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H9N2 Avian Influenza Virus Antibody Titers in Human Population in Fars Province, Iran

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ABSTRACT

Among the avian influenza A virus subtypes, H5N1 and H9N2 viruses have the potential to cause an influenza pandemic because they are widely prevalent in avian species in Asia and have demonstrated the ability to infect humans. This study was carried out to determined the seroprevalence of H9N2 avian influenza virus in different human populations in Fars province, which is situated in the south of Iran. Antibodies against H9N2 avian influenza virus were measured using hemagglutination-inhibition (HI) test in sera from 300 individuals in five different population in Fars province, including poultry-farm workers, slaughter-house workers, veterinarians, patients with clinical signs of respiratory disease, and clinically normal individuals, who were not or rarely in contact with poultry. Mean antibody titers of 7.3, 6.8, 6.1, 4.5, and 2.9 and seroprevalences of 87%, 76.2%, 72.5%, 35.6%, and 23% were determined in those groups, respectively. Higher prevalences were detected in poultry-farm workers, slaughter-house workers, and veterinarians, possibly due to their close and frequent contact with poultry.

INTRODUCTION

Influenza is a highly contagious, acute illness that has afflicted humans and animals since ancient times. Influenza viruses belong to the Orthomyxoviridae family and are grouped into types A, B and C, according to antigenic characteristics of the core proteins (Fouchier Ron *et al*., 2005; Swayne & Suarez, 2000; Swayne, 2007). Influenza A viruses infect a large variety of animal species, including humans, pigs, horses, sea mammals, and birds, occasionally producing devastating pandemics in humans, such as in 1918, when over twenty million deaths occurred worldwide. In the 20th Century, the sudden emergence of antigenically different strains in humans, termed antigenic shift, has occurred on four occasions, as follows, in 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and 1977 (H1N1), each resulting in a pandemic (Taubenberger & Morens, 2006; Potter, 2006; Palese, 2004; Nicholson, 2003; Edwin, 2006). Currently, epidemics occur throughout the world in the human population due to infection with influenza A viruses of subtypes H1N1 and H3N2 or with influenza B virus (Palese, 2004; Nicholson, 2003; Edwin, 2006; Alexander & Brown, 2000). Since 1996, the viruses H7N7, H5N1 and H9N2 have been transmitted from birds to humans, but have apparently failed to spread in the human population (Alexander & Brown, 2000). The emergence of an avian virus in the human population prompted an epidemiological investigation to determine the extent of human-to-human transmission of the virus and risk factors associated with infection (Rowe *et al*., 1999). Human infections with wild-type strains of these viruses could occur in the United States in poultry and

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turkey farm workers and in travelers returning from countries in which avian influenza viruses are prevalent in birds, such as Thailand, Vietnam, and China. Laboratory-acquired infections could also occur in vaccine researchers working with wild-type or candidate vaccine viruses, including cold-adapted viruses (Chen *et al*., 2003a,b; Subbarao *et al*., 2003; Fedorko & Nelson, 2006). Avian viruses replicate in the respiratory tracts of mammals, whereas, in birds, they replicate in the intestinal tract as well. Infected mammals presented no significant disease signs and produced low levels of humoral antibodies; however, challenge experiments in ferrets indicated that they were immune. These studies suggest that influenza A viruses currently circulating in avian species represent a source of viruses capable of infecting mammals, thereby contributing to the influenza A antigenic pool from which new pandemic strains may originate (Hinshaw *et al*., 1981). Fars province is an active pole of the poultry industry in Iran, and where 26%, 14%, and 10% of broiler, layer, and broiler breeder farms of Iran are located. The aim of this study was to evaluate LPAIV H9N2 exposure of the human population of the Fars province using the hemagglutination inhibition test.

MATERIALS AND METHODS

Serum samples

Human serum samples were randomly obtained from 300 individuals in five different human population (poultry-farm workers, slaughter-house workers, veterinarians, patients with clinical signs of respiratory disease, and individuals that have no or rare contact with poultry) in Fars province. All participants were encouraged to participate in the study by the veterinary information agency, which informed them about the public health importance of this research. In each population, 60 individuals were sampled. Samples were maintained at room temperature and transported to the testing laboratory within 24 h. Blood samples were centrifuged for serum separation. Antibodies to H9N2 avian influenza virus present in the serum samples were detected using the hemagglutination-inhibition (HI) assay.

HI assay

The (HI) assay is the standard method for serologic detection of influenza virus infection in humans. The obtained sera were treated with RDE (receptor destroying enzyme) by diluting one part of serum with three parts of enzyme and incubated overnight in 37°C water bath. The enzyme was inactivated by 30-min incubation at 56°C, followed by the addition of six parts of 0.85% physiological saline solution to obtain a final dilution of 1/10. HI assays were performed in U-bottom 96-well microtiter plates with 0.5% turkey erythrocytes (Rowe *et al*., 1999).

RESULTS

Samples were considered negative if titers were ≤ 20. Positive samples had at least one serum sample with titer > 20 or at least 3/15 with titer = 20. Mean antibody titers were 7.3, 6.8, 6.1, 4.5, and 2.9 $\log_2$ in poultry-farm workers, slaughter-house workers, veterinarians, patients with clinical signs of respiratory disease, and normal individuals, respectively, and the seroprevalences were found to be 87%, 76.2%, 72.5%, 35.6% and 23%, respectively, in these groups. The results were statistically analyzed by one-way ANOVA, and no significant variation ($p>0.05$) in H9N2 avian influenza virus antibody titesr or seroprevalence of H9N2 AIV were found among poultry-farm workers, slaughter-house workers and veterinarians. However, significant differences ($p<0.05$) were observed between these groups and two other groups (patients with clinical signs of respiratory disease and normal individuals).

DISCUSSION

In the present study, H9N2 AIV antibody titers in poultry-farm workers, slaughter-house workers, veterinarians were in the range of 3 to 8 $\log_2$ HI, while the two other groups (patients with clinical signs of respiratory disease and normal individuals) H9N2 AIV antibody titers ranged between 0 and 6 $\log_2$ HI. This could be due to the frequent and close contact of those groups with H9N2 avian influenza virus circulating in Iranian poultry farms, which may result in different stages of infection in these groups. The patients with clinical signs of respiratory disease and normal individuals did not have contact with poultry at all or only rare contact. In virological and serological surveys of H9N2 subtype of influenza A virus in chickens and humans in Shenzhen city, approximately 26% of human sera and only 7% of chicken sera were seropositive, and the study concluded that human H9N2 virus infection probably derived from the H9N2 chicken virus (Cheng *et al*., 2002). In a serological study to assess the epidemic status of avian influenza A (H9N2) virus

in chickens and men in Guangzhou area, it was shown that anti-H9N2 antibody was found in 12.8% of the chickens and 5.1% of the poultry-farm workers (Li *et al*., 2004). The results of a sero-epidemiological survey on avian (H9N2) virus in humans, chickens, and pigs showed that approximately 19% of humans presented antibodies against the H9N2 virus, and 5 strains of influenza A (H9N2) virus were isolated from the patients (Guo *et al*., 1999). In another study, HI and neutralization titers of H9N2 virus in the serum of a convalescent patient reached 400 and ≤640, respectively. An HI antibody titer of 25 against H9N2 virus was also detected in the serum of patient's mother. The main hypotheses are that the mother had contact with birds, especially chickens carrying H9N2 virus, and then transmitted it to the patient, or the patient herself directly breathed air with H9N2 virus particles (Guo *et al*., 2000). Peiris *et al*. (1999) reported the clinical features of two cases of human infection with influenza A virus subtype H9N2 in Hong Kong, and showed that serum samples from blood donors in Hong Kong had neutralizing antibodies suggestive of prior infection with influenza H9N2 (Peiris *et al*., 1999). Jia *et al*. (2009), from a total of 583 sera from farmers in Xinjiang with positive titers equal to or greater than 160, showed that 10 (1.7%) were positive for H9 virus infection. In another study carried out by Meijer *et al*. (2006), with a cut-off of ≤40, found that 2 (6%) of A (H7- infected individuals, 36 (7%) of 508 poultry-exposed individuals, and 4 (6%) of 63 individuals exposed to A (H7)-infected individuals presented A (H7) specific antibodies (Meijer *et al*., 2006).

The higher prevalence detected in poultry-farm workers, slaughter-house workers and veterinarians as compared to patients with respiratory signs and normal individuals, as detected in the present study, was possibly due to the close and frequent contact of those groups with H9N2 avian influenza virus, which is endemic in Iranian poultry farms.

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