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Seroprevalence of H9N2 Avian Influenza Virus in Human Population in Boushehr Province, Iran

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ABSTRACT

Among the avian influenza A virus subtypes, the 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 understand the seroprevalence of H9N2 avian influenza virus in different human population in Boushehr Province which is situated at the South of Iran. Antibodies to H9N2 avian influenza virus in sera from 300 individuals in five different human population in Boushehr province (poultry farm-workers, slaughter-house workers, veterinarians, patients with clinical signs of respiratory disease and normal general citizens were not in contact to poultry at all or only had rare contact) were measured using Hemagglutination-Inhibition (HI) test. The mean antibody titer was found 5.63, 5.1, 4.85, 3.5, 2.73 and seroprevalence was 90, 78.3, 71.6, 46.6 and 25% in these groups, respectively. A higher prevalence was detected in poultry farm-workers, slaughter-house workers and veterinarians possibly enabled by the close and frequent contact of these groups with poultry industry.

Key words: Avian influenza virus, Boushehr Province, H9N2, human, Iran, seroprevalence

INTRODUCTION

Influenza is a highly contagious, acute illness which has afflicted humans and animals since, ancient times. Influenza viruses are part of the Orthomyxoviridae family and are grouped into types A, B and C according to antigenic characteristics of the core proteins (Ron *et al*., 2005; Swayne and Suarez, 2000; Swayne, 2007). 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 and Morens, 2006; Potter, 2006; Palese, 2004; Nicholson, 2003; Kilbourne, 2006). 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 and 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 turkey farm workers and in travelers returning from countries in which avian influenza viruses are prevalent in birds, such as Thailand, Vietnam and China. Replication of the avian viruses occurred in the respiratory tracts of mammals, whereas, in birds, they replicate in the intestinal tract as well. The infected mammals had 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). The aim of this study was to evaluate LPAIV H9N2 exposure of human population in Boushehr province by the hemagglutination inhibition test.

MATERIALS AND METHODS

Serum samples: Human serum samples were randomly obtained from 300 individuals over 25 years old in five different human population (poultry farm-workers, slaughter-house workers, veterinarians, patients with clinical signs of respiratory disease and normal general citizens (persons were not in contact to poultry at all or only had rare contact)) in Boushehr province from May to September of 2009. From each population 60 individuals were sampled. Samples were maintained at room temperature and transported to the testing laboratory within 24 h. If a delay in transport of samples was expected, samples were held for 24 h and then the serum was decanted and the serum samples were frozen at -20°C prior to laboratory submission. Antibodies to H9N2 avian influenza virus present in the serum samples were detected using the haemagglutination inhibition assay.

HI assay: The Hemagglutination Inhibition (HI) assay, the standard method for serologic detection of influenza virus infection in humans.The sera were treated with Receptor Destroying Enzyme (RDE) by diluting one part serum with three parts of this enzyme and were incubated overnight in a 37°C water bath. The enzyme was inactivated by a 30 min incubation at 56°C followed by addition of six parts of 0.85% physiological saline for a final dilution of 1/10. HI assay were performed in U-bottom 96-well microtiter plates with 0.5% Turkey erythrocytes (Rowe *et al*., 1999). Samples were considered negative if titers were = 1/8. Positive samples had at least one serum sample with titer > 1/8 or at least 3/15 with titer = 1/8.

RESULTS AND DISCUSSION

The mean antibody titers were 5.63, 5.1, 4.85, 3.5, 2.73 $\log_2$ in poultry farm-workers, slaughter-house workers, veterinarians, patients with clinical signs of respiratory disease and normal general citizens (Fig. 1) and the seroprevalences were found to be 90, 78.3, 71.6, 46.6 and 25%, respectively in these groups (Fig. 2). The results were analyzed statistically with one-way ANOVA method and found no significant variation ($p>0.05$) in H9N2 avian influenza virus antibody titer and seroprevalence of H9N2 AIV among the poultry farm-workers, slaughter-house workers, veterinarians, although between these groups and two other groups (patients with clinical signs of respiratory disease and normal general citizens) significant variation ($p<0.05$) were observed. In each group between the mean HI antibody titer and seroprevalence, significant variation ($p<0.05$) were observed.

In the present study, in poultry farm-workers, slaughter-house workers, Veterinarians the H9N2 AIV antibody titer were found in the range of 3 to 8 $\log_2$ HI, however in two other groups (patients with clinical signs of respiratory disease and normal general citizens) the H9N2 AIV antibody titers were found in the range of 0 to 6 $\log_2$ HI. The high HI Ab titers were present in poultry farm workers, because the persons in this group had the highest occupational exposure, whether birds of these areas were seropositive for H9N2. The presence of HI antibody titers in patients with respiratory disease and normal person groups could be due to probable contact with

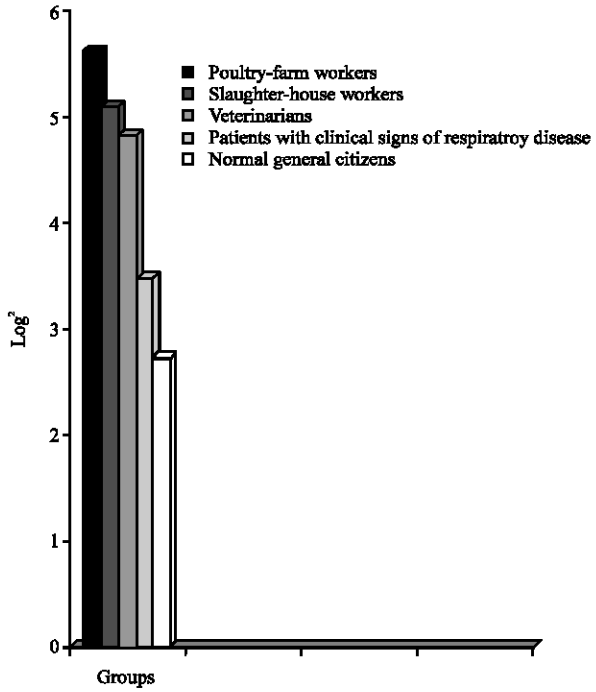

Fig. 1: Comparison of mean HI Ab titers among different groups

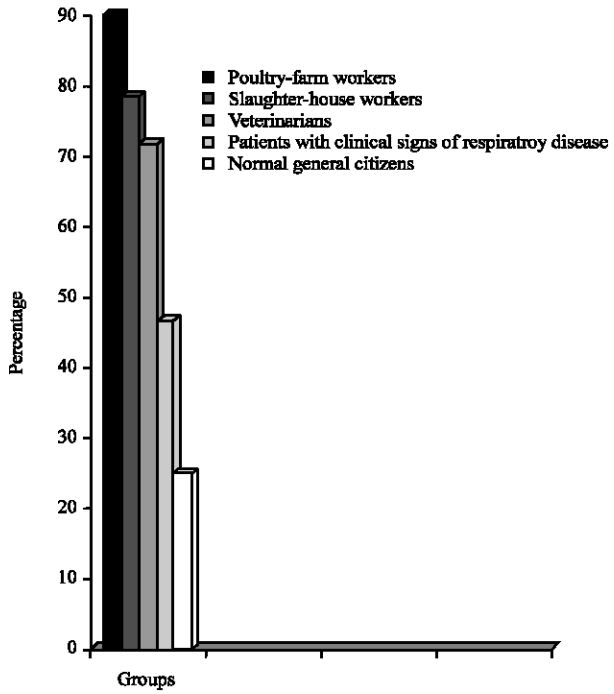

Fig. 2: Comparison of seroprevalence (%) among different groups

H9N2 avian influenza virus. In seroepidemiologic survey of H5N1 and H9N2 in Guangdong population, the positive rate of antibody to H5N1 was 3.03% in the occupational exposure group and 2.34% in general citizens group; that of H9N2 was 9.52% in the occupational exposure group

and 3.76% in the general citizens group (Lu *et al*., 2008). In virological and serological surveys for H9N2 subtype of influenza a virus in chickens and men in Shenzhen City, approximately 26% of human sera and only 7% of chicken sera were seropositive and concluded that the human H9N2 virus infection probably derived from chicken H9N2 virus (Cheng *et al*., 2002). In serological study to understanding the epidemic status of avian influenza A (H9N2) virus in chickens and men in Guangzhou area, showed that the 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 seroepidemiological surveys for avian (H9N2) virus in human, chickens and pigs showed that the approximately 19% of human had antibody to H9N2 virus and 5 strains of influenza A (H9N2) virus were isolated from the patients (Guo *et al*., 1999). In another study, the HI and neutralization titers to H9N2 virus in convalescent serum of the patient reached 1:400 and > or = 1: 640, respectively. The HI antibody titer 1:25 to H9N2 virus was also detected in the serum of patient's mother. The greatest possibility was that her mother had contacted with birds, especially chickens carrying H9N2 virus, then transmitted to her or she breathed in the air borne with H9N2 virus particles directly (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 antibody suggestive of prior infection with influenza H9N2 (Peiris *et al*., 1999). A higher prevalence was detected in poultry farm-workers, slaughter-house workers, Veterinarians than two other groups in our study possibly enabled by the close and frequent contact of these groups with H9N2 avian influenza virus that is endemic in Iranian poultry farms.

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