SEROPREVALENCE OF H3N2 INFLUENZA A VIRUS IN PIGS FROM PARANÁ (SOUTH BRAZIL): INTERFERENCE OF THE ANIMAL MANAGEMENT AND CLIMATIC CONDITIONS

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ABSTRACT

Since the beginning of the 20th Century, Influenza can be understood as an illness associated to a viral infection, and its etiological agent can be better characterized. From then on, the swine species has occupied a prominent place in the Influenza epidemiology, for it allows the circulation and replication of viruses of potentially human and avian origin. The aim of the present study was to determine the current seroprevalence for the H3N2 virus subtype in two types of pig-raising systems in the state of Paraná-Brazil. The first one represents pig farms, with an adequate health, nutritional, and environmental management; and the second one represents pig runs, without an adequate health, nutritional, and environmental management. To accomplish these aims, 675 blood samples of pigs were analyzed by HI (hemagglutination inhibition) assay and the interference of the pig management and climatic conditions of each region were analyzed. The results showed that 46% of the pig farms are positive, as opposed to 6% of the pig runs; and 20% of the animals on pig farms are serum positive, as opposed to 3% of the animals in pig runs. Likewise, the samples coming from colder areas in Paraná presented, as a rule, a high positivity index. The results allow concluding that the virus is present in pig-raising facilities, and the strategies for preventing future epidemics and epizooties must consider the role of swine as an important factor in the epidemiology of influenza.

INTRODUCTION

The influenza virus was isolated in humans in 1933 by Smith and his collaborators, two years after Shope - in the USA - had demonstrated that swine influenza was caused by a virus (Van Epps 2006). That virus would be characterized, in the current proposal, as the type-A virus, which infects humans and several animal species. In 1936, Shope (Shope 1936) showed the incidence of antibodies against the virus in pigs and humans. Since the end of last century it has been shown that the pig is an important factor in the process of adaptation of the influenza virus to humans for, in its acute form, this virus could undergo ‘reassortment’, a form of genetic rearrangement, and after that it could replicate in humans with high efficiency (Reid & Taubenberger 2003). The characteristic of the segmented RNA genome of this virus makes this type of mutation possible - also called antigenic shift (Wright et al. 2007). The punctual
mutations, in not more than 3% of the genome, also common in other viruses, can cause changes across the viral structure, and the most antigenically significant are those that change the viral Neuraminidase (NA) and Haemagglutinin (HA) (Andreasen & Sasaki 2006). It is these two glycoproteins, embedded in the viral envelope, that allow classifying the type-A influenza virus into sub-types, and are often related to the viral pathogenicity. Up to now, 16 different kinds of HA and 9 different NA’s (Webster & Hulse, 2004) have been described. Restriction of influenza virus in certain species happens mainly because of the affinity of different viral subtypes, through viral hemagglutinin with receptors found in host cells. In eukaryotic cells, a varied range of glycoconjugates are profusely found in the plasma membrane and, among the carbohydrates present in the membrane glycoconjugates, the sialic acids stand out, which constitute a family of 9-carbon complex carbohydrates usually linked to other carbohydrates through α-cetosidic links, which occur in nature in about 50 types (De Fátima et al. 2005). The viral hemagglutinin is the main responsible for such restriction among hosts, mainly due to its role in the recognition and adsorption to the host’s cell. In order that such adsorption provokes a conformational change in the hemagglutinin, which is a trimer, it must be triggered off by the action of host’s proteases in a hemagglutinin site called ‘cleavage site’ (Tong et al. 1998). The endosome’s pH formed after adsorption and virus penetration is decisive for the fusion of the viral envelope with the cell membrane culminating in the success of the infection, when it is possible to observe the conformational changes of hemagglutinin that allow such fusion (Wharton et al. 1995).

Webster et al. (2006) have proposed that the emergence of highly pathogenic sub-types of the virus is related to the possibility of replication of the virus in several animal species and mainly to the density of the population in which the virus is present. According to the authors, these were relevant components in the emergence of the highly pathogenic H5N1 sub-type, which since 1997 has been causing several epizooties, raising the risk of a new influenza pandemic.

In pigs, the prevalence mainly of the H3N2 sub-type, besides the H1N1 sub-type, has been observed and, unlike in humans, it presents few antigenic and genetic changes over time (Peiris et al. 2001). Several studies suggested that swine workers have a higher risk of presenting infections with influenza viruses common to humans.
and pigs (Gray et al. 2007). The risk of reassortment can increase in situations where the pig takes part in the epidemiology of some sub-types and, as far as H3N2 is concerned, with evidence that that possibility is real and current (Zhou et al. 2000).

Given the risks that pigs represent in the epidemiology of influenza, the present study aims at determining the seroprevalence in pigs for human H3N2 sub-type in the state of Paraná and correlating the data on the provenance of the samples - such as health-food management, climate, and especially density - with the obtained results. A discussion is proposed to allow correlating, in the event of high prevalence in pigs, the risks of generating a pandemic and the increased susceptibility of these workers.

MATERIAL AND METHODS

Pig serum samples were collected in several counties in the State of Paraná, Brazil, from properties divided into runs (mostly with small number of animals without adequate health and nutritional management) and farms (properties with an adequate nutritional and health management). Out of a total of 675 samples, 482 came from 74 pig farms, and 193 came from 90 pig runs. Blood was aseptically collected by venipuncture, and the serum was separated for testing. Animals of various ages and both sexes were randomly sampled (samples obtained from Brazilian National Program for Swine Health (Programa Nacional de Sanidade Suídea - PNSs) of the Ministry of Agriculture, Cattle Raising, and Provision (Ministério da Agricultura Pecuária e Abastecimento, MAPA).

Haemagglutination inhibition (HI) tests were carried out to determine the titer of serum antibodies in the microplates of 96 V-bottom wells, following the standard procedure of the WHO- Manual on Animal Influenza Diagnosis and Surveillance, and using Kaolin instead of RDE and turkey red blood cells (WHO 2002).

Standard hyper-immune serum (A/PANAMA/2007/99/H3N2-CDC-WHO) was used as positive control and the viral sub-type of the HI test was the human H3N2 (nº 223) granted by the virology laboratory of the HC-UFPR (The UFPR-University Hospital), which presented the highest titer in the Hemagglutination (HA) test using turkey instead of rooster red blood cells.

The red blood cells were used in suspension at 0.75%, after three successive 5-minute washes at 1200 rpm in PBS, in a refrigerated centrifuge at 8°C. An HI reading
was done after two hours, and only samples with a titer equal or higher than 1:10 were considered positive.

For the risk factor analysis, the categorical variables were reduced to as few categories as possible, ideally two. Univariate odds ratios (univariable, unilevel) were calculated taking into account two types of pig-raising systems and the temperature of the area. The analyses were carried out using Fischer test and the Statview® Program.

RESULTS AND DISCUSSION

The results were grouped under two different conditions: ‘pig runs’ and ‘pig farms’. To be considered positive, the property had to present at least one sample with titration equal or higher than 1:10 in the HI test. 46% of serum samples tested was positive in 74 farms analyzed in at least one sample. As for the pig runs, their positivity was 6%. This first result points at an interesting condition: that of the density of animals possibly influencing the results, showing that influenza is a respiratory disease common in swine industry. In the farms, 20% prevalence of animals positive for H3N2 was observed, and the small incidence of 3% in pigs on the positive tested runs, (P<0.05). It is important to observe that under conditions of greater density, even with an ideal management, the influenza viruses are more able to spread, though not producing clinical signs in the animals, with conclusive diagnosis, as is the case of the tested properties, where there was no record of clinic influenza in the animals.

Our data are consistent with those of Elazhary et al. (1985), which analyzed serum samples from 350 pigs with a history of respiratory problems from 11 farms in Canada (Quebec) and found 46.6% positivity (the sub-type is not specified). Likewise, Poljak et al. (2008) concluded that from 2005 on, the incidence of the H3N2 sub-type superimposed that of H1N1 in pigs, when 40.8% of the tested farms were positive in Ontario, Canada. Maldonado et al. (2006) in Spain - in properties with high density of pigs - found 83% of properties positive for at least one sub-type (H1N1, H1N2, and H3N2), and 76.3% of the evaluated animals were seropositive.

With a representative sample of the State of Minnesota, Choi et al. (2002) found 33.7% positive sera for influenza, and 26.9% out of these were represented by the H3N2 sub-type. In tests carried out in pig-raising properties in Malaysia by Suriya et al.
(2008), 12.1% of the animals were positive for H3N2 and 41.4% of the properties were positive, with a strong correlation with their sizes.

Brown et al. (1995) also established a seroprevalence of 39% for H3N2 in pigs tested from 1991-1992 in England. In pig-raising properties in Korea, Pascua et al. (2008) found a higher seroprevalence of H1N1 and a smaller prevalence of H3N2, and viral isolates pointing at a possible selective pressure when using vaccines. The constant and contiguous coexistence of man and pig is a relevant factor when considering the possibility of replication of the same viral sub-types in both species. Analyzing serum from 128 patients in the U.S., Ramirez et al. (2006) showed that in 49 of them, who had frequent contact with pig production, the prevalence of antibodies against the influenza H1N1 sub-type was higher, especially when management techniques - such as the use of gloves - were not employed. Likewise, Myers et al. (2006) concluded that there was an increased susceptibility to infection by influenza in workers involved in the pig production industry, detecting seropositivity in veterinarians, swine workers, and meat handlers.

On these farms, the detection of 20% of the animals infected could justify the application of control measures in order to prevent associated clinical manifestations in pigs. Likewise, its role in the generation of a possible pandemic would justify the adoption of preventive measures concerning the contact and manipulation of them by farm employees, minimizing the chances of infection in both species. Samples of H3N2 influenza isolated from pigs in the U.S. between 1977 and 1999 show that the reassortment between human and pig strains may occur in properties characterized by close coexistence (Karasin et al. 2000). It is also important to observe the virus spreading difficulty when, even under inadequate conditions of both health and nutrition management, the population density is low, as happens on runs. Analyzing 150 properties in Europe, Maes et al. (2000) concluded that high density and winter were decisive conditions for finding 40% of properties positive for the H3N2 virus.

As the samples tested in the present work virtually represented the entire state of Paraná, one can infer the influence that the climate would be having on the occurrence of that infection in pigs. In Figure 1, one can observe the average minimum temperatures historically recorded in the state of Paraná. In the region of Guarapuava, with an average of 12°C, 13 out of 47 samples tested were serum reagent (27.6%
prevalence), a proportion that is repeated in all tested regions within this range of temperature. In the north region of the state, with an average of 16°C to 18°C, it can be observed in the areas of Londrina and Maringá, for example, that only 2 out of the 50 samples tested were positive (4% prevalence). Likewise, in the Umuarama area, toward the northwest, with an average minimum temperature of 18°C, only one out of the 40 samples evaluated was positive (2.5%). When associated to the regions of the state, these prevalence show that, in those areas where minimum temperatures are lower (10 to 14°C), the number of serum-reagent animals was higher (P<0.05).

In summary our results demonstrated that H3N2 viruses are present in our region and suggest that it would be prudent to establish vigilant surveillance in pigs and in workers who have occupational exposure. The analysis of serologically positive samples for the H3N2 sub-type of the influenza virus indicates that some factors are crucial for its presence and maintenance in a pig-raising unit. Among all the variables, the density of the population subject to infection is a facilitating factor for, as in human beings, the virus must find a large number of susceptible individuals, and with the

![Figure 1. Minimum temperatures historically recorded in the state of Paraná.](http://www.simepar.br/tempo/clima/parana (07/04/2009))
possibility of high morbidity on these farms, mutation may appear more frequently. The lower temperatures also seem to favor the installation of the infection and the presence of the virus. By the fact that it is a sub-type with zoonotic potential, these results may suggest the need for associated control strategies for both (pigs and swine workers), since pigs have an important role in the reassortment of a new sub-type of influenza virus that, though not presenting high pathogenicity, could be a whole new sub-type to humans and, because of this condition, it has the potential for presenting high morbidity generating epidemics and epizooties at various levels.

REFERENCES


