

Age Distribution Profile of H9N2 Avian Influenza Virus Antibody Titers in Humans

M.M. Hadipour, M.R. Hadipourfard, S. Pazira, M. Jafarpour and F. Azad
Department of Clinical Sciences, School of Veterinary Medicine,
Islamic Azad University, Kazerun Branch, Kazerun, Iran

Abstract: To understanding the serological status of H9N2 avian influenza virus in human population according to age bias, we studied 240 serum samples from male persons with ages between 5-70 years of age in different regions of Fars province, Iran. Haemagglutination-Inhibition assay was used for evaluation of H9N2 antibody titers in persons sera. None of these persons received any of influenza A vaccines. All persons clinically normal without any influenza-like illness and were encouraged to participate in this study by veterinary information agency. Age distribution was as follows: 5-15 years, 8.0%; 16-26 years, 12.0%; 27-37 years, 16.0%; 38-48 years, 20.0%; 49-59 years, 22.0%; and 60-70 years, 22.0%. The increasing seropositive rate with increased years of age could be due to frequent exposures during the life periods.

Key words: Age, antibody, influenza, H9N2, human

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 twenty million deaths occurred worldwide (Taubenberger and Morens, 2006; Potter, 2006; Palese, 2004; Nicholson, 2003; Edwin, 2006). In late 1997 during the H5 outbreak, several subtypes 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, 2003; Edwin, 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 subtypes 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 subtype is practiced, routinely. The aim of this study was to investigate seropositivity against H9N2 influenza virus among male persons with ages between 5-70 years of age in different regions of Fars province, Iran.

MATERIALS AND METHODS

Sample collection: A seroepidemiological investigation was conducted in October 2010 by taking 4 mL blood samples from 240 participants (40 samples from each age group) according to age bias. The participants were all men in a 5-70 age range in different regions of Fars province, Iran. Samples were accompanied with questionnaires on demographic information and data on vaccination history, occupational type exposure, employment history and recent influenza symptoms. All persons were encouraged to participate in this study by veterinary information agency, which informed them about the public health importance of this research.

Serology: Blood samples were maintained at room temperature and transported to the shiraz veterinary research laboratory within 24 h. Blood samples were centrifuged for serum separation. Sera were separated and

stored at -20°C until used. Human sera were tested using the Hemagglutination Inhibition (HI) assay, as described by World Health Organization (WHO, 2002), against the locally circulating H9N2 strain. The samples were tested to investigate antibodies against the A/Chicken/Iran/772/99 (H9N2) virus using a modified HI adapted by Meijer *et al.* (2006). Sera were inactivated at 56°C and treated with RDE following heme-adsorption with packed horse erythrocytes. HI assay was performed using 2HA units and 1% horse erythrocytes in V-bottom plates. The seropositive rate was defined as the percentage of serum titers ≥ 40 by HI. A $p < 0.05$ was considered significant correlation between age and HI titer.

RESULTS AND DISCUSSION

None of participants received any of influenza A vaccines. All persons clinically normal without any influenza-like illness. The seropositive rate among participants according to age distribution profile was as follows: 5-15 years, 8.0%; 16-26 years, 12.0%; 27-37 years, 16.0%; 38-48 years, 20.0%; 49-59 years, 22.0%; and 60-70 years, 22.0%. With increasing the participant age, the HI titer increased too. A significant correlation ($p < 0.05$) was observed between the participant age and their sera HI titer. 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 significant correlation between SPR and participant age in the present study was possibly due to frequent exposure of adults and older persons during their life. 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 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. 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.

CONCLUSION

Frequent exposure of humans to H9N2 avian influenza virus during their life result in increasing the antibody titers in their sera. A massive vaccination programme should be used in human population at different years of age (especially in persons with high occupational exposure) for protecting them against the H9N2 avian influenza virus which is endemic in Iranian poultry farms and it has public health significance.

ACKNOWLEDGMENT

We thank the veterinary information agency staff who encouraged persons for participating in this study. We also thank the razi veterinary laboratory staff for their helps in sample collection and technical assistance.

REFERENCES

- Alexander, D.J. and I.H. Brown, 2000. Recent zoonoses caused by influenza A viruses. *Rev. Sci. Tech.*, 19: 197-125.
- 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. *Iranian. 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. *Chinese J. Exp. Clin. Virol.*, 16: 319-321.
- Edwin, D., 2006. Influenza pandemics of the 20th century. *Emerg. Infect. Dis.*, 12: 129-149.
- 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 influenza 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. *Brazilian J. Poult. Sci.*, 12: 153-156.
- Hinshaw, V.S., R.G. Webster, B.C. Easterday and W.J. 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, L. Zhan, R.L. Dang, Y.H. Ma, X.J. Wang, T. Liu, G.P. Yang, Q.L. Wen, J.H. Richardus, S. Lu and W.C. Cao, 2009. Serological reports of human infections of H7 and H9 avian influenza viruses in northern China. *J. Clin. Virol.*, 44: 225-229.
- 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., 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 Path.*, 31: 247-252.
- Palese, P., 2004. Influenza: old and new threats. *Nat. Med.*, 10: 82-87.
- Peiris, M., K.Y. Yuen, C.W. Leung, K.H. Chan, P.L. Ip, R.W. Lai, W.K. Orr and K.F. Shortridge, 1999. Human infection with influenza H9N2. *Lancet*, 354: 916-917.
- Potter, C.W., 2006. A history of influenza. *J. Appl. Microbiol.*, 91: 572-579.
- Rowe, T., R.A. Abernathy, J. Hu-Primmer and W.W. Thompson, X. Lu, W. Lim, K. Fukuda, N.J. Cox and J.M. Katz, 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.
- World Health Organization (WHO), 2002. Manual on animal influenza diagnosis and surveillance 2002. Retrieved from: www.WHO/CsR/NCS/2002.5.