 through convenience sampling in nine provinces (Tables 1, 2). Wild boar were hunted in "El Palmar" National Park in Entre Ríos Province, through a multi-stakeholder management program targeting wild boar and other invasive mammals (Gürtler et al. 2017), and in different cattle ranches in Buenos Aires and Río Negro Provinces, where wild boar coexists with livestock (cattle, sheep, and swine) raised under extensive and semi-extensive systems. All the research was performed under provincial approved hunting licence and rewilding projects. Legislation numbers and links to the rewilding projects are the following: Río Negro: Exp. 085206-SAYDS-2015 (Secretary of Sustainable Development and Environment of Argentina), Corrientes: https://rewildingargentina.org/iberá-project/#pecari.

Entre Ríos: Res 289/19 Argentina Ambiental: https://argentinambiental.com/legislacion/nacional/resolucion-28919-plan-control-mamiferos-exoticos-invasores-jurisdiccion-del-parque-nacional-palmar/. Wild boar were killed by authorized hunters and blood samples were obtained by jugular venipuncture. Hunters were specially trained before sample collection.

Peccary samples were obtained through Rewilding Argentina Foundation, which operates a collared peccary reintroduction program in Corrientes Province (Hurtado 2017). Peccaries were originally translocated from zoos and animal rescue centres located in six different provinces, and they were sampled during their quarantine period before reintroduction (Hurtado 2017; Zamboni et al. 2017). Blood samples were obtained by venipuncture of the external saphenous vein. All procedures were approved under the reintroduction project by the provincial environmental agency.

For the serological analysis, blood samples were centrifuged, and the obtained sera were frozen (− 20°C) until processing. Sera samples were analysed in SENASA's central laboratory. The presence of antibodies against TGEV/

Table 1. Information About Sampling Location and Serology Results for TGEV and PRCV in Wild Boar.

Location	Year	*N*	TGEV	PRCV
Río Negro	2014	4	Negative	Negative
	2015	41	Negative	Negative
	2016	34	Negative	Negative
Buenos Aires	2016	39	Negative	Negative
Entre Ríos	2017	42	Negative	Negative

Table 2. Information About Sampling Location and Serology Results for TGEV and PRCV in Collared Peccary.

Location	Year	*N*	TGEV	PRCV
Salta	2019	7	Negative	Negative
Tucumán	2016	14	Negative	Negative
La Rioja	2017	22	Negative	Negative
Corrientes	2016–2017	8	Negative	Negative
	2017	7	Negative	Negative
	2020	4	Negative	Negative
Córdoba	2017	8	Positive (1)	Negative
	2017	7	Negative	Negative
Mendoza	2018	10	Positive (2)	Negative

PRCV was determined by means of the Ingezim Corona Differential 2.0 ELISA kit (Ingenasa S.A., Spain) (Vengust et al. 2006). This kit includes a recombinant antigen and two specific monoclonal antibodies: one to recognize generic epitopes to TGEV and PRCV and the other to recognize a specific epitope from TGEV. The ELISA was carried out according to the manufacturer's instructions and optical density (OD) for the cut-off values was calculated for each group. Uncertainty in TGEV and PRCV prevalence was modelled under the hypothesis that these viruses are present in the wild boar and peccary populations, although possibly at very low prevalence. Then, a Beta distribution with a Uniform [0, 1] prior was included in Monte Carlo simulations (10,000 iterations) for both viruses in these populations. All the analyses were done using the programming language R (R Core Team 2019).

Three collared peccaries showed evidence of exposure to TGEV. For the peccaries from Cordoba Province, the cut-off value for TGEV was below OD = 1870, and the positive sample had a value of OD = 0.893. For the two positive peccaries from Mendoza Province, the cut-off value was below OD = 0.943, and the values obtained for those animals were of OD = 0.577 and OD = 0.517. Antibodies against PRCV were not detected in any of the peccaries. Simulation results yielded a mean prevalence of 1.1% (95% CI 0.03–4.03) for TGEV and 1.1% (95% CI 0.03–4.18) for PRCV. In wild boar, prevalence was 0.62 (95% CI 0.02–2.3) both for TGEV and PRCV. Three collared peccaries (3.4%) showed evidence of exposure to TGEV. Antibodies against PRCV were not detected in any of the peccaries.

The detection of antibodies against a swine coronavirus in wild Suina highlights the importance of epidemiological surveillance in wildlife populations and in captive animals before their reintroduction. Also, their potential role in maintaining and eventually spreading the pathogen and associated disease to native wildlife and the domestic pig population warrants further investigations.

Worldwide, other studies have reported seroprevalence of swine coronaviruses in wild boar, mostly in European countries (Vengust et al. 2006; Roic et al. 2012). Here, antibodies against TGEV or PRCV were not detected in wild boar. Similarly, previous research did not detect antibodies against TGEV or PRCV in wild boar from Buenos Aires Province (Carpinetti et al. 2017). This could be explained, at least in part, by the small sample size or, alternatively, by the fact that the disease is still absent in the wild boar population.

Information regarding seroprevalence against TGEV and PRCV in other wild Suina is scarce, and notably, only one study assessed exposure to TGEV in *Tayassu* peccaries from Bolivia (Karesh et al. 1998), without positive results.

It should be noted that the serological cross-reactivity between TGEV and PRCV can be significant even when using TGEV/PRCV differential blocking ELISAs (Magtoto et al. 2019). Two of the three TGEV-positive peccaries were from the same captive group, suggesting either transmission between them or exposure to a common source of infection. Although the observed TGEV prevalence in peccaries was low, our results underline the importance of closely monitoring not only free ranging, but also captive populations before their reintroduction as an integral component of a disease risk analysis (Jakob-Hoff et al. 2014).

In conclusion, the detection of antibodies against TGEV in collared peccaries suggests covert circulation of TGEV in captive and/or free roaming populations of this vulnerable species. The results generated will serve as baseline information for future risk assessments and further research on the ecology of coronaviruses in wild Suina. Additional research is warranted to better understand the role of free-ranging and captive Suina in the epidemiology and genetic evolution of TGEV, PRCV and other coronaviruses.

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Availability of Data and Materials

Raw data were generated at the School of Veterinary Science, University of Buenos Aires. Derived data supporting the findings of this study are available from the corresponding author on request.

Code Availability

Not applicable.

Declarations

Conflict of interest The authors have no competing interests to declare.

Ethical Approval The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Peccaries' sampling was approved under the reintroduction project by the provincial environmental agency. No ethical approval was required as the case notification data was accessed from the public domain (SENASA).

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