Efficacy of vector-based avian influenza H9 vaccine against of H9N2 viruses

Usman Elahi1, Abdul Rehman2, Hajra Qadir3, Anum Safdar3, Sajid Umar4,5*

1Department of Agricultural Sciences, University of Bonn, Germany, 2Friedrich-Loeffler-Institute of Epidemiology Berlin, Germany, 3Department of Pathobiology PMAS Arid Agriculture University, Rawalpindi, Pakistan, 4National Veterinary School Toulouse, France

Abstract: A recombinant fowl pox-avian influenza (AI) H9 vaccine (rFP-AI-H9) expressing the haemagglutination of A/chicken/Tunisia/12/2010 (H9N2) was prepared and its efficacy was evaluated against escalating doses of LPAI H9N2 AI virus in white leg horn layer chickens. In this study, 4 weeks old white leg horn layer chickens were vaccinated by subcutaneous route with rFP-AI-H9 (10² PFU) and challenged 3 week later by the oronasal route with field isolate H9N2 AI virus. Vaccinated chickens were fully protected from clinical disease signs after challenge with H9N2 AI virus whereas unvaccinated chickens showed clinical disease signs after 2 days post-infection (dpi), shedding virus in buccal and cloacal routes. Significant reduction of virus in oral swabs was observed in vaccinated birds. The rFP-AI-H9 vaccine induced haemagglutination inhibition antibodies detectable with homologous H9N2 antigen. Protective immunity could last for 40 weeks post-immunisation. Together, these results confirm the excellent level of protection induced by rFP-AI-H9 in chickens against LPAI H9N2 isolates of Pakistan.

Key words: H9N2 virus, Fowlpox, recombinant vaccine, chicken

Introduction

Avian influenza (AI) H9N2 virus is considered a low-pathogenic virus with widespread circulation in poultry in Asia [1, 2]. LPAI H9N2 virus can pose a significant zoonotic threat like H5N2 [3]. The H9N2 viruses have been isolated from domestic as well as wild avian species [4, 5] and have been found circulating in multiple avian species in Eurasia resulting in great economic losses in the poultry industry [6-9]. In recent years, H9N2 virus has gained significant importance because of its continuous rapid spread among domestic birds [10-12]. This virus persists in chicks and spreads to non-affected flocks through feral-oral route without showing much of severe clinical signs [13]. Sporadic outbreaks of this subtype of the virus have been reported noticeably in Pakistan, India and Iran [14, 15]. Thus, the development of highly effective influenza virus vaccine for chickens would be of benefit to both human and veterinary health. Inactivated vaccines have been used to control the spread of highly pathogenic H5 and H7 avian influenza viruses (AIV) in several countries [16, 17]. An inactivated vaccine derived from A/chicken/Pakistan/AG519/98 (H9N2) was used to prevent H9N2 avian influenza in 2003 in Pakistan [18]. The killed vaccines having matrix and nucleoprotein as the antigen is being used in Pakistan. One or both of these proteins are used as antigen for antibody tests. Therefore, vaccinated birds cannot be distinguished from naturally infected birds using this approach. This loss of the ability to do surveillance has been a major problem. The protective immunity, evaluated in specific pathogen free SPF chickens demonstrated that immunization with rFPV-HA-NA could induce complete protection against challenge with H5N1 [19]. Fowl pox virus (FPV), a member of the family Poxviridae, with a large double strand of DNA genome and host range limited to avian species, have been used to successfully express protective immunogen genes from several avian viruses, and vaccination with the recombinant FPV was able to protect chickens from infection with the corresponding viruses [20]. The construction and immunogenicity of a recombinant FPV co-expressing H5 haemagglutinin (HA) and N1 neuraminidase (NA) have been reported previously [19, 21, 22]. In the present study, the immune efficacy, safety, the minimum immunizing dose, the time of immunity induced and the immune duration of the vector-based vaccine was evaluated in commercial layer chicken.

Materials and Methods

Commercial layer chickens used in the study were obtained from a local hatchery and housed in experimental sheds of Veterinary research institute Lahore. A/chicken/Pakistan/UDL-01/08 is a novel genotype of LPAI H9N2. The virus was propagated in allantoic cavities of nine-to-ten-day old SPF embryonated chicken eggs and titrated using standard methods. A recombinant fowl pox virus (rFPV-HA-NA) vaccine containing a cDNA copy of HA and NA gene (A/chicken/Tunisia/12/2010 (H9N2) was used in this study. All birds were declared serologically naïve and free from influenza virus by haemagglutination inhibition (HI) and virus isolation (VI) in eggs using standard methods [5].

Experimental design

A total of thirty commercial layer chickens (4 weeks old) were randomly assigned three groups, one group was immunized with rFPV-HA-NA vaccine (10⁷ PFU) by wing web puncture with a double needle used for commercial vaccination of poultry with FPV. The other two unvaccinated groups were used as control (control negative and infection control). All the chickens were bled weekly after immunization to harvest serum samples for determination of antibody titer. The challenge was conducted three weeks after vaccination with 10⁶ EID₅₀ of A/chicken/Pakistan/UDL-01/08 isolate by the intranasal route. The chickens were monitored daily for two weeks for death and infections. Buccal swabs were collected for virus re-isolation daily up to 8 days post-infection (PI). Vaccinated control birds bed periodically to harvest serum for the determination of immunity duration. Some vaccinated birds were infected again with the H9N2 virus at 30 and 35th week post vaccination.

Serological tests

All the serum samples collected weekly after immunization and challenge were stored at-20°C, so that all tests were conducted simultaneously. The HI test was performed as described previously (OIE 2008) using 4HA units of A/chicken/Pakistan/UDL-01/08 virus as antigen; the HI titers were determined as the reciprocal of the highest serum dilution in which inhibition of hemagglutination was observed [22].
Determination of virus shedding

Buccal swabs were collected daily from all birds from 1 to 8 DPI to assess virus shedding in 1mL of PBS containing 1% gentamycin and kept at -80 °C until further used. RNA was extracted using the QiAamp viral RNA isolation kit (Qiagen, Valencia, CA, USA) according to the instructions of the manufacturer. The virus titer of each sample was determined by using quantitative real time reverse transcriptase polymerase chain reaction (qRT-PCR) as described in previous studies [5]. qRT-PCR reactions targeting the influenza virus M gene [17] were conducted using Quantitect SYBR Green RT-PCR Kit (Qiagen, Germany). The quantitative RT-PCR reactions were performed on a light cycler® 480, Real-Time PCR system (Roche diagnostics, Switzerland) with the following program: the reverse transcription step conditions for primer sets were one cycle at 50°C for 30 min, 95°C for 15 min followed by 45 cycles of 95°C for 15s, 56°C for 15s, and 72°C for 15s. A standard curve for virus quantification was established with RNA extracted from the challenge virus, the C, values of samples were converted into EID₉₀/mL as described in previous studies [17]. The virus titer was represented by the mean ± S.D. of the virus titre per ml of sample.

Statistical analysis

Statistical analysis and graphical presentation were performed using GraphPad Prism 6 software (GraphPad Software Inc. La Jolla, CA, USA) and values were expressed as the mean ± standard deviation of the mean (SDM). Student’s t test was used to analyze virus titres in swabs and HI titer. The number of birds shedding virus was tested for statistical significance using Fisher’s exact test. For statistical purposes, all qRT-PCR negative oropharyngeal and cloacal swabs were given a numeric value of 10⁻⁰ EID₉₀/mL for AIV. All HI-negative serum was given a value of 3 log₂. These values represent the lowest detectable level of antibodies in these samples based on the methods used. Statistical significance was set at p < 0.05 unless otherwise stated.

Results

Serum samples were collected after immunization for detection of HI antibody response. HI antibody (log₂ 7.6) was observed one week post immunization (p.i.), of chickens vaccinated with rFPV-HA-NA vaccine (Fig.1). In comparison, no detectable HI antibodies were detected in the chickens of unvaccinated control. Meanwhile, morbidity, mortality and reduction in virus shedding were also detected following experimental infection. Vaccinated chickens showed significant reduction in virus-shedding, and protected from clinical disease signs and death after being challenged with H9N2, while the control challenged chickens showed clinical disease (Fig.2).

Discussion

A unique genotype of H9N2 virus, having some genome similarity to H7N3 virus, is circulating in poultry industry in Asian countries [17]. Although the inactivated whole virus AI vaccines have shown efficacy against the outbreak of LPAIV, they induced immune response to the group specific influenza A nucleoprotein antigen that may negate the detection of natural infection [18]. Several studies were conducted on the basis of developments of a novel vaccine strategy. HA and NA are the major surface glycoproteins of influenza virus A primarily involved in the induction of specific humoral immunity. Functionally distinct roles have been attributed to the humoral response elicited by these two viral proteins. A variety of vaccines derived from HA and NA genes of AIV, including recombinant virus vaccine, sub-agglutination HA protein and DNA vaccines [23,24] have been shown experimentally effective for immunization against influenza. The immunogenicity of fowl pox virus expressing HA and NA genes of avian influenza virus have been discussed in a previous paper [19]. The recombinant rFPV–HA–NA vaccine has several advantages in comparison with the inactivated vaccine and the recombinant NDV vaccine. The use of rFPV–HA–NA vaccine should prevent confusion between vaccinated birds and infected birds for surveillance purposes, which is a problematic issue with the use of whole-virus influenza vaccines, and also there is no residue problem resulting from the use of adjuvant in the inactivated vaccines. In comparison with the recombinant NDV vaccine [20], which is easy to be produced and applied, the recombinant rFPV–HA–NA vaccine induced much longer antibody duration in the vaccinated chickens. Furthermore, the recombinant rFPV–HA–NA vaccine could provide protection against both AI and fowlpox [25]. This paper has focused on evaluating the immune efficacy of the recombinant fowl poxvirus vaccine. Humoral immunity plays an important role in the immunology of avian influenza. HI antibodies could neutralize the infection of virus and NI antibodies could restrict the replication of infective viruses [26-28]. All the chickens immunized with rFPV–HA–NA vaccine were protected against the challenge with H9N2. HI antibodies play a key role in protecting against AIV by neutralizing the infective ability of challenge viruses [29]. The level of HI antibody induced by the whole virus inactivated vaccine is correlated to the observed protection and a level that can be considered a cut off for susceptibility, is 4log₂. For the recombinant virus vaccine, the criteria may not be applicable. From our results, the HI antibody titers as low as 3log₂, the complete protection could be achieved after vaccination with this vaccine. This result was consistent with report that recombinant vaccinia virus expressing the HA gene of AIV could induce complete protection against lethal challenge, accompanied by an HI antibody titer of 1–2 log 2 [30]. These results suggested that cellular immunity is also very important for the protection of chickens against AIV challenge. When the antibody level is insufficient to induce protection, cellular immunity can reduce the morbidity and mortality rates of immunized chickens to a certain extent, although it cannot protect chickens from infection with the challenge virus. Even though no HI antibody could be detected, the chicken immunized with this vaccine would survive the challenge...
with H9N2. This result was consistent with the document that the recombinant vaccine virus expressed HA gene of AIV could induce complete protection against the challenge accompanied by the HI antibody titer of 1-2 log2 [31]. Results confirmed that cellular immunity of AI is very important for protection of chicken from the challenge. When the antibody is insufficient to induce protection, the cellular immunity could decrease the mortality and morbidity of immunized chickens to a certain extent, though it could not protect chicken from infection by the challenge virus. Bublot et al. [32] reported that one injection of TROVAC-H5 protects chickens against AI-induce mortality and morbidity for at least 20 weeks. Our data from the present study show that a single inoculation of the rFPV–HA–NA vaccine could protect chickens from H9N2 virus challenge for at least 35 weeks. More importantly, the rFPV–HA–NA vaccine was capable of inducing strong antibody responses to the HA, which were detected as early as 1 week after vaccination. Other reason for enhanced immunity may be supplement of NA protein in the vaccination could offer the prospect of broader heterovariant immunity [22, 33, 34] When compared to inactivated vaccines, the recombinant fowl pox vaccine virus has three advantages. First, it does not interfere with surveillance studies monitoring natural infection in which antibodies to NP are used as marker. Secondly, the domestic poultry are, when necessary, vaccinated against fowl-poxx; the use of fowl pox vector has another important advantage that it can be used to produce multivalent vaccines providing protection against both AI and fowl-poxx. Finally the recombinant vaccine avoids the adjuvant, a component of inactivated vaccine, which makes it much safer than the later for public health. In conclusion, our results demonstrate the development of a safe and effective vaccine to prevent the outbreak of H9 subtype AI.

References


