

SHORT COMMUNICATION

Seroprevalence and Risk Factors for Swine Influenza Zoonotic Transmission in Swine Workers from Northwestern Mexico

G. López-Robles¹, M. Montalvo-Corral¹, G. Caire-Juvera¹, G. Ayora-Talavera² and J. Hernández¹

¹ Laboratorio de Inmunología, Centro de Investigación en Alimentación y Desarrollo, A.C. Hermosillo, Sonora, Mexico

² Centro de Investigaciones Regionales "Dr. Hideyo Noguchi", Universidad Autónoma de Yucatán, Mérida, México

Keywords:

swine influenza; risk factors; Mexican swine workers; zoonoses

Correspondence:

J. Hernández. Laboratorio de Inmunología, Centro de Investigación en Alimentación y Desarrollo A.C., Carretera a la Victoria km 0.6., P.O. Box 1735, CP 83000, Hermosillo, Sonora, México.

Tel.: +52 662 289 2400 Ext. 294;

Fax: +52 662 280 0944;

E-mail: jhdez@ciad.mx

Received for publication February 3, 2011

doi:10.1111/j.1865-1682.2011.01250.x

Summary

A cross-sectional study was conducted to evaluate the transmission of swine influenza through occupational exposure and to assess some risk factors for zoonotic transmission in workers from commercial farms in Mexico. Seroprevalence to swine influenza subtypes was determined by hemagglutinin inhibition assay and was higher in exposed (E), in comparison with unexposed (UE) participants ($P < 0.05$). Percentages of seropositivity between UE and E were 28.57% and 19.35% to A/NewCaledonia/20/99 (H1N1), 68.25% and 33.87% to A/Panama/2001/99-like (H3N2), 1.58% and 12.9% to A/Sw/England/163266/87 (H3N2), respectively. No antibodies were detected against A/Sw/Wisconsin/238/97 (H1N1) in the UE subjects, and only 3.22% were positive in the E group ($P < 0.05$). A significant association between elevated antibody titres to swine influenza virus (SIV) H3N2 and the exposition to swine [OR 3.05, 95% (CI) 1.65–5.64] and to geographic location [OR 8.15, 95% (CI) 1.41–47.05] was found. Vaccination appeared as a protective factor [OR 0.05, 95% (CI) 0.01–0.52]. Farms with high number of breeding herd were associated with increased anti-SIV antibodies in the E group [OR 3.98, 95% (CI) 1.00–15.86]. These findings are relevant and support the evidence of zoonoses in swine farms and point out the need to implement preventive measures to diminish the occurrence of the disease and the potential emergence of pathogenic reassortant strains.

Introduction

The influenza virus belongs to the *Orthomyxoviridae* family and has a segmented RNA genome with negative polarity. The virus has a double-lipid envelope derived from cell host and an average diameter of 120 nm. The classification of Influenza A viruses is based on the nature of its surface glycoproteins, hemagglutinin (HA or H) and neuraminidase (NA or N). At present, 16 H subtypes and 9 N have been discovered in different species (Fouchier et al., 2005). Recognition of target cells in the specific host and the spread of infection are among glycoproteins main functions (Thacker and Janke, 2008).

Since the first outbreaks in Mexico and the USA in February–March 2009, a new influenza pandemic hit human population with high rates of transmission. The aetiological agent was a particular swine originated influenza virus, H1N1 (S-OIV), with a reassortant combination of swine, human and avian gene segments. The HA, NP and NS were from swine influenza, PB2 and PA were from American avian origin, and PB1 was from human origin. The NA and M had an Eurasian avian–porcine origin. Based on this evidence, a quadruple recombination of the virus was assumed (Neumann et al., 2009; Smith et al., 2009). Possible viral ancestors were circulating in commercial farms in United States since 1998 (Zhou et al., 1999;

Olsen, 2002; Vincent et al., 2009). The emergence of the novel virus from a pig source highlighted that animal reservoirs are a key source for the generation of pandemic influenza strains, as also suggested for the pandemic events in 1918 and 1957 (Belshe, 2005).

Swine influenza is a frequent disease in pigs and important zoonoses. The pig has been implicated as the intermediate host in the interspecies transmission and reassortment of influenza strains (Webster et al., 1992). The occurrence of human infections with pig influenza viruses has been documented in people with close contact to swine (Olsen et al., 2002; Ayora-Talavera et al., 2005; Myers et al., 2006; Gray et al., 2007). The State of Sonora is located in the north-western region of Mexico and is the largest pork producer in the country; in our interest, swine workers are a particular population with occupational exposition to SI, and they were evaluated in this study to determine serological data and assess potential risk factors involved in the transmission of the swine influenza virus (SIV).

Materials and Methods

Study design and subjects

A cross-sectional study was conducted in 15 swine commercial farms during the years 2007–2008. A total of 125 subjects participated in the study. They included 62 swine workers (exposed, E) and 63 controls (unexposed, UE) that were recruited from 3 cities (Hermosillo, Ciudad Obregón and Navojoa). The persons from the E group were all the workers of the selected farms that consented to participate in the study. Unexposed controls were involved in other activities not related to the swine industry. Sample size was determined using the Cannon and Roe (1982), designed to estimate the sample size required at an expected prevalence rate of the disease. A 30% prevalence of swine disease was used to select the number of hogs included in the study, and the confidence level was established at 95%.

The study was reviewed and approved by the ethical committee of Centro de Investigación en Alimentación y Desarrollo (Research Center for Food and Development), and informed written consent was obtained from all participants. They completed a questionnaire adapted from Gray et al. (2007), to obtain personal data such as age and city of origin, and working conditions, such as use of protective gear, exposure to swine, occurrence of flu-like illness and biosafety measures in farms.

Biological samples

Throat swabs and blood samples from E and UE individuals were collected. The swabs were preserved in Hank's

medium supplemented with 10% glycerol and antibiotics and kept at -70°C until processing. Blood samples were collected in tubes without anticoagulant. Serum was separated and stored at -20°C until processing.

Real time RT-PCR

RNA extraction of the samples was conducted using spin columns QIAmp RNA mini kit (QIAGEN, Inc., Valencia, CA, USA). Genomic RNA of SIV was detected by rRT-PCR using QIAGEN[®] One Step RT-PCR Kit (QIAGEN, Inc.), using previously described matrix gene target oligonucleotides and 0.4 μl probe (Spackman et al., 2002). The rRT-PCR conditions were one cycle at 50°C for 30 min, one cycle at 95°C for 10 min, forty cycles at 95°C for 15 s and at 60°C for 10 s.

Hemagglutinin inhibition assay

Blood samples from E and UE individuals were collected for the detection of antibodies against swine and human influenza virus subtypes H1N1 and H3N2 by the hemagglutinin inhibition (HI) assay (Ayora-Talavera et al., 2005). Based on their availability at our laboratory, four influenza strains were used: A/NewCaledonia/20/99 (H1N1), A/Panama/2001/99-like (H3N2), A/Swine/England/163266/87 (human lineage segments H3N2) and A/Swine/Wisconsin/238/97 (H1N1). Samples with antibody titres $\geq 1 : 32$ were considered seropositive.

Statistical analysis

Descriptive analyses were used to compute the percentages of the characteristics of the population and other covariates. Chi-squared tests were used to examine differences in the selected characteristics by E/UE group. To evaluate the possible associations between the presence of the disease and some potential risk factors, the hemagglutination inhibition assay antibodies against SIV were independently used as the outcome variables and dichotomized into positive versus negative presence of antibodies. Crude and adjusted odds ratios (ORs) and 95% Confidence Intervals (CIs) were calculated by bivariate logistic regression analyses for each SIV (H1N1 or H3N2). The analyses were conducted using the following as independent variables in each bivariate model: E or UE to swine, geographic location, smoking and received influenza vaccine. We also included seropositive for human influenza as independent variables, to evaluate the effect of cross-reactivity. An unadjusted model and a model adjusted for age were developed for each of the dependent variables. The confounding effect of age was included in the models because the two groups were not matched according to

age. For the 'Geographic location' variable, the 'Ciudad Obregon' category was removed, because no subjects tested positive to antibodies, and this issue distorted the results. Analyses were conducted using Stata version 9.2 (StataCorp LP, College Station, TX, USA).

Results

Characteristics of the study subjects

Some demographic characteristics were evaluated in the two studied groups, and other occupational features were considered specifically in swine workers (Table 1). Study participants were all men with a higher age in the E group ($P < 0.0001$). There were some missing data for age and smoking status, because some of the participants did not provide those data on the questionnaire. There were differences between the two groups in the variables City of residence ($P < 0.0001$), live in a swine farm or did in the past ($P < 0.0001$), the last contact with swine ($P < 0.0001$) and smoking ($P < 0.05$). The remaining variables were similar statistically ($P > 0.05$).

Prevalence of influenza

No influenza viruses were detected by qRT-PCR. However, serological evidence of previous exposure to swine and human influenza virus was found (Table 2). Geometric mean titres of antibodies to swine influenza (H1N1: 1.21 and 2.77; H3N2: 4.21 and 19.3) were significantly different between UE and E groups, respectively ($P < 0.05$). Interestingly, only a person from the UE group was positive for the swine influenza H3N2, and no antibodies were detected for both the swine and human subtypes. The E group had a lower percentage of seropositives for human viruses in relation to the UE group. There was a higher seroprevalence of H3N2 of human and porcine origin in both groups ($P < 0.05$). Two persons in the E group were positive for swine influenza H1N1 and one of them was also positive for the same subtype of human virus. Eight subjects in the E group tested positive for swine influenza H3N2 and 5 of them were also positive for human influenza H3N2. These data could indicate cross-reactivity between viruses, although this issue cannot be fully assured.

Risk factors for swine influenza

Table 3 shows the odds ratios and 95% confidence intervals (CI) for elevated antibodies against the SIVs H1N1 and H3N2. A significant association between elevated antibody titres to SIV H3N2, and the exposition to swine [OR: 3.05, 95% (CI) 1.65–5.64] and to geographic location [OR 8.15, 95% (CI) 1.41–47.05] was found when

Table 1. Characteristics of the exposed and unexposed subjects included in the study

Variables	Exposed, <i>n</i> (%) <i>n</i> = 62	Unexposed, <i>n</i> (%) <i>n</i> = 63	<i>P</i> -Value*
Age, years			
15–29	15 (24.2)	31 (49.2)	0.000
30–49	26 (41.9)	12 (19.0)	
50–65	15 (24.2)	5 (7.9)	
Missing	6 (9.7)	15 (23.8)	
Mean age	38.1	32.8	
City of residence			
Hermosillo	21 (33.9)	31 (49.2)	0.000
Cd. Obregon	21 (33.9)	32 (50.8)	
Navojoa	20 (32.2)	–	
Live in a swine farm or did in the past			
Yes	23 (37.1)	0	0.000
No	39 (62.9)	63 (100)	
Years worked in swine farms			
Never	0	63 (100)	0.491
<1	11 (17.7)	–	
1–10	32 (51.6)	–	
>10	19 (30.7)	–	
Use personal protection equipment when work with swine			
B, C, HD, G	8 (12.9)	–	–
B, C	44 (71.0)	–	
B	10 (16.1)	–	
Shower			
At entry and egress of the farm	24 (38.7)	–	–
Never	3 (4.8)	–	
Only at entry or egress	35 (56.4)	–	
Production stage in which the subject works			
Farrowing	19 (30.6)	–	–
Weaning	24 (38.8)	–	
Fattening	19 (30.6)	–	
When was the last contact with swine?			
Never	0	33 (52.4)	0.000
>1–12 month	3 (4.8)	28 (44.4)	
<7 days	59 (95.2)	2 (3.2)	
Smoke			
No/Quit smoking	39 (69.2)	53 (84.1)	0.013
Yes	8 (12.9)	10 (15.9)	
Missing	15 (24.2)	0	
Received influenza vaccine			
Yes	22 (35.5)	17 (27.0)	0.179
No/Unsure	40 (64.5)	46 (73.0)	

B, boots; C, coverall; G, gloves; HD, hands disinfection.

* χ^2 test.

adjusted for age. These results may relate to the swine density of the farm and the number of breeding herds [OR 3.98, 95% (CI) 1.00–15.86]. In addition, vaccination appeared as a protective factor [OR 0.05, 95% (CI) 0.01–0.52] for the presentation of the H3N2 virus. There were no risk factors associated with elevated antibody titres to SIV H1N1. These results could be related to the fact that

Table 2. Seroprevalence of human and swine influenza per exposed/unexposed group

Groups	H1N1, <i>n</i> (%)			H3N2, <i>n</i> (%)		
	Swine	Human	Both	Swine	Human	Both
Exposed, <i>n</i> = 62	2 (3.22)	12 (19.35)	1 (1.61)	8 (12.9)	21 (33.87)	5 (8.06)
Unexposed, <i>n</i> = 63	0 (0)	18 (28.57)	0 (0)	1 (1.58)	43 (68.25)	0 (0)
Total, <i>n</i> = 125	2 (1.60)	30 (24)	1 (0.8)	9 (7.2)	64 (51.2)	5 (4)

H1N1, A/NewCaledonia/20/99 and A/Sw/Wisconsin/238/97; H3N2, A/Panama/2001/99-like and A/Sw/England/163266/87.

Table 3. Odds ratios and 95% confidence intervals (CI) for elevated hemagglutination inhibition assay antibodies (enrollment sera) against swine influenza viruses

Independent variables subgroups (%)	<i>n</i>	Swine H1N1		Swine H3N2	
		Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Exposed to Swine	125				
Yes (49.6)	62	–	1 (0.14–6.87)	9.18 (1.11–75.79)*	3.05 (1.65–5.64)*
No (50.4)	63	Reference	Reference	Reference	Reference
Geographic location	72				
Hermosillo (72.2)	52	–	2.03 (0.35–11.84)	4 (0.95–16.83)	8.15 (1.41–47.05)*
Navajoa (27.8)	20	Reference	Reference	Reference	Reference
Smoking	110				
No/Quit smoking (80)	88	4.14 (0.24–68.98)	3.89 (0.22–66.29)	1.39 (0.63–3.05)	2.15 (0.49–9.41)
Yes (20)	22	Reference	Reference	Reference	Reference
Received influenza vaccine	125				
Yes (31.2)	39	0.44 (0.02–7.33)	0.49 (0.02–8.54)	0.10 (0.02–0.55)*	0.05 (0.01–0.52)*
No/Unsure (68.8)	86	Reference	Reference	Reference	Reference
Human influenza H1N1	125				
No (76)	95	3.24 (0.19–53.45)	3.92 (0.21–71.61)	4.55 (1.13–18.21)*	4.96 (0.99–24.64)
Yes (24)	30	Reference	Reference	Reference	Reference
Human influenza H3N2	125				
No (48.8)	61	0.95 (0.05–15.57)	1.12 (0.06–20.65)	1.20 (0.30–4.75)	1.23 (0.24–6.25)
Yes (51.2)	64	Reference	Reference	Reference	Reference

OR, odds ratio; 95% CI, 95% confidence interval; –, no values were calculated; **P* < 0.05; Data adjusted by age.

there were only two positive samples in the E group. Antibodies to human influenza did not increase the risk for swine influenza for any of the two subtypes. Three of 22 (13.63%) smokers and 6 of 88 (6.81%) non-smokers tested seropositive to H3N2 SIV (data not shown); however, the ORs did not increase, probably due to the small sample size in the smokers' category.

Discussion

The present work was carried out in the largest State producer of pork in México (Sagarpa, 2011), and considering that swine workers are a confined population with increased probabilities to acquire swine flu (Olsen et al., 2002; Myers et al., 2006; Gray et al., 2007). We evaluated some demographic characteristic, health status and vaccination in farms to identify risk factors that could explain

the serological data. Other studies that evaluate risk factors in swine workers have reported elevated odds for farmers of SIV H1N1 [OR 54.9, 95%, (CI) 13.0–232.6] and H1N2 [OR 13.5, 95%, (CI) 6.1–29.7] in the United States (Gray et al., 2007) and to H1N1 in Luxembourg (OR 2.3, 95% CI 1.1–5.0) (Gerloff et al., 2011). We found our results in concordance with the mentioned studies, only for the H3N2 virus. In agreement with a previous report (Gray et al., 2007), vaccination appeared as a protective factor [OR 0.22, 95% (CI) 0.07–0.70]. The results of our study are the first evidence of SIV transmission and risk factors among swine workers and pigs in México.

The seropositivity observed in the E group was higher for H3N2 SIV than for H1N1 SIV, this could be explained by the fact that swine workers are more frequently in contact with the H3N2 virus, which is the

predominant circulating subtype in swine commercial farms from Sonora (G. López-Robles, unpublished results). We also observed that the E group had higher seroprevalence of SIV than the UE group, and these data are consistent with the study reported by Gray et al. (2007) but not with the data reported by Gerloff et al. (2011). A possible explanation for these results is the fact that the Gerloff et al. study was carried out after the pandemic event, and apparently there is cross-reactivity among pandemic A/H1N1 and SIV viruses, as the aforementioned and other authors have reported (Kyriakis et al., 2009).

Also, some characteristics of farms such as geographic location [OR 3.48, 95% (CI) 1.21–9.97], and number of breeding herd on the farm [OR 3.98, 95% (CI) 1.00–15.86], were associated with increased anti-SIV antibodies. The new industrial organization method for animal production in crowded swine farms has been argued to increase disease infections and to enhance the circulation of swine pathogen and transmission in the animal–human interface (Graham et al., 2008). This form of food production is not likely to change in the mid-term. Therefore, and as mentioned by other researchers (Gray and Kayali, 2009), we emphasize the urgent need for SIV occupational E workers to receive annual immunizations and educational prevention as part of the public health programs. Furthermore, it is important to improve work conditions, specially the use of protective equipment. Only 12.9% of farmers answered affirmatively that they used gloves during swine manipulation, which agrees with Gray et al. (2007), they observed that 14% of the workers wore gloves; although it was not possible to find any association in our study, it has been demonstrated by others as a risk factor for SI transmission (Ramirez et al., 2006).

In the analysis of our overall findings, we acknowledge that one of the limitations in this study was the small sample size. The generalization of our results is limited to Mexican workers. All the subjects worked in the farms that accepted to participate were included in the study; however, the UE subjects were not randomly selected, which may introduce a selection bias. It would be interesting to evaluate the seroprevalence with a higher sample size of our population and to use a more recently North American swine H3N2 strain. A primary strength of this study is that the results for SIV transmission and the risk factors for swine workers are the first evidence on this issue in México. The technique used to hemagglutinin inhibition assay was equally applied to both E and UE groups, taking care of the quality control; therefore, observation bias was minimized.

Our findings are relevant and support the evidence of zoonoses in swine farms and point out the need to imple-

ment preventive measures such as vaccination and to diminish the occurrence of the disease and the potential emergence of pathogenic reassortant strains. It is also important to promote the inclusion of susceptible groups in the prevention strategies and immunization protocols for influenza.

Acknowledgements

We thank to the farm owners and workers for their kindly participation and for all the facilities proportioned. We also thank to Karina Espinoza and Monica Resendiz for technical assistance. This study was funded by FONDO MIXTO Sonora-CONACYT project 6621.

References

- Ayora-Talavera, G., J.M. Cadavieco-Burgos, and A.B. Canul-Armas, 2005: Serologic evidence of human and swine influenza in Mayan persons. *Emerg. Infect. Dis.* 11, 158–161.
- Belshe, R.B., 2005: The origins of pandemic influenza – lessons from the 1918 virus. *N. Engl. J. Med.* 353, 2209–2211.
- Cannon, R.M., and R.T. Roe, 1982: *Livestock Disease Surveys: A Field Manual for Veterinarians*. Australian Government Publishing Service, Canberra.
- Fouchier, R.A., V. Munster, A. Wallensten, T.M. Bestebroer, S. Herfst, D. Smith, G.F. Rimmelzwaan, B. Olsen, and A.D. Osterhaus, 2005: Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. *J. Virol.* 79, 2814–2822.
- Gerloff, N.A., J.R. Kremer, E. Charpentier, A. Sausy, C.M. Olinger, P. Weicherding, J. Schuh, K. Van Reeth, and C.P. Muller, 2011: Swine influenza virus antibodies in humans, western Europe, 2009. *Emerg. Infect. Dis.* 17, 403–411.
- Graham, J.P., J.H. Leibler, L.B. Price, J.M. Otte, D.U. Pfeiffer, T. Tiensin, and E.K. Silbergeld, 2008: The animal–human interface and infectious disease in industrial food animal production: rethinking biosecurity and biocontainment. *Public Health Rep.* 123, 282–299.
- Gray, G.C., and G. Kayali, 2009: Facing pandemic influenza threats: the importance of including poultry and swine workers in preparedness plans. *Poult. Sci.* 88, 880–884.
- Gray, G.C., T. McCarthy, A.W. Capuano, S.F. Setterquist, C.W. Olsen, and M.C. Alavanja, 2007: Swine workers and swine influenza virus infections. *Emerg. Infect. Dis.* 13, 1871–1878.
- Kyriakis, C.S., C.W. Olsen, S. Carman, I.H. Brown, S.M. Brookes, J.V. Doorselaere, and K.V. Reeth, 2009: Serologic cross-reactivity with pandemic (H1N1) 2009 virus in pigs, Europe. *Emerg. Infect. Dis.* 16, 96–99.
- Myers, K.P., C.W. Olsen, S.F. Setterquist, A.W. Capuano, K.J. Donham, E.L. Thacker, J.A. Merchant, and G.C. Gray, 2006: Are swine workers in the United States at increased risk of infection with zoonotic influenza virus? *Clin. Infect. Dis.* 42, 14–20.

- Neumann, G., T. Noda, and Y. Kawaoka, 2009: Emergence and pandemic potential of swine-origin H1N1 influenza virus. *Nature* 459, 931–939.
- Olsen, C.W., 2002: The emergence of novel swine influenza viruses in North America. *Virus Res.* 85, 199–210.
- Olsen, C.W., L. Brammer, B.C. Easterday, N. Arden, E. Belay, I. Baker, and N.J. Cox, 2002: Serologic evidence of H1 swine Influenza virus infection in swine farm residents and employees. *Emerg. Infect. Dis.* 8, 814–819.
- Ramirez, A., A.W. Capuano, D.A. Wellman, K.A. Leshner, S.F. Setterquist, and G.C. Gray, 2006: Preventing zoonotic influenza virus infection. *Emerg. Infect. Dis.* 12, 996–1000.
- SAGARPA, 2011: Servicio de información agroalimentaria y pesquera. Available at <http://www.siap.sagarpa.gob.mx/> (accessed May 3, 2011).
- Smith, G.J., D. Vijaykrishna, J. Bahl, S.J. Lycett, M. Worobey, O.G. Pybus, S.K. Ma, C.L. Cheung, J. Raghvani, S. Bhatt, J.S. Peiris, Y. Guan, and A. Rambaut, 2009: Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. *Nature* 459, 1122–1125.
- Spackman, E., D.A. Senne, T.J. Myers, L.L. Bulaga, L.P. Garber, M.L. Perdue, K. Lohman, L.T. Daum, and D.L. Suarez, 2002: Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. *J. Clin. Microbiol.* 40, 3256–3260.
- Thacker, E., and B. Janke, 2008: Swine influenza virus: zoonotic potential and vaccination strategies for the control of avian and swine influenzas. *J. Infect. Dis.* 197(Suppl 1), S19–S24.
- Vincent, A.L., W. Ma, K.M. Lager, M.R. Gramer, J.A. Richt, and B.H. Janke, 2009: Characterization of a newly emerged genetic cluster of H1N1 and H1N2 swine influenza virus in the United States. *Virus Genes* 39, 176–185.
- Webster, R.G., W.J. Bean, O.T. Gorman, T.M. Chambers, and Y. Kawaoka, 1992: Evolution and ecology of influenza A viruses. *Microbiol. Rev.* 56, 152–179.
- Zhou, N.N., D.A. Senne, J.S. Landgraf, S.L. Swenson, G. Erickson, K. Rossow, L. Liu, K. Yoon, S. Krauss, and R.G. Webster, 1999: Genetic reassortment of avian, swine, and human influenza A viruses in American pigs. *J. Virol.* 73, 8851–8856.