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Evaluation of Antibody Titers to H9N2 Influenza Virus in Hospital Staff in Shiraz, Iran

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Abstract: This study was carried out to understanding the seroprevalence of H9N2 avian influenza virus among hospital staff in Namazi hospital in Shiraz which is situated at the Southwest of Iran. A total of 300 serum samples from hospital staff and 300 control serum samples from people who came for physical check-up (control group) were collected. Hemagglutination-Inhibition (HI) tests were conducted to determine the individual serological status. The seropositive rate (SPR, defined as the proportion with HI titer = 1:40) of antibodies to H9N2 influenza virus and its Geometric Mean Titer (GMT) were calculated and compared among these groups.

Key words: H9N2, antibody, hospital staff, antibodies, influena virus, Iran

INTRODUCTION

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 20 million deaths occurred worldwide (Taubenberger and Morens, 2006; Potter, 2006; Palese, 2004; Nicholson *et al*., 2003; Kilbourne, 2006). In late 1997 during the H5 outbreak, several sub-types were identified in domestic poultry in addition to H5N1 of which H9N2 was the most prevalent.

Domestic chickens, the source of most infections, appeared to act as an intermediate host in avian-to-human transmission. Pigs which are readily infected by avian and human viruses may act as an intermediate host both for transmission and in facilitating genetic reassortment between avian and human viruses as may have occurred prior to the emergence of the 1957 H2N2 and 1968 H3N2 pandemic viruses (Palese, 2004; Nicholson *et al*., 2003; Kilbourne, 2006; 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).

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). A number of different sub-types of Avian Influenza (AI) viruses have emerged in humans including H5N1, H7N2, H7N7 and H9N2. These influenza viruses are excreted in the infected birds and in their respiratory secretions.

Transmission to humans can result from close contact with infected (dead or live) poultry, droppings or contaminated surfaces. The suspected organs of influenza virus entry to humans are assumed to be the mouth, nose, eyes and lungs. Avian influenza H9N2 infections have been reported in the Middle East causing widespread outbreaks in commercial chickens in Iran, Saudi Arabia and Pakistan (Alexander, 2006).

An outbreak of H9N2 infection in poultry farms was first reported in 1998 in Iran (Nili and Asasi, 2002) which is now endemic and vaccination against this sub-type is practiced, routinely. The aim of this study was to investigate, seropositivity against H9N2 virus among healthcare personnels in Namazi hospital.

MATERIALS AND METHODS

Serum samples: A total of 300 serum samples were collected from Namazi hospital staff and 300 serum samples were collected from people who came for physical check-up.

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. Samples were maintained at room temperature

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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 plate with 0.5% turkey erythrocytes (Rowe *et al*., 1999). HI titer ≥40 was interpreted as positive. The Geometric Mean Titer (GMT) is defined as the geometric mean of the positive HI titers. The Seropositive Rate (SPR) is defined as the percentage of HI titers ≥40.

RESULTS AND DISCUSSION

A total of 300 hospital staff and 300 control cases (control group) were tested. The χ^2-test was used to compare differences in discrete variables and Student's t test was used to analyze the HI titer results. All analyses were performed with SPSS version 17. $p<0.05$ was considered statistically significant. With HI ≥40 as the cut-off value for seropositivity, the SPR of the hospital staff was significantly higher than that of the control group (32.6 vs. 2.5%, $p<0.001$). There was statistically significant difference in GMT antibodies to H9N2 influenza virus between the hospital staff and control group (58.3 vs. 26.2, $p<0.001$).

Since 1998, an epidemic of avian influenza occurred in the Iranian poultry industry and now this virus is endemic in Iranian poultry farms (Nili and Asasi, 2002). The higher SPR was observed in the present study was possibly due to the close and frequent contact of hospital staff with patients occupationally infected with H9N2 avian influenza virus (especially poultry farm workers, slaughter-house workers, veterinarians and villager patients) which may result in different stages of infection in these groups.

In the serological study of H9N2 avian influenza virus in five human population in Fars province Iran, the seroprevalence were determined 87, 76.2, 72.5, 35.6 and 23% in poultry farm workers, slaughter-house workers, veterinarians, patients with clinical signs of respiratory disease and normal general citizens, respectively (Hadipour, 2010). Alizadeh *et al.* (2009) reported that the seroprevalence of avian influenza (H9N2) in slaughter-house workers and poultry farm workers were 51.6 and 24.6%, respectively. In virological and serological surveys of H9N2, sub-type 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 sub-type 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. 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. The SPR of antibodies against the H9N2 virus in the hospital staff was higher than that in the general population, reflecting a higher contact risk. Prevaccination surveillance of the immune status of different risk groups may help to prioritize which groups should be vaccinated first.

CONCLUSION

The SPR of the antibodies to H9N2 influenza virus of the hospital staff was significantly higher than that of the control group (32.6 vs. 2.5%, $p<0.001$). However, the GMT

of antibodies to H9N2 influenza virus of the hospital staff was significantly different from that of the control group ($p<0.001$). The SPR of antibodies against the H9N2 avian influenza virus in the hospital staff was higher than that in the general population, reflecting a higher contact risk.

REFERENCES

Alexander, D.J. and I.H. Brown, 2000. Recent zoonoses caused by influenza A viruses. Rev. Sci. Tech., 19: 197-225.

Alexander, D.J., 2006. An overview of the epidemiology of avian influenza. Vaccine, 6691: 1-8.

Alizadeh, E., S.M. Hosseini, M.T. Kheiri, V. Mazaheri, R. Bashar and M. Tabatabaeian, 2009. Avian Influenza (H9N2) among poultry workers in Iran. Iran. J. Microbiol., 1: 3-6.

Cheng, X., J. Liu, J. He and F. Shan, 2002. Virological and serological surveys for H9N2 subtype of influenza A virus in chickens and men in Shenzhen city. Chin. J. Exp. Clin. Virol., 16: 319-321.

Guo, Y., J. Li and X. Cheng, 1999. Discovery of men infected by avian influenza A (H9N2) virus. Chinese J. Exp. Clin. Virol., 13: 105-108.

Guo, Y., J. Xie and M. Wang, 2000. A strain of nfluenza A H9N2 virus repeatedly isolated from human population in China. Chinese J. Exp. Clin. Virol., 14: 209-212.

Hadipour, M.M., 2010. H9N2 Avian influenza virus antibody titers in human population in Fars Province, Iran. Braz. J. Poult. Sci., 12: 161-164.

Hinshaw, V.S., R.G. Webster, B.C. Easterday and W.J. Jr. Bean, 1981. Replication of avian influenza A viruses in mammals. Infect. Immun., 34: 354-361.

Jia, N.A., S.J. de Vlas, Y.X. Liu, J.S. Zhang and L. Zhan *et al*., 2009. Serological reports of human infections of H7 and H9 avian influenza viruses in northern China. J. Clin. Virol., 44: 225-229.

Kilbourne, E.D., 2006. Influenza pandemics of the 20th century. Emerg. Infect. Dis., 12: 9-14.

Li, C.H., X.Z. Zhou and M.X. Li, 2004. Discoveries of avian influenza A(H9N2) virus in chickens and men infected by H9N2 virus in Guangzhou area. Chinese J. Exp. Clin. Virol., 18: 213-214.

Meijer, A., A. Bosman, E.E. van de Kamp, B. Wilbrink, M. Du Ry van Beest Holle and M. Koopmans, 2006. Measurement of antibodies to avian influenza virus A(H7N7) in humans by hemagglutination inhibition test. J. Virol. Methods, 132: 113-120.

Nicholson, K.G., J.M. Wood and M. Zambon, 2003. Influenza. Lancet, 362: 1733-1745.

Nili, H. and K. Asasi, 2002. Natural cases and an experimental study of H9N2 avian influenza in commercial broiler chickens of Iran. Avian Pathol., 31: 247-254.

Palese, P., 2004. Influenza: Old and new threats. Nat. Med., 10: 82-87.

Peiris, M., K.Y. Yuen, C.W. Leung, K.H. Chan and P. Ip *et al*., 1999. Human infection with influenza H9N2. Lancet, 354: 961-971.

Potter, C.W., 2006. A history of influenza. J. Appl. Microbiol., 91: 572-579.

Rowe, T., R.A. Abernathy, J.H. Primmer, W.W. Thompson and X. Lu *et al*., 1999. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. J. Clin. Microbiol., 37: 937-943.

Taubenberger, J.K. and D.M. Morens, 2006. 1918 Influenza: The mother of all pandemics Emerg. Infect. Dis., 12: 15-22.