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ORIGINAL ARTICLE

Serological study of influenza viruses in veterinarians working with swine in Mexico

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Abstract Humans and swine are both affected by influenza viruses, and swine are considered a potential source of new influenza viruses. Transmission of influenza viruses across species is well documented. The aim of this study was to evaluate the seroprevalence of different influenza virus subtypes in veterinarians working for the Mexican swine industry, using a hemagglutination inhibition test. All sera tested were collected in July 2011. The data were analysed using a generalized linear model and a linear model to study the possible association of seroprevalence with the age of the veterinarian, vaccination status, and biosecurity level of the farm where they work. The observed seroprevalence was 12.3%, 76.5%, 46.9%, and 11.1% for the human subtypes of pandemic influenza virus (pH1N1), seasonal human influenza virus (hH1N1), the swine subtypes of classical swine influenza virus (swH1N1), and triple-reassortant swine influenza virus (swH3N2), respectively. Statistical analysis indicated that age was associated with hH1N1 seroprevalence ($P < 0.05$). Similarly, age and vaccination were associated with pH1N1 seroprevalence ($P < 0.05$). On the other hand, none of the studied factors were associated with swH1N1 and swH3N2 seroprevalence. All of the pH1N1-positive sera were from vaccinated veterinarians, whereas all of those not vaccinated tested negative for this subtype. Our findings suggest that, between the onset of the 2009 pandemic and July 2011, the Mexican veterinarians working in the swine industry did not have immunity to the pH1N1 virus; hence, they would have been at risk for infection with this virus if this subtype had been circulating in swine in Mexico prior to 2011.

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Introduction

Influenza A virus (H1N1) was isolated for the first time in swine in 1930 [1]. This virus belongs to the family *Orthomyxoviridae*, which includes two other types, B and C [2–4].

Influenza A viruses infect a large variety of species, including fowl, swine, humans, and horses. Swine play a very important role in interspecies transmission [5]. The interspecies barriers to transmission of influenza virus between humans and swine are not rigorous [6, 7]. Influenza virus infection in swine is relevant, as these animals are capable of expressing the necessary cellular receptors to facilitate recombination of viruses from different species, including humans (N-acetylneuraminic acid-α2,6-galactose) and birds (N-acetylneuraminic acid-α2,3-galactose) [8]. Some avian-type porcine viruses acquire the ability to recognize human receptors, thereby increasing the likelihood of their transmission to humans. As a result, swine are considered the “mixing vessel” for the generation of new reassortant viruses with pandemic potential [9].

In Mexico, records from the period of 2009-2011 made by the General Agency of Epidemiology, through the

National Epidemiological Surveillance System, indicate that the number of human deaths each year due to infection with non-typed influenza virus was 1744 (1.6%), 410 (0.4%), and 135 (0.1%), respectively [10].

The initial outbreak of the new influenza virus in 2009 had a mortality rate of 0.6% [11], which increased to 2.2% in Mexico [12]. In Mexico, the cases of this new influenza virus, pH1N1, occurring in 2009, were most frequent in the 15- to 50-year-old age group. A possible explanation for this epidemiological distribution could be that adults, particularly those over 60 years of age, have some type of cross-reactive antibody response to the pandemic strains and thus avoided infection [13].

Influenza viruses have been evolving continually. Recent studies have revealed complex relationships among antigenic evolution, genetic evolution, natural selection and the frequent reassortment of influenza viruses [14, 15]. The most common subtypes circulating in humans include H1N1, H1N2, and H3N2, which cause seasonal influenza [16, 17]. It is probable that the influenza viruses circulating in swine, H1N1, H3N2, and H1N2, are closely related to human strains [18]. Subtypes H1N2, H1N1, and H3N2 are the most frequently circulating subtypes in a large number of countries, but neither the human nor the swine H1N2 subtype has been reported in Mexico [19, 20]. Diverse studies have shown that the exposure to swine by personnel working with them serves as a bridge between animals and humans [8, 21]. Although there have been sporadic infections in humans with the classical H1N1 swine and triple-reassortant viruses, only a few cases have been documented in exposed workers [21]. Occupational exposure to swine considerably increases the risk of infection with swine influenza virus subtypes and the pandemic 2009 virus [18, 22]. At a global level, there are antecedents of the transmission of the pandemic virus from humans to swine [22–24]. On the other hand, despite the fact that positive serology and sporadic isolations of the swine influenza virus have been reported for human infections in several countries [16, 25, 26], the seroprevalence in swine-specialist veterinarians in Mexico is not known. The objective of this study was to determine the seroprevalence of human influenza viruses pH1N1 (pandemic) and hH1N1 (seasonal), as well as the swine influenza viruses swH1N1 and swH3N2, in swine-specialist veterinarians in Mexico.

Materials and methods

Study population

We processed a total of 81 serum samples obtained from veterinarians at the Meeting of the Association of Mexican Veterinarians Specialized in Swine (AMVEC in Spanish) held in Puerto Vallarta, Jalisco, Mexico, in July 2011. Of these samples, 79 were obtained from swine specialist veterinarians who came from different federal states of Mexico, whereas the other two were obtained from international attendees (one from the Netherlands and one from Costa Rica). The Executive Council of AMVEC authorized blood sampling. Samples were obtained from veterinarians who provided consent to participate in the study. Samples were collected in Vacutainer® tubes at an approximate volume of 5 mL; the sera were centrifuged and stored at -20°C until hemagglutination inhibition (HI) tests were performed. All participants provided consent for the publication of results; personal data were handled confidentially. Each sampled individual answered a questionnaire, which was then used for the analysis of variables.

Viruses

The following influenza subtypes were used as antigens: seasonal human influenza (hH1N1) A/Mexico/INER1/2000 (H1N1) (GenBank accession number JN086908), pandemic influenza (pH1N1) A/Mexico/LaGloria-3/2009 (H1N1) (GenBank accession number CY077595), classical swine (swH1N1) A/swine/New Jersey/11/76 (H1N1) (GenBank accession number K00992), and a triple-reassortant swine influenza virus (swH3N2) A/swine/Minnesota/9088-2/98 (H3N2) (GenBank accession number AF153234). Viruses were grown according to a previously described protocol [27].

Hemagglutination inhibition assay

We used the procedure established by the World Health Organization [28], with the following modifications: hemagglutinating units (HAU) were adjusted to 8. Sera were inactivated at 56 °C and adsorbed with kaolin and 5% chicken erythrocytes. Briefly, the serum was diluted using twofold serial dilutions from 1:20 to 1:5120. Afterwards, the diluted sera were mixed with 8 HAU of each virus and incubated for 30 min at ambient temperature (21 °C). At the end of this incubation, 0.5% chicken erythrocytes were added, and the mixture was left to incubate for an additional 30 min at ambient temperature. Readings were taken at the end of the incubation period. Titers of sera were considered positive if they were $\geq$1:80.

Statistical analysis

Antibody titers were normalized using the $\log_2$ transformation. A generalized linear model was used to assess seropositivity and its association with different factors. During the survey, we collected the following data: sex, age, number of years of veterinary practice with swine, vaccination history against seasonal human influenza and/or pandemic influenza virus, units of porcine production managed, production region in Mexico (northwestern, northeastern, central-western, central, and southeastern), and the level of biosecurity on the farms (low, medium, or high). Only the following variables were statistically analysed: sex, vaccination, and level of biosecurity (because only these variables had complete data). The missing variables were included only in the descriptive analysis. All statistical analyses were performed using JMP® 9.0 software (SAS Institute Inc., Cary, NC, USA).

Results

The number of seropositive subjects, the average titer, the seroprevalence, and the percentage seropositive for pH1N1, hH1N1, swH1N1, and swH3N2 are presented in Table 1. Among the participating veterinarians, 79% were men, with an average age of 42.2 years (range 20-63 years), 21% were women, and their average age was 35.8 years (range 23-53 years). Among the final sample of 81 surveyed veterinarians, 79 were Mexicans and two were foreigners; their average age was 40.8 years, and the average time of exposure was 15.2 years. These data are shown in Table 1.

Statistical analysis revealed that in the entire group of veterinarians (vaccinated and unvaccinated, n = 81), the age and vaccination effects had a significant association with pH1N1 seropositivity ($P < 0.049$, and $P < 0.0001$, respectively). These data indicate that a higher age of the interviewed veterinarians was associated with a larger number of samples with antibodies and that the vaccinated individuals tested positive. Age was significantly associated with hH1N1 seropositivity ($P < 0.0002$), and this finding indicates that seropositivity for human influenza viruses increased with age; this observation is independent of whether people work with swine, as these viral subtypes circulate in the human population. Regarding swH1N1, only a marginal effect was found ($P = 0.057$) to be associated with seroprevalence, suggesting that older veterinarians have had more contact with swine over time and therefore have a higher probability of a humoral immune response against swH1N1. For subtype swH3N2, none of the assessed variables were significantly correlated with seropositivity ($P > 0.05$). The result was not significant, as this subtype is less common in swine [29]; thus, there is a lower probability of transmission to humans.

Among vaccinated veterinarians (vaccinated individuals, n = 29), for subtype pH1N1, age had a significant association ($P = 0.048$). In Mexico, there is a vaccination campaign that considers the elderly as candidates for vaccination; hence, the data are consistent with the observation that there is a higher seropositivity for the pandemic virus in older veterinarians. For subtype hH1N1, the biosecurity level was significant ($P = 0.0094$). Although this variable was statistically significant, it does not correspond to an expected biological effect. For subtype swH1N1, age was significant ($P = 0.012$), and the age effect corresponds to that described previously. For subtype swH3N2, none of the variables were significant ($P > 0.05$) in the studied group.

When analysing only unvaccinated veterinarians (unvaccinated, n = 52), age was statistically significant for subtype hH1N1 ($P = 0.0006$); in this group, the level of significance is consistent with expected behaviour, as exposure to the seasonal virus is greater with time, whereas for subtypes swH1N1 and swH3N2, none of the variables were statistically significant ($P > 0.05$). The P-values for the different effects among the viruses are shown in Table 2.

Positive sera from swine analysed for subtypes hH1N1 and swH1N1 yielded higher titers (1:1280) as compared to swH3N2 and pH1N1 (1:160 and 1:320 respectively). The frequency and average of the antibody titers for each subtype are shown in Table 3. On the other hand, Tukey test results indicated that the titer of the antibodies for subtype hH1N1 did not differ significantly from those observed for subtype pH1N1. However, the titer recorded for hH1N1 differed significantly from those for swH1N1 and swH3N2 ($P < 0.05$). No significant differences were observed among the values for pH1N1, swH1N1, and swH3N2 ($P > 0.05$) (Table 3).

Table 4 summarizes the results of tests of the antibody response when two, three, or four subtypes were present simultaneously. For pH1N1, when in combination with subtype hH1N1, there were 10 veterinarians with a positive humoral response to these two subtypes in which the antibody titer against the hH1N1 virus was five times higher than that against pH1N1.

In seven of the veterinarians in whom antibodies were found against both pH1N1 and swH1N1 simultaneously, the swH1N1 virus generated a response with titers two times higher than those of pH1N1. In combination with swH3N2, there were three veterinarians who were positive for this combination of subtypes, and in the three cases, the titer was higher for subtype pH1N1.

In individuals with antibodies against both hH1N1 and swH1N1, there were 33 veterinarians with a positive response; among these veterinarians, 16 had higher titers for subtype hH1N1. When swH3N2 simultaneously was present, there were eight veterinarians with a positive response; among these veterinarians, seven had higher titers for subtype hH1N1.

In individuals with antibodies against both swH1N and swH3N2, there were six veterinarians with a positive

Table 1 Number seropositive, average titer, seroprevalence and percentage of veterinarians according to variable and virus subtype

Variable	*n*	pH1N1	hH1N1	swH1N1	swH3N2
Total	81				
Seropositive		10	62	38	9
Seroprevalence % [95% CI]		12.3 [6.5-20.3]	76.5 [62.6-81.2]	46.9 [34.5-55.2]	11.11 [5.6-18.9]
Average titer		1:168	1:260	1:189	1:98
Biosecurity level at the farm					
Low	5	0 (0)	3 (60)	2 (40)	0 (0)
Medium	15	1 (6.7)	11 (73.3)	6 (40)	3 (20)
High	61	9 (14.7)	48 (78.7)	30 (49.2)	6 (9.8)
Influenza vaccination					
Yes	29	10 (34.5)	22 (75.9)	15 (51.7)	4 (13.8)
No	52	0 (0)	40 (76.9)	23 (44.2)	5 (9.6)
Sex**					
Men	64 (79)	9 (11.1)	52(64.2)	31 (38.3)	9 (11.1)
Women	17 (21)	1 (1.2)	10 (12.3)	7 (8.6)	0 (0)
Mean age	40.8	47.6	43.3	43.1	45.1
Age (ranges)**					
20-30 years		0	7	5	2
31-41 years		2	18	9	2
42-52 years		6	28	18	4
53-63 years		2	9	6	1
Mean exposed years**	15.2	23.6	17.1	18.1	16.7
Region**					
Northwestern	8 (9.9)	2 (2.5)	6 (7.4)	4 (4.9)	1 (1.2)
Northeastern	1 (1.2)	0 (0)	1 (1.2)	1 (1.2)	0 (0)
Central-western	37 (45.7)	5 (6.2)	31 (38.3)	18 (22.2)	3 (3.7)
Central	28 (34.5)	1 (1.2)	18 (22.2)	12 (14.8)	4 (4.9)
Southeast	3 (3.7)	0 (0)	3 (3.7)	1 (1.2)	0 (0)
International	2 (2.5)	1 (1.2)	2 (2.5)	1 (1.2)	1 (1.2)
Units of porcine production managed**					
0	9	0	6	3	0
1	23	2	13	7	1
2	23	2	11	7	3
3	27	4	12	8	3
4	10	0	5	4	1
5	17	1	8	7	1
>6	9	1	6	2	0

Missing data are due to failure to fully answer the questionnaire. ** these variables were not included in the statistical analysis

[ ] = confidence interval. () = percentage of positive veterinarians to each variable

response; among these veterinarians, four had higher titers of subtype swH1N1.

At no time were the titers to subtype swH3N2 higher than those to the other subtypes.

In the case where veterinarians generated antibodies simultaneously against three subtypes, seven were positive against pH1N1, hH1N1, and swH1N1; in this case, two veterinarians had higher titers to subtype swH1N1. On the other hand, three veterinarians had antibodies against pH1N1, hH1N1, and swH3N2; in this case, subtype hH1N1 was the one with the highest titer compared to the other two. In addition, three veterinarians had antibodies against pH1N1, swH1N1, and swH3N2; in this case, the titers against swH1N1 were the highest. Three veterinarians had antibodies against hH1N1, swH1N1, and swH3N2; here, the titers against subtype hH1N1 were the highest.

Table 2 Level of significance of the studied effects according to virus subtypes analyzed in the different groups of veterinarians

Variable	pH1N1	hH1N1	swH1N1	swH3N2
All veterinarians (n = 81)				
Age	[0.0491]*	[0.0002]*	[0.0569]°	[0.3386]
Biosecurity level at the farm	[0.2742]	[0.1977]	[0.6749]	[0.2705]
Vaccinated	[<0.0001]*	[0.4215]	[0.7853]	[0.5959]
Vaccinated veterinarians (n = 29)				
Age	[0.0476]*	[0.3229]	[0.0122]*	[0.1716]
Biosecurity level at the farm	[0.4486]	[0.0094]**	[0.1071]	[0.6056]
Unvaccinated veterinarians (n= 52)				
Age	[NC]	[0.0006]*	[0.6357]	[0.8748]
Biosecurity level at the farm	[NC]	[0.6338]	[0.4272]	[0.4945]

[P value], *, Statistically significant; ° = Marginal; NC = noncomputable; **, this effect although registered as significant, does not have epidemiological relevance, as it is not a dependent variable

Table 3 Average and frequency of antibody titers against the four influenza subtypes in the sampled population

Titer	pH1N1	hH1N1	swH1N1	swH3N2
<40	62	5	33	57
1:40	9	14	10	15
1:80	3	16	18	7
1:160	5	22	14	2
1:320	2	11	3	-
1:640	-	12	2	-
1:1280	-	1	1	-
Average*	$(7.1)^{a,b}$	$(7.6)^{a}$	$(7.0)^{b}$	$(6.4)^{b}$

* Average based on log2 transformed data

Different superscript letters indicate statistically significant difference ($P < 0.05$)

Finally, three veterinarians had antibodies against pH1N1, hH1N1, swH1N1, and swH3N2 simultaneously; in this case, the subtype swH1N1 generated the highest titer.

Discussion

Serological evidence of transmission of swine influenza virus subtypes among swine specialist veterinarians is considered positive when the titer is at least 1:40 [30]. The possibility of cross-reactivity among hH1N1, pH1N1 and, swH1N1 exists. However, starting with a dilution of 1:160, hH1N1 becomes the most frequently observed virus. It has also been observed that the same sample can be positive for all three subtypes; however, the antibody titers against one subtype were much higher than those against the other subtypes, and similar titers for the three subtypes were never observed. A person can have antibodies against one or more subtypes at the same time, and the existence of cross-reactivity with different subtypes is also possible; however, the higher titers indicate a specific response and appear to rule out cross-reactivity among the subtypes. Instead, the presence of antibodies against more than one subtype may be a result of a recent infection [22]. In the present study, a titer of $\geq$ 1:80 was considered the cutoff point, thereby increasing the specificity of the HI test used [27].

Influenza infection is a global endemic disease [31]. However, there are no previous reports of serological studies on veterinarians in Mexico. In the present serological evaluation, we used only lineages that circulate in Mexico. Other studies in Mexico have used strains of European and North American lineages, such as A/Bayern/7/95 (H1N1) (GenBank: EF566037.1), A/Sydney/5/97 (H3N2) (GenBank: EF566075.1), A/Swine/Wisconsin/238/97 (H1N1) (GenBank: AF222033.2), A/Swine/Minnesota/593/99 (H3N2) (GenBank: AF251427.2), A/NewCaledonia/20/99 (H1N1) (GenBank: AY289929.1), A/Panama/2007/99 (H3N2) (GenBank: DQ487340), and A/Swine/England/163266/87 (H3N2) (GenBank: CY115996) [32, 33]; therefore, a higher seropositivity was observed in the present study.

In studies by López Robles et al., Ayora-Talabera et al., and Fragaszy et al [22, 32, 33], the average seroprevalence for subtype swH1N1 was lower than that for swH3N2, and these researchers concluded that this subtype circulates more frequently than swH1N1. This conclusion is in contrast to our assessment that antibodies against subtype swH1N1 were present in veterinarians at higher frequency and with higher titers than those against swH3N2, similar to what has been observed in swine on farms [29].

In Iowa, a seroprevalence of 12.4% against swH1N1 has been observed in individuals exposed to swine, and this was reportedly associated with variables such as sex, age, years of porcine production management, number of days working with swine, use of protection equipment, recent

Table 4 Combination of subtypes that simultaneously generated a humoral response in the veterinarians, the number of times each subtype was associated with the highest titer, and the average titer of antibodies

Subtypes that simultaneously generated positive results	Times each subtype was associated with the highest titer (average)*	Total average antibody titer (log_2)			
		pH1N1	hH1N1	swH1N1	swH3N2
Two subtypes					
pH1N1-hH1N1 (10)	2 (7.82) - 5 (8.92)	7.22	7.82	NA	NA
pH1N1- swH1N1 (7)	2 (7.82) - 2 (9.82)	7.46	NA	7.75	NA
pH1N1-swH3N2 (3)	3 (7.98) - 0	7.98	NA	NA	6.65
hH1N1-swH1N1 (33)	16 (8.57) – 8 (8.45)	NA	7.84	7.2	NA
hH1N1-swH3N2 (8)	7 (8.46) - 0	NA	8.19	NA	6.44
swH1N1-swH3N2 (6)	4 (8.32) - 0	NA	NA	7.82	6.65
Three subtypes					
pH1N1-hH1N1-swH1N1 (7)	1 (8.32) – 3 (8.98) – 2 (9.82)	7.46	8.32	7.75	NA
pH1N1-hH1N1-swH3N2 (3)	1 (8.32) – 1 (9.32) – 0	8.65	7.98	NA	6.65
pH1N1-swH1N1-swH3N2 (3)	1 (8.32) – 1 (10.32) – 0	7.98	NA	8.3	6.65
hH1N1-swH1N1-swH3N2 (3)	1 (9.32) – 1 (8.32) – 0	NA	7.65	7.32	6.32
Four subtypes					
pH1N1-hH1N1-swH1N1-swH3N2 (3)	1 (8.32) – 1 (9.32) – 1 (10.32) - 0	7.98	8.65	8.32	6.65

*Average is based on log2 transformed data, and the missing data correspond to equal titers. NA = not applicable

exposure, number of swine on the farm, and type of farm [34]. In Wisconsin, different variables were analysed, and age (≤50 years) and vaccination history were significantly associated factors, whereas the number of working hours was not [30]. In these previous studies, a titer of 1:40 was used as the cutoff, in contrast to the 1:80 used in the present study. In July 2009, a study was conducted on a farm in Alberta, Canada, and it was determined that workers exposed to swine had a seroprevalence of 67% against subtype pH1N1 (including permanent staff, researchers, and students) [35]. This finding is different from our results of seroprevalence in veterinarians (12.3%), indicating that veterinarians may be less susceptible than personnel exposed to swine on a daily basis. In veterinarians, a seroprevalence of 22.7% against subtype swH3N2 has been reported in Germany for the period from December 2007 to April 2009 [16].

We found seropositivity against the four analysed subtypes. The average antibody titer was highest for subtype hH1N1, followed by subtype swH1N1. Therefore, our results imply that seropositivity for hH1N1 is achieved by transmission of the infection among humans, whereas seropositivity for pH1N1 virus originated from vaccination, and seropositivity for swH1N1 was due to exposure to swine [16, 21, 32].

In our study, we observed lower seroprevalence (11.1%) for the swH3N2 virus. However, since seropositivity was observed, the possibility of transmission of the swine virus to humans cannot be ignored. We detected antibodies against swH3N2 in veterinarians who work with swine; however, there is the possibility of cross-reactivity with viruses H3N2 that circulate in humans, and there are studies supporting this notion [36].

Limitations of the present study include the use of old strains and the omission of a control group of non-veterinarians. In addition, we obtained more male samples than female samples because there are more men than women involved in porcine production in Mexico. The largest number of seropositive samples was for hH1N1, indicating that this subtype is circulating among the population of veterinarians; however, the pH1N1-positive results corresponded to veterinarians who had been vaccinated against this subtype. We observed that most of the veterinarians who answered the questionnaire work at farms with a high biosecurity level. In addition, the largest number of positive samples corresponded to subtype hH1N1. Regarding biosecurity, there were no significant effects; however, numerically, there was a higher proportion in high-biosecurity farms. According to our classification, farms with a higher level of biosecurity correspond to farms with a larger number of animals. The high density of swine favors dissemination of infections. Although it is true that with higher biosecurity (an arbitrary and not objective parameter) the entry of pathogens is diminished, it does not mean that pathogens already inside the farm are reduced, especially in farms where many infections are endemic, as is the case of swine influenza virus.

The number of positive results for influenza virus obtained in this study was higher in the central and central-western regions of the country; these two regions comprise more than 70% of the porcine production of the country [37].

One of the recommendations given at the time of the pandemic was that persons with flu-like symptoms should stay home from work. In Mexico, it was recommended that workers in contact with swine receive the vaccination against human influenza each year. In Mexico, the populations that are usually vaccinated are children and the elderly. According to the results obtained in the present study, the seroprevalence against the pH1N1 virus in the swine-specialist veterinarians (12.3%) was lower than that in the general population (20%) [38]. The results of this study are consistent with those of other studies in which there is evidence of dissemination of the virus (swine influenza H1N1 and swine influenza H3N2) from swine to veterinarians [18, 39].

Age and vaccination were significantly associated with pH1N1 seropositivity. Similarly, age was significantly associated with hH1N1 seropositivity and swH1N1 seropositivity. None of the variables were significantly associated with swH3N2 seropositivity, as sera positive for swH3N2 were scarce. These findings fail to establish a relationship among age, vaccination, biosecurity level, and sex. Despite having worked directly with swine for years, the veterinarians' level of infection for the latter subtype was low. Even though seropositivity was not significant, veterinarians could still be infected with this subtype. The lack of a significant effect of subtype swH3N2 suggests that there was no cross-reactivity antigenicity exists in the veterinarians vaccinated with the vaccinal component H3N2, which is usually included in the vaccine applied to humans.

Our results indicate that among veterinarians working with swine in Mexico, the risk of infection with influenza viruses pH1N1, hH1N1, and swH1N1 is higher in older veterinarians; however, the pH1N1 and hH1N1 seropositivity values are not known for those working on swine farms.

The present findings could prove instrumental in determining the seroprevalence of swine influenza virus among veterinarians in Mexico and in establishing associated risk factors. The pH1N1-positive results from veterinarians are due to vaccinations, whereas unvaccinated veterinarians tested negative for this subtype. Hence, it can be concluded that after the 2009 influenza pandemic, the veterinarians working in porcine production in Mexico have not been at risk for possible transmission of the pandemic virus to swine, at least, not at the moment. Studies by Nelson and coworkers have clearly indicated the transmission of the influenza virus from humans to swine [14, 23, 24]; however, in our work, the detection of antibodies to the pH1N1 subtype indicates that these originated from vaccination, at least in this group of veterinarians.

If veterinarians were transmitting the pandemic virus, the number of positive samples to this subtype would be similar to or higher than that of seasonal hH1N1 influenza, but this phenomenon did not occur. This hypothesis was proposed after the 2009 pandemic and may be rejected according to the present results.

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Compliance with ethical standards

This article does not contain any studies with animals performed by any of the authors.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Universidad Nacional Autónoma de México and the Consejo Nacional de Ciencia y Tecnología (Mexico).

Informed consent Informed consent was obtained from all individual participants included in the study.

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