



Seroprevalence of influenza A in pigs in small-scale units in Nuevo León, Mexico



Anahis Marlene Olvera-Rivera ^a

Adrián Rosas-Taraco ^a

José Pablo Villarreal-Villarreal ^b

Heidi Giselle Rodriguez-Ramirez ^{b*}

^a Universidad Autónoma de Nuevo León. Facultad de Medicina, Departamento de Inmunología. Gonzalitos 235 Norte, Mitras Centro, 64460 Monterrey, Nuevo León, México.

^b Universidad Autónoma de Nuevo León. Facultad de Medicina Veterinaria y Zootecnia. *Campus* de Ciencias Agropecuarias, Colonia Ex-Hacienda el Canadá, General Escobedo, Nuevo León, México.

*Corresponding author: rdzmvz@gmail.com

Abstract:

Influenza is a respiratory illness that affects both humans and pigs. Traditional farming systems are characterized by deficiencies in biosecurity, which leads to the coexistence of animals of different species, favoring the presence and spread of influenza. This study determined the seroprevalence of influenza A virus in backyard pig production units in Nuevo León, Mexico. To this purpose, 207 sera were included and analyzed using a commercial ELISA kit from IDEXX. A seroprevalence of 9.2 % was obtained; additionally, animals living on a dirt floor have a higher risk of seropositivity. In conclusion, backyard animals maintain contact with the influenza A virus.

Keywords: Orthomyxovirus, Swine, Small scale, Seroprevalence, Influenza A, Backyard.

Received: 18/03/2025

Accepted: 25/08/2025

The influenza virus is an orthomyxovirus, which is divided into three types depending on the M1 protein they express: A, B, and C. So far, only type A viruses have been able to produce pandemics. The influenza A virus is considered a pathogenic agent with zoonotic potential, holding special importance in public health; there are various studies that have proven infection in humans with viruses of porcine origin⁽¹⁻³⁾. In addition to the above, the last influenza pandemic, which occurred in 2009, was recognized as being caused by a virus of swine origin⁽⁴⁾. Pigs are known as “mixing vessels” because they simultaneously present the N-acetylneuraminic acid, α 2,3-galactose, and α 2,6-galactose molecules, which are necessary for cell attachment by viruses of avian and human origin, respectively⁽⁵⁾. For both humans and pigs, the influenza virus affects the respiratory system, and its clinical signs include fever, runny nose, cough, sneezing, and can even lead to the development of pneumonia⁽⁴⁾. For the pig industry, this virus generates economic impacts due to its complications, along with a decrease in intake and weight gain⁽⁶⁾.

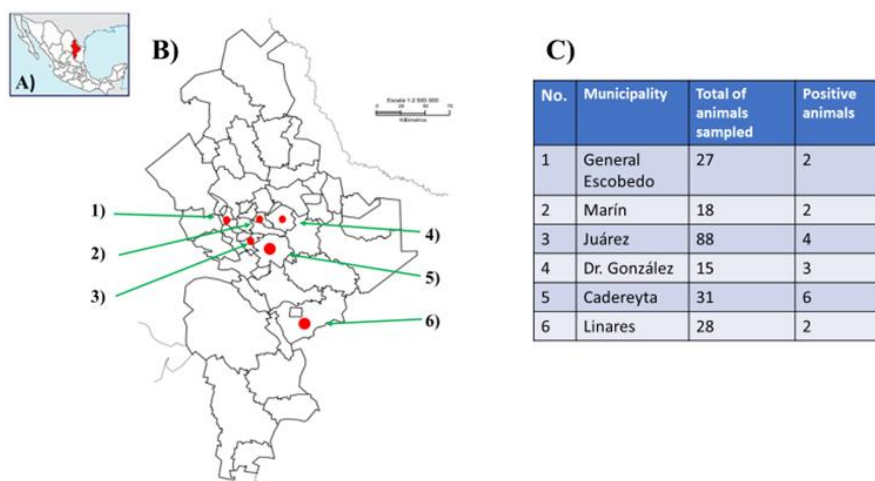
In Mexico, small-scale systems prevail in rural or suburban areas as a secondary economic activity that serves as support for family income⁽⁷⁾; these production units are characterized by deficiencies in biosecurity measures, as well as regular veterinary care for the prevention of diseases or treatment of existing ones, coupled with an inadequate diet^(8,9). Among the biosecurity deficiencies found by various authors are the lack of separation from other animal species, such as dogs, cats, ruminants, horses, and poultry; this can maximize the possibility of interspecies contagion⁽¹⁰⁾. The fact that this agent has zoonotic potential, as well as repercussions on livestock production and public health, coupled with the lack of information regarding exposure in pigs in small-scale systems in Nuevo León, places this virus as a virus of interest. Therefore, this study aimed to determine the seroprevalence against influenza A virus in pigs in small-scale units, without a history of vaccination, in the state of Nuevo León.

The small-scale production unit was defined as one in which pig farming was carried out to support the family’s economy, self-consumption, and occasional sale, in the home where the family lives. The minimum sample size was calculated using the WinEpi platform, considering the following: an expected prevalence of 8 %⁽¹¹⁾, a confidence level of 95 %, and an acceptable error of 5 %, with an unknown population, which resulted in a total of 114 samples to be included. The samples were collected from August 2016 to August 2017. Pigs of different ages and possible production stages were included. Lactating piglets and pregnant sows were not included in the study due to the presence of maternal antibodies and the risk of abortion, respectively.

For sampling, each animal was physically restrained, and approximately 4 mL of blood was obtained from the jugular vein, using a system of vacuum tubes. The samples were collected in red tubes without anticoagulants to allow the clot to form. The samples were centrifuged at 1,000 rpm for 10 min at 4 °C, then the serum was separated, and 500 µL aliquots were prepared, which were stored at -20 °C until further processing. A commercial kit (IDEXX Swine Influenza Virus Ab test) was used for detecting antibodies against influenza A, following the manufacturer's protocol. The kit used detects antibodies that recognize the nucleoprotein of the virus (NP), specific to the swine-origin virus; thus, the detection is limited to the influenza A virus, without distinguishing the subtype based on the hemagglutinins it carries. In the literature, it has been reported that this commercial kit has a sensitivity of 95.5 % and a specificity of 99.6 %⁽¹²⁾. The result was read at 650 nm with an Epoch BioTek® spectrophotometer.

Some characteristics present in the environment of the production units were noted for further analysis as possible risk factors, including the presence of domestic birds, contact with wild birds (lack of infrastructure to avoid contact), and the floor material (dirt, wood, or concrete) corresponding to the area intended for pigs. None of the animals in the present study had been vaccinated against influenza A. The total number of positive animals was represented as a percentage. The statistical analysis was performed using the Chi-square test, and those that were significant were included in a binomial logistic regression model using SPSS software. A *P*-value < 0.05 was considered significant.

Twenty production units were found, in which it was possible to sample 207 animals of varying ages; the owners could not precisely specify these ages due to a lack of records. The animals included in this study were found in the municipalities of General Escobedo, Juárez, Cadereyta, Marín, Doctor González, and Linares (Figure 1). The owners reported that the animals were mainly crosses of the Landrace, Duroc, and Yorkshire breeds, without keeping a record. The only animals included, for which the breed was known, were eight Vietnamese pigs. Likewise, the owners reported that the objective of farming pigs is to generate an additional income through sales to their neighbors and for self-consumption; therefore, these animals lacked an age or weight limit before being slaughtered.

Figure 1: Municipalities in which the production units were located

Positive production units accounted for 55 % (11/20). Despite having no history of vaccination, 9.2 % of the animals were positive for the presence of influenza antibodies. ELISA results were validated using negative controls and positive controls included in the kit; a negative sample/control absorbance ratio of less than 0.60 was considered positive for the presence of antibodies. Although the animals may have originated from other production units where they received vaccination, this data was not obtained during the study and will be considered in subsequent projects.

Seroprevalence against the influenza virus has been studied by different working groups, with a wide range of results. A review and meta-analysis study focusing on small-scale units found an average seroprevalence of 18.28 % and a presence of the virus of 1.32 %⁽¹³⁾; a constant was the lower presence of the virus compared to the presence of antibodies in animals that did not exhibit clinical signs. The kit used for this study detects specific antibodies against the NP of the influenza A virus, so seroprevalence for different subtypes was not distinguished.

Of the three environmental variables evaluated to determine their association with seropositivity (Table 1), the type of floor was significant; however, when analyzed in the logistic regression model, it was not significant ($P=0.129$). The type of floor has been studied as a factor that favors the presence of other pathogens in pigs, such as gastrointestinal parasites⁽¹⁴⁾; although this was not significant, there is a trend. A possible explanation for this trend is that dirt floors have a greater capacity to retain water compared to concrete floors, and the influenza virus remains viable for a longer time in humid environments, so its presence has been described in drinking water, wastewater, surface water⁽¹³⁾, and surfaces of production units⁽¹⁵⁾. Nevertheless, further studies are required to prove this.

Table 1: Analysis of frequencies of environmental variables

Variable	Analysis	Frequency (%)	P-value
Type of floor	Concrete	146/207 (70)	0.012
	Dirt	52/207 (25)	
	Wooden	9/207 (5)	
Domestic birds	Present	126/207 (60.87)	0.78
	Absent	81/207 (39.13)	
Wild birds	Present	207/207 (100)	>0.999
	Absent	0/207	

Figure 2: Representative image of the conditions of small-scale production units

Contact with domestic birds was not found to be a significantly associated factor either. In addition to this, the presence of wild birds was a constant in all production units, since there was no infrastructure to prevent their contact with them. Birds are an important reservoir of influenza viruses, and in other studies, they have been shown to be a risk factor for contact with the virus for pigs⁽¹⁶⁾. Among the domestic birds found in this study are chickens, turkeys, guinea fowls, pigeons, quails, geese, and ducks. An interesting approach would be the analysis regarding the presence of web-footed birds or waterfowl, as these are the most important reservoirs of the virus⁽¹⁷⁾. In this study, the presence of domestic birds was only recorded as positive, with no difference between the species present in each production unit.

In Nuevo León, there are small-scale pig production units in which contact with the influenza virus can occur among animals raised in those places, since, despite lacking vaccination, 9.2 % of them tested positive for the presence of specific antibodies for the NP protein of the influenza A virus. Of the three variables studied, a statistical association could not be verified.

Although small-scale production units are a support to the family economy, there are several opportunities for improvement in terms of hygiene and biosecurity measures.

Acknowledgements

We are grateful to PRODEP for the financial support for this project, with the approval of the project DSA/103.5/16/10510 UANL-PTC-962. Anahis M. Olvera-Rivera thanks PRODEP for the support granted as a scholarship within this project. Likewise, we express our gratitude to Raúl Sarmiento-Escamilla for his technical support during the execution of this project.

Conflicts of interest

The authors of this paper declare that there is no conflict of interest.

Literature cited:

1. Saavedra-Montañez M, Castillo-Juárez H, Sánchez-Betancourt I, Rivera-Benitez JF, Ramírez-Mendoza H. Serological study of influenza viruses in veterinarians working with swine in Mexico. *Arch Virol* 2017;162(6):1633–1640.
2. Bowman AS, Walia RR, Nolting JM, Vincent AL, Killian ML, Zentkovich MM, *et al.* Influenza A(H3N2) virus in swine at agricultural fairs and transmission to humans, Michigan and Ohio, USA, 2016. *Emerg Infect Dis* 2017;23(9):1551–1555.
3. Anjorin AAA, Sausy A, Muller CP, Hübschen JM, Omilabu SA, Snoeck CJ. Human seasonal influenza viruses in swine workers in Lagos, Nigeria: Consequences for animal and public health. *Viruses* 2023;15(6).
4. Abdelwhab EM, Mettenleiter TC. Zoonotic animal influenza virus and potential mixing vessel hosts. Multidisciplinary Digital Publishing Institute Aktiengesellschaft. *Viruses* 2023;15(4):980.
5. Ito T, Nelson J, Couceiro SS, Kelm S, Baum LG, Krauss S, *et al.* Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. *J Virology* 1998;72(9):7367-7373.

6. Zimmerman JJ. Disease of swine. 11th ed. USA: Wiley-Blackwell; 2019.
7. Montero LEM, Martínez GRG, Herrada LMA, Ramírez HG, Espinoza HMS, Martínez RR. Alternativas para la producción porcina a pequeña escala. 1st ed. México: Universidad Nacional Autónoma de México; 2015.
8. Baumberger C, Di Pillo F, Galdames P, Oyarzun C, Marambio V, Jimenez-Bluhm P, *et al.* Swine backyard production systems in central Chile: characterizing farm structure, animal management, and production value chain. *Animals* 2023;13(12):2000.
9. Bejarano-Martínez MG. Determinación de parásitos gastrointestinales y factores de riesgo en cerdos de traspatio, ubicados en el área metropolitana de Monterrey y región periférica [Tesis maestría]. Escobedo, Nuevo León: Universidad Autónoma de Nuevo León; 2020.
10. Blagodatski A, Trutneva K, Glazova O, Mityaeva O, Shevkova L, Kegeles E, *et al.* Avian influenza in wild birds and poultry: Dissemination pathways, monitoring methods, and virus ecology. *Multidisciplinary Digital Publishing Institute Aktiengesellschaft, Pathogens* 2021;10(5):630.
11. Tinoco YO, Montgomery JM, Kasper MR, Nelson MI, Razuri H, Guezala MC, *et al.* Transmission dynamics of pandemic influenza A(H1N1) pdm09 virus in humans and swine in backyard farms in Tumbes, Peru. *Influenza Other Respir Viruses* 2016;10(1):47–56.
12. Goodell CK, Prickett J, Kittawornrat A, Johnson J, Zhang J, Wang C, *et al.* Evaluation of screening assays for the detection of influenza a virus serum antibody in swine. *Transbound Emerg Dis* 2016;63(1):24–35.
13. Chauhan RP, Gordon ML. A systematic review of influenza A virus prevalence and transmission dynamics in backyard swine populations globally. *Porcine Health Management*. BioMed Central Ltd; 2022.
14. de Araújo HG, da Silva JT, Álvares FBV, Ferreira LC, Azevedo SS, Vilela VLR. Prevalence and risk factors associated with swine gastrointestinal nematodes and coccidia in the semi-arid region of northeastern Brazil. *Trop Anim Health Prod* 2020;52(1):379–385.
15. Neira V, Rabinowitz P, Rendahl A, Paccha B, Gibbs SG, Torremorell M. Characterization of viral load, viability and persistence of influenza a virus in air and on surfaces of swine production facilities. *PLoS One* 2016;11(1).

16. Chauhan RP, Gordon ML. A systematic review of influenza A virus prevalence and transmission dynamics in backyard swine populations globally. *Porcine Health Management* 2022;8(1):10.
17. Oawa M, Kawaoka Y. Cross talk between animal and human influenza viruses. *Annual Review of Animal Biosciences*. Annual Reviews Inc. 2013;(1):21–42.