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Technical note

Detection of pathogens of epidemiological importance in feral pigs from Chihuahua and Durango, Mexico

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Abstract:

This study aimed to evaluate in feral pigs the presence of *Salmonella* spp (Spp), porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), swine influenza virus (SIV), porcine epidemic diarrhea virus (PEDV), *Mycoplasma hyopneumoniae* (Mhyo) and *Actinobacillus pleuropneumoniae* (App). The samples were obtained from pigs in the states of Chihuahua and Durango, Mexico. The tests analyzed for

the animals from Chihuahua were nasal swabs for SIV, rectal swabs for Spp and PEDV, serum for PRRSV and PCV2, lung, liver, and lymph nodes for Spp, SIV, PRRSV, and PCV2, as well as serum for serological tests. From animals in the state of Durango, the following was collected: nasal swabs for SIV, rectal swabs for Spp and PEDV, and serum for PCV2 for molecular and serological studies. The molecular results in both states showed samples positive for PCV2, 73.3 % in Chihuahua and 91.3 % in Durango; likewise, two positive samples were obtained for Spp in the state of Chihuahua (13.3 %) and one in Durango (6.6 %), for SIV there were two positive (8.7 %) in Durango. For PRRSV and PEDV, samples were negative in both states. Serological results in pigs from the two states showed positivity for PCV2, Spp, and App. Samples were negative for PRRSV, PEDV, and Mhyo in both states. It is important to highlight the molecular and serological detection of feral pigs positive for various infectious agents important in pig production with zoosanitary repercussions on public health, which implies an epidemiological relevance of these animals in the context of "one health".

Keywords: Feral pigs, Salmonellosis, Influenza, Porcine Circovirus type 2, PRRS, Porcine epidemic diarrhea.

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Feral pigs are animals that live in the wild and are widely distributed worldwide. They are considered invasive species due to the damage they cause to agriculture, livestock farming, and natural resources and because they interfere with other species[1-2]. In Mexico, the National Commission for the Knowledge and Use of Biodiversity (CONABIO, for its acronym in Spanish) has reported the presence of these animals mainly in the northern states, particularly in the Santa Elena Canyon in Chihuahua and in the La Michilía Reserve located in the state of Durango, also in the Laguna de Términos in Campeche and some areas of Central Mexico.

Several studies have shown that feral pigs can be an important source of bacterial diseases, such as brucellosis, viral diseases, such as Aujeszky, and parasitic diseases, such as trichinellosis, which represents a risk to public and animal health[3-6]. Currently, in Mexico, there is little information on the sanitary aspect of these animals; the available information indicates that the presence of swine influenza, leptospirosis, salmonellosis, and brucellosis has been detected in the states of Baja California Sur and Durango[7-8]. To expand the health information available so far, the present study determined the presence and frequency of *Salmonella* spp (Spp), porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), swine influenza virus (SIV), porcine epidemic diarrhea

virus (PEDV), *Mycoplasma hyopneumoniae* (Mhyo) and *Actinobacillus pleuropneumoniae* (App) in samples collected from feral pigs in the states of Chihuahua and Durango, Mexico.

During the period from 2019 to 2020, with the support of the feral pig control program in the Santa Elena Canyon, Chihuahua, and in the La Michilía Biosphere Reserve, Durango, 15 and 23 animals, respectively, of both sexes and different sizes were opportunistically captured. After capture, the animals were sacrificed at the trapping site according to the guidelines of NOM-033-SAG/ZOO-2014. Immediately after slaughter, biological samples were collected from the animals: nasal and rectal swabs, which were stored in minimum essential medium (MEM) as a means of transport until their analysis. It was determined the presence of SIV in the nasal swabs and the presence of Spp and PEDV in the rectal swabs by PCR.

Blood samples were taken from all pigs from the anterior vena cava using a 16G x 4-inch needle and placed in a tube with separator gel, then centrifuged at 1,500 rpm for 10 min, and the serum was stored in refrigeration until analysis for PRRSV and PCV2 by PCR and antibodies of PRRSV, PCV2, App, Mhyo, Spp, and PEDV. In the case of the animals captured in Chihuahua, it was also possible to obtain organs such as lung, liver, and lymph nodes, collecting a fragment of approximately 5 cm of each one, which were collected in new plastic bags that were stored in freezing at -20 °C until the analysis of Spp, SIV, PRRSV, and PCV2. All determinations were carried out in the diagnostic laboratory of the Department of Medicine and Zootechnics of Pigs of the Faculty of Veterinary Medicine and Zootechnics of the National Autonomous University of Mexico.

For molecular studies, nucleic acids were extracted from nasal, rectal, serum, and tissue samples using the commercial kit (QIAamp cador Pathogen Mini Kit QIAGEN) following the manufacturer's instructions. Subsequently, the real-time polymerase chain reaction (PCR) was performed, for which a commercial kit (GeneReach Pockit) was used for each of the agents mentioned above; these kits were used under the protocol for each of the agents with their respective positive and negative controls; the interpretation as positive was with a CT equal to or less than 35 according to the supplier. Serological tests were performed using commercial kits for Spp (Idexx Laboratories Inc), PCV2 (BioNote Inc.), PRRSV (Civtest Suis PPRS A/S Hipra), PEDV (ID Screen PEDV indirect), Mhyo (Civtest Suis MHYO Hipra) and App (ID Screen App Screening indirect); the methodology and interpretation were performed according to the supplier's instructions.

For the information analysis, a descriptive statistic was made due to the non-homogeneity between the samples, their small number, and the absence of other information on the sampled animals, obtaining the percentages of positive samples for each etiological agent in each state.

In the two states analyzed, 11 positive samples for PCV2 (73.3 %) in Chihuahua and 21 (91.3 %) in Durango were detected by molecular means; likewise, two samples positive for Spp in the Chihuahua group (13.4 %) and one for Durango (6.6 %) were obtained. In the case of SIV, there were two positive samples (8.7 %) in Durango. In the case of PRRSV and PEDV, the samples were negative in both states (Table 1).

Table 1: Number of samples positive and negative for different pathogens in feral pigs from the states of Chihuahua and Durango: 2019-2020

Agent	**Chihuahua**		**Durango**	
	(+)	(-)	(+)	(-)
SIV	0	15	2	21
Salmonella spp	2	13	1	-
PEDV	0	15	0	23
PRRSV	0	15	0	23
PCV2	11	4	21	2

SIV= swine influenza virus; PEDV= porcine epidemic diarrhea virus; PRRSV= porcine reproductive and respiratory syndrome virus; PCV2= porcine circovirus type 2.

Serology results showed 12 (80 %) samples positive for PCV2 and 13 for *Salmonella* spp (56.5 %), respectively; 2 (13.3 %) samples positive for Spp in Chihuahua and 23 (100 %) for Durango were also identified. For App, there were two positive samples (2.6 %) for Chihuahua and 23 (100 %) for animals from Durango. Samples were negative for PRRSV, PEDV, and Mhyo in all samples, Table 2.

Table 2: Number of positive and negative samples by serology for different pathogens in feral pigs from the states of Chihuahua and Durango: 2019-202

Agent	**Chihuahua**		**Durango**	
	(+)	**(-)**	**(+)**	**(-)**
Salmonella spp	3	12	23	0
PCV2	12	3	13	10
PRRSV	0	15	0	23
PEDV	0	15	0	23
Mhyo	0	15	0	23
App	2	13	23	0

PCV2= porcine circovirus type 2; PRRSV= porcine reproductive and respiratory syndrome virus; PEDV= porcine epidemic diarrhea virus; Mhyo= *Mycoplasma hyopneumoniae*. App= *Actinobacillus pleuropneumoniae*.

The high number of positive samples detected in serum and organs for PCV2 in both states suggests that this virus actively circulates in the sampled feral pig population, which is consistent with what has been reported in other similar reports[9-11]. The above may suggest that feral pigs could be a reservoir for domestic pigs or vice versa. Regarding SIV, despite being a widely distributed disease in the world, only two positive samples were found in Durango; nevertheless, success in antigen detection is complicated because the virus has a very short excretion period, and sampling must be performed when the pig is in a febrile period[12].

The results of this study coincide with previous studies[13-14] in which the detection of this virus is also affected by external factors such as interaction with other species[15]. In the case of Spp, it is known that the bacteria can be eliminated intermittently in feces for long periods, so possibly, when the animals were captured, they were not eliminating it. It must be considered that large populations are also required to maintain the infection in the environment, so there is a possibility that the bacteria is not present in feral pigs. The results showed an uneven behavior as a high frequency was detected in pigs from Durango, but a low frequency in Chihuahua, possibly due to the type of habitat that influences the availability of water and food, and this is a stressor that causes the pig to eliminate the pathogen and have more frequent contact with the bacteria, feces, and water[1].

In the case of PRRS and PED, the negative results suggest that these agents have little or no circulation in feral pigs, which coincides with what has been reported by several authors[14,16,17], since they require specific epidemiological conditions for their spread within a herd, such as overpopulation, which happens constantly in technified pig farms; feral pigs are low-density populations, which reduces the possibility of contact with these viruses, so transmission would not be possible. Regarding Mhyo, not finding positive samples may indicate that this agent is probably not present, but it should be considered that there may be subclinical infections detectable only with histopathology[3,18]. There are studies with data positive for Mhyo, but it may be due to geography, time of collection, and number of samples[19]. In the case of App, the results show the presence of this agent, which is consistent with other studies[9,14] where a high frequency of this disease is reported, and if it is added that this kit has high sensitivity and detects the 12 relevant serotypes in domestic pigs, it can be inferred that this agent circulates in these populations[20-21].

This work demonstrated the presence of animals positive for several important agents in commercial pig production and public health, as had already been reported in a previous study in Baja California Sur. It is relevant to comment on the implication of the above because these animals move long distances and in herds searching for food and water, which leads to their interaction with other wild and domestic species and humans, representing a risk as it is a reservoir of various infectious agents. Although CONABIO indicates the presence of this species throughout Mexico, the total population is unknown, so it would be

good to conduct more research to know exactly where they are present, estimate the population in each of those places to be able to implement control programs such as the one carried out by La Michilía, and at the same time carry out studies with more targeted designs and with a larger sample size to continue with the detection of infectious agents present in these animals. The above will allow to know a more accurate situation in Mexico, where the information is virtually zero.

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Conflict of interest

The authors state that they have no conflict of interest in this study.

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