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Short Article

Serological evidence of swine influenza in Brazil

Daniela S. Rajão,[a] Fabiana Alves,[a] Helen L. Del Puerto,[b] Gissandra F. Braz,[a] Fernanda G. Oliveira,[a] Janice R. Ciacci-Zanella,[c] Rejane Schaefer,[c] Jenner K. P. dos Reis,[a] Roberto M. C. Guedes,[d] Zélia I. P. Lobato,[a] Rômulo C. Leite[a]

[a]Preventive Veterinary Medicine Department, Veterinary School, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. [b]Department of General Pathology, Institute of Biological Science, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. [c]Virology Laboratory, EMBRAPA Suínos e Aves, Concórdia, Brazil. [d]Veterinary Clinic and Surgery Department, Veterinary School, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

Correspondence: Daniela S. Rajão, Preventive Veterinary Medicine Department, Veterinary School, Universidade Federal de Minas Gerais, 6627 Presidente Antônio Carlos Ave, POBox 567, Campus UFMG, Belo Horizonte, Minas Gerais, Brazil 31270-901. E-mail: rajao.ds@gmail.com

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The aim of this work was to detect serum antibodies specific to influenza viruses in swine in Brazil. Serum samples of 355 pigs from 17 herds in Minas Gerais state were tested by hemagglutination inhibition (HI) for antibodies against H1N1 swine (SIV) and human influenza viruses, and H3N2 SIV. HI revealed that 158 animals (44·5%) and 11 herds (64·7%) were positive for H1N1 SIV, 36 animals (10·1%) and four herds (23·5%) were positive for H3N2 SIV, and 136 animals (38·3%) and 10 herds (58·8%) were positive for H1N1 human. This study indicates that swine influenza is disseminated throughout Minas Gerais state, Brazil.

Keywords Hemagglutination inhibition test, herd, influenza, pigs, swine.

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Introduction

Swine influenza virus (SIV) is an *Orthomixovirus* that causes an acute respiratory disease in pigs. The disease is characterized by sudden and explosive outbreaks, with high morbidity and low mortality.[1] Common clinical signs are hyperthermia, anorexia, coughing, and nasal discharge.[2] Since its first report in 1931,[3] three subtypes of influenza A virus have been circulating in swine populations worldwide (H1N1, H1N2, and H3N2). However, these SIV subtypes differ in origins and genetic characteristics in different continents and regions.[1]

Classical swine H1N1 influenza virus (cH1N1) was the predominant SIV subtype circulating in the United States,[1] but the introduction of a H3N2 subtype in 1998 led to reassortments that resulted in the circulation of other H1N1 viruses, H3N2 viruses, and novel subtypes like H1N2.[4] In Europe, avian-like H1N1 has become the predominant subtype infecting swine populations, but reassortant swine H3N2 virus is also endemic among European pig herds.[5]

Swine influenza virus infection in Brazilian swine population is not well characterized, and only a few data demonstrate evidence of SIV infection by serological diagnosis.[6,7] Minas Gerais is the fourth largest swine-producing state in Brazil, accounting for 12·9% of the country's pig population. Therefore, the objective of this study was to evaluate the presence of anti-swine and anti-human influenza virus antibodies in swineherds in Minas Gerais state so as to demonstrate the circulation of SIV in Brazil.

Materials and methods

A total of 355 serum samples from a diagnostic laboratory sera panel were used for this study. Blood samples were collected between January and March 2009 previously to the H1N1 pandemic occurrence, by jugular puncture, centrifuged after clot formation, and the serum kept at −20°C until used. Sample size was based on financial and availability limitations. At least 10 breeding-age animals (sows and gilts) were sampled per farm, from 17 commercial herds randomly distributed in Minas Gerais state, Brazil. All herds were farrow-to-finish operations with all-in-all-out system, located in pig densely populated areas, and with no SIV vaccination history. No respiratory signs were reported in any sampled pig. This study did not have an ethics committee approval because all samples were sent by the herd owners, previously to the start of the study, to the diagnostic laboratory for diagnostic purposes unrelated to this study. All herd owners gave their consent for the use of the sera in this study.

Hemagglutination inhibition test (HI) was performed as previously described.[8] Briefly, sera were heat inactivated at 56°C, followed by a treatment with a 20% Kaolin suspension and adsorption with 0·5% rooster red blood cells (RBC) suspension to remove non-specific inhibitors and natural serum agglutinins. For HI against H3N2 SIV, sera were treated with trypsin–potassium periodate (KIO4), as previously described.[9] The initial serum dilution was 1:10 using phosphate-buffered saline (PBS; pH 7·4), and then each sample was twofold diluted to a final dilution of 1:10,240 in 96-well V-bottom plates. Samples were tested for HI activity against 4 hemagglutination units of H1N1 SIV reference strain (A/swine/Iowa/15/1930), H3N2 SIV reference strain (A/swine/Iowa/8548-2/98), and H1N1 human reference strain (A/WSN/1933) grown in specific pathogen-free 10-day-old embryonated chicken eggs (passage number 3). HI antibody titer of each sample was determined as the reciprocal of highest dilution in which no hemagglutination was observed, and a sample was considered positive if it had HI titer equal or above the cutoff value of 1:40, as lower titers may be due to non-specific reactions. A herd was considered positive when at least one of the animals sampled were positive. Ninety-five percent confidence intervals (CIs) were calculated for herd and animal percentages, and descriptive statistic was calculated for antibody titer in positive and negative herds.

Results

Hemagglutination inhibition results are summarized in Table 1. Of the 355 sera tested, 158 (44·5%) had antibodies against H1N1 SIV, 36 (10·1%) against H3N2 SIV, and 136 (38·3%) against H1N1 human. Of the 17 herds tested, 11 (64·7%) were considered positive for H1N1 SIV, four (23·5%) for H3N2 SIV, and 10 (58·8%) for H1N1 human, and the percentages of infected and non-infected animals in positive farms for each virus are shown in Figure 1. The percentage of breeding females positive for multiple antigens was calculated (Table 2). A higher percentage of animals positive to both H1N1 viruses (20·84%) than to only one of them (16·05% to H1N1 SIV and 11·83% to H1N1 human) was found. A few animals were positive for both SIV viruses (3·38%), and a small percentage was also positive for all three antigens (4·22%). Mean HI titers for positive and negative herds are shown in Table 3.

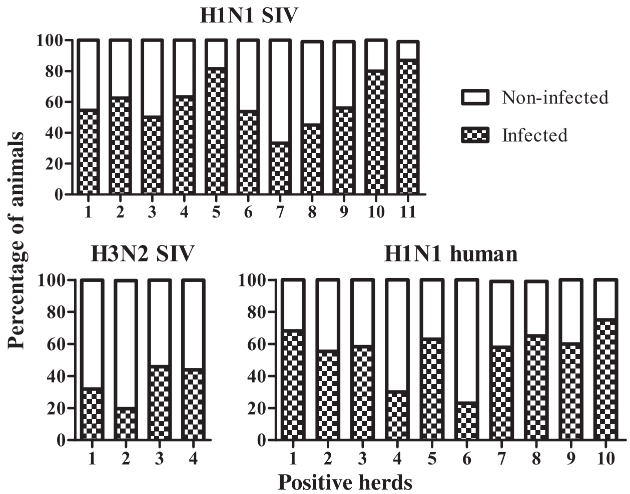

Figure 1. Percentage of infected and non-infected animals in positive herds. Hemagglutination inhibition was performed with H1N1 swine influenza virus (SIV), H3N2 SIV, and H1N1 human influenza virus.

Discussion

Our results demonstrate a high occurrence of anti-H1N1 influenza antibodies in swine in Minas Gerais state's herds, which are likely to be due to previous infection because no vaccines are available in the country. However, anti-H3N2 SIV antibodies occurrence was lower than those found for H1N1 swine and human viruses, and even positive herds

Table 1. Pig- and herd-level seroprevalence of H1N1 and H3N2 swine influenza viruses (SIV) and H1N1 human influenza virus in Minas Gerais state, Brazil

Variables	H1N1 SIV		H3N2 SIV		H1N1 human	
	Pig	Herd	Pig	Herd	Pig	Herd
Number of samples tested	355	17	355	17	355	17
Positive number	158	11	36	4	136	10
Prevalence, % (CI 95%)	44·5 (39·33–49·67)	64·7 (41·98–87·42)	10·1 (6·97–13·23)	23·5 (3·34–43·66)	38·3 (33·24–43·36)	58·8 (35·4–82·2)

CI, confidence interval.

Table 2. Percentage of animals with antibodies to multiple influenza virus antigens in Minas Gerais state, Brazil

	H1N1 SIV only	H3N2 SIV only	H1N1 human only	H1N1 SIV + H3N2 SIV	H1N1 SIV + H1N1 human	H3N2 SIV + H1N1 human	All 3 antigens
Number of animals	57	4	42	12	74	5	15
Percentage (%)	16·05	1·13	11·83	3·38	20·84	1·41	4·22

Table 3. Hemagglutination inhibition titers of positive and negative herds against H1N1 and H3N2 swine influenza viruses (SIV), and H1N1 human influenza virus in Minas Gerais state. Brazil

Variables	Positive herds			Negative herds		
	H1N1 SIV	H3N2 SIV	H1N1 human	H1N1 SIV	H3N2 SIV	H1N1 human
Number of herds	11	4	10	6	13	7
Minimum titer	29.97	14.64	19.10	11.89	10.34	10.68
Median	46.30	22.36	46.08	13.19	11.49	12.03
Maximum titer	146.72	26.70	118.19	16.62	14.32	20.00
Mean titer	59.34	21.02	47.50	13.58	11.59	13.27
Standard deviation	16.77	13.27	16.82	11.45	10.91	12.61
Standard error	11.69	11.52	11.79	10.57	10.25	10.91
CI 95%	41.90–83.98	13.41–32.97	32.76–68.92	11.78–15.66	11.00–12.22	10.71–16.44

CI, confidence interval.

showed a high percentage of non-infected animals. So far, this is one of the first studies to show SIV infection in Brazilian herds, and the first to show anti-influenza antibody prevalence in Minas Gerais state. Influenza virus has been identified previously in Brazilian pigs in São Paulo[6] and in Paraná[7] states.

The rates of 44·5% and 38·3% animals with antibodies against H1N1 swine and human viruses, respectively, found in the present study are similar to those in prevalence studies for H1N1 virus in the United States (66·3%),[10] Italy (46·4%), and Spain (38·5%).[5] However, the prevalence of anti-SIV H1N1 antibodies in another study in Spain seemed to be lower than the findings in the present study, and evidences show that a novel recently emerged H1N2 SIV is widespread in that country.[11] The rate of 10·1% of H3N2-positive animals found here was similar to that of 20% found in a recent study in Paraná state, Brazil,[7] and also similar to that found in Ireland (4·2%) previously.[5] However, it was significantly lower than those rates found in Italy (41·7%) and Spain (38%),[5] and in the United States (33·7%).[10]

The proportion of animals with antibodies to both H1N1 viruses was higher than the proportion of animals with antibodies to only one of them. Some animals were positive for both swine influenza viruses. In addition, a percentage of animals were also positive for all three antigens. Thus, different influenza virus strains are cocirculating in the Brazilian swine population and causing mixed infection, which may result in genetic reassortment between these viruses.

This study also showed herd-level prevalences of 64·7% for H1N1 SIV and 58·8% for human H1N1, which are close to the reported in Korean herds (71·5%).[12] In addition, a herd-level prevalence of 80·1% in saws was found in Canada,[13] which is slightly higher than our results. For H3N2, the herd-level prevalence was lower (23·5%) than that of H1N1 viruses, and also lower than in the recent study in Paraná state (46%).[7] Thus, HI results presented in this work indicate that influenza viruses are circulating in Minas Gerais state and may be endemic in Brazilian swine population.

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