Evaluation of the Effectiveness of Avian Influenza Vaccines in Native Chicken Using Two by Two Contingency Table

M Indradji and Sufiriyanto

Faculty of Animal Science, Jenderal Soedirman University
JL. Dr. Soeparno No 60 Purwokerto, Central Java 53123, Indonesia
Corresponding author email: Mohandas_08@yahoo.com

Abstract. The aim of this study was to evaluate the effectiveness of vaccination program analysis method using the 2 x 2 contingency table to provide better assessment to the vaccination program management and implementation. This study used survey methods. A total of 230 serum samples from vaccinated chickens and 20 serum samples from unvaccinated chickens were used. The blood serum samples were then examined with the Hemagglutination Inhibition Test/HI to measure antibody levels. The data were analyzed using a 2 x 2 contingency table. Results showed that the level of vaccine protection was 68.92% with 31.31% vaccines failure rate, the level of natural protective immunity in samples was 0%, 100% vaccines specificity and the effectiveness of the vaccine was 71.20%.

Keywords: Vaccination, contingency table, hemaglutination inhibition.

Abstrak. Penelitian bertujuan untuk mengevaluasi keberhasilan program vaksinasi dengan menggunakan tabel kontingensi 2 x 2 yang diharapkan dapat digunakan untuk menilai manajemen program vaksinasi yang lebih komprehensif. Penelitian ini menggunakan metode survei, dengan total 230 sampel serum dari ayam divaksinasi dan 20 sampel serum dari ayam yang tidak divaksinasi. Sampel serum darah ayam kemudian diperiksa dengan uji hemaglutinasi inhibisi/HI untuk mengukur tingkat antibodi. Data dianalisis dengan menggunakan tabel kontingensi 2 x 2. Hasil menunjukan bahwa tingkat proteksi vaksin yang diaplikasikan sebesar 68,92 % dengan tingkat kegagalan vaksin 31,31 %. Tingkat kekebalan alami yang protektif pada sampel sebesar 0 %, spesifitas vaksin 100 %, dan efektifitas vaksin sebesar 71,20 %.

Kata kunci: Vaksinasi, tabel kontingensi, inhibisi hemaglutinasi.

Introduction

The outbreak of avian influenza A (H5N1) viruses in poultry throughout Asia including Indonesia causes major economic problems. These viruses have been isolated worldwide from both domestic and wild species. The largest numbers of viruses have been isolated from feral water birds including ducks, geese, terns, shearwaters, and gulls as well as from a wide range of domestic avian species such as turkeys, chickens, quail, pheasants, gheese, and ducks (Doan et al., 2005; Suarez et al., 2007; Khan et al., 2009).

Bio-security is the first defense in the prevention and control of avian influenza (AI). Its use has been highly successfull in keeping avian influenza out of commercial poultry worldwide. As controlled marketing and rescheduling reduce the bird density in an area, controlled immunization with an inactivated vaccine can reduce the susceptibility of the population. Vaccination is the second line of defense against AI (Dharmayanti et al., 2005).

Vaccination program to prevent Avian Influenza has been a regular program on a farm, many vaccine products have been available on the market Therefore, it is a necessary prudence in the use of appropriate vaccine product. There are still many doubts on the effectiveness of protection that the used vaccines may contribute, whether the vaccine could protect chickens against pandemic, or even it can cause disease outbreaks with new strain.

To answer these doubts, it is important to know whether the vaccination is able to protect chickens or not, it would require testing or evaluation of the effectiveness (level of protection) of the vaccine, by examining the level of immunity produced in chickens after vaccination.

In the present paper, the effectiveness of management of the vaccination program was evaluated using statistical calculation for epidemiology, namely 2 x 2 contingency tables (Gary, 2010) that have been popular in analysis of epidemiologic studies. This method provides not just a description of information of optimum levels of antibody concentrations after vaccination, but also describe the level of immunity and immunological phenomena in the group or population dynamics.

Materials and Methods

The material used for the study were adult chickens (aged more than six months) of 250 individuals (10% sample was taken from the amount of chicken that got AI vaccination in AI vaccination programs of free-range chickens in Banyumas, within the scope of one village) that were kept by the farmers in the village of Ajibarang, Ajibarang District, Banyumas. The samples were obtained from chickens that were vaccinated 4 weeks before AI, and a total of 20 native chickens that were not vaccinated as a control. The vaccine used in this study was an inactive vaccine Avian Influenza(H5N2), Qian Yuan Biological Production, Zhengzhao City Co. Ltd. China.

The samples of the chicken were taken in a non random, in this study known as the sample by the judgments, this sampling method was used because of the rapid changes/dynamics in the chicken population, which can occur due to buying and selling, high mortality, consumption of the owner or other causes. The chicken

blood serum samples were taken and examined four weeks after vaccine application. Blood serum samples were submitted and checked in BPPV Wates ,Yogyakarta, to determine the HI titer. The HI test was extremely reliable, provided reference antisera were available to all subtypes. The disadvantages of the HI test include the need to remove nonspecific inhibitors which naturally occur in sera, it needs to standardize antigen each time a test is performed, and the need for specialized expertise in reading the results of the test. However, the HI assay remains the test of choice for WHO to do global influenza surveillance.

The experiment was conducted by survey method to obtain the data (samples) and laboratory to determine the level of vaccine protection in blood serum samples with titers Hemaglutination Inhibition test (HI) to measure the concentration of antibodies. The observed output variable was the rate (level) of titer(concentration)HI(antibody), as an input variable were the vaccinated and not vaccinated chickens.

The evaluation of the vaccination program was calculated with the model of 2 x 2 contingency table (Gary, 2010). The table was used to measure quantitative data protection level of the vaccine. According to Suardana et al. (2009), good protection (protective) that can inhibit AI infection in chickens, if the titer (concentration) equals to or above 2^4 antibodies and poor protection (not protective) if the titer is below 2^4. The assessment of analysis was done using 2 x 2 contingency table (Table 1).

The calculations to measure the effectiveness of the vaccine was performed by using a contingency table as follows: the results of the analysis was in the form of percentage (0-100%).

Table 1. Two by two contingency table

Protection level	Status of Chickens		
	Vaccinated (+)	Not Vaccinated (-)	Total
Good protection (+)	A	B	A+B
Poor protection (-)	C	D	C+D
	A+C	B+D	A+B+C+D=N

A/(A+C) x 100%, was called the sensitivity that was used to measure the level of protection of the vaccine in chicken, it showed the ability of chickens to respond to protective antibodies from the vaccine given. C/(A+C) x 100%, was called false-negative rate to measure the failure rate of protection of the vaccine in chicken, it showed the ability of chickens to respond to the formation of chicken antibodies from the vaccine, but the concentration was not protective. B/(B + D) x 100%, was called false-positive rate to measure the level of natural immunity of chicken, indicates a concentration of protective antibodies in chickens, although chickens did not get vaccination. D/(B+D) x 100%, is called specificity, to measure individual response to the application of poultry vaccines, it showed the actual immune status of the population without stimulation of chicken vaccine. Specificity of the vaccine meant that the measured antibodies in chickens was only awakened by a vaccine that had been given. (A+D)/N x 100%, was called accuracy, was used to determine the effectiveness of the vaccine on the application in the field, the figures obtained can show the ability to objectively assessed vaccine protection from antibody status in a population, where the measured included the unvaccinated population.

Results and Discussion

Immunity from the sample distribution. Of the 250 samples of chicken blood serum being examined, whether the chicken was vaccinated or not, the distribution of immunity was as follows 158 heads (60.32%) had a protective antibody, and 92 heads (39.68%) were not protective (Table 2). The presence of protective levels of antibodies meant that the chickens would be able to survive from the attack of AI, while not protective, in the event of disease the chicken would be sick. The sample data on the control chickens (not vaccinated) also showed that 8 out of 20 samples (40%) had measurable levels of antibodies, which meant that the existence of natural immunity that had been owned by free-range chicken or chicken immunity without vaccination. But the immunity level was very low (2^1), whereas the antibody of 12 samples were unmeasurable, so it needed to get attention because native chicken could be suspected to be reservoirs of AI or spreaders (shedder) of AI virus. While it was not measurable, antibodies might indicate that the chicken populations had not been exposed to AI viruse. Measurable antibodies can occur because of viral infection in low doses, and a reminder that the AI virus has existed (exist) in the population, then it should be recommended the implementation of AI vaccination in this population.

Sensitivity of the vaccine. Sensitivity is the probability of vaccine protection of protective antibody titers compared to the total number of native chickens in the vaccine, numbered 158 heads. The test results showed a sensitivity rate of 68.92%. This suggested that the level of vaccine protection was quite good, and it was probably caused by individual factors of chicken, feed variation, the virus itself, the environment and specific immune of the chickens.

Table 2. The calculation of vaccine effectiveness.

Protection level	Status of Chickens		Total
	Vaccinated (+)	Not Vaccinated (-)	
Good protection (+)	158	0	158
Poor protection (-)	72	20	92
	230	20	250

Sensitivity	A/(A+C)x100%	= 158/230x100%	=68.92%
The failure rate	C/(A+C)x100%	= 72/230x100%	=31.31%
Natural protection.	B/(B+D)x100%	= 0/20x100%	=0%
Specificity	D/(B+D)x100%	= 20/20x100%	=100%
Accuracy	(A+D)/Nx100%	= 178/250x100%	=71.20%

The chicken antibody titers after the vaccine was considered successful if the value was greater than or equaled to 2^4 and the range was considered to be able to protect chickens against AI disease. The blood sampling for HI titers should be carried out one month to two months after vaccination. Four weeks were required to antigen reaction (vaccine)with immunoglobulins to form antibody (Priyono, 2004).

The level of immunity or antibodies showed the ability of the body for protection against infectious agents. This examination is important for field research in places where individuals who were vaccinated and who have never been vaccinated that was randomly chosen, but it should be emphasized that the ability of the vaccine is not determined by the stimulation of serum antibody alone but more towards the addition of protection against a certain disease. Tizzard (2000) says that the immune response or the sensitivity of an animal can be determined by finding specific antibodies in blood serum due to animal or livestock exposed to or infected with a specific antigen.

False-negative rate/vaccine failure. In this study the false negative rate or failure rate of AI vaccine protection was calculated based on the comparison between the number of vaccinated chicken that indicated the level of immunity or HI titers below 2^4, or not protective, of the total number of native chickens that were vaccinated, multiplied by 100%. The results obtained amounted to 31.31%, this can be caused by many factors. The factors that caused the failure of vaccination were that, the minimum dose of vaccine was still lacking, chickens had been infected so there was no immunity, the vaccine was damaged due to improper treatment or improper storage (2-8°C), the increased virulence of AI in the environment of LPAI to HPAI, stress conditions of treatment, or the possibility of subjective factors that were not the same AI virus between vaccine virus to the virus in the field because the virus easily experienced gene mutation. The vaccinators and their skills also played a role in the success of a vaccination.

The failure of vaccination is an evasion of immune response or failure of eradication of the virus from the host because of antigenic variation, the AI virus antigen has several different surfaces such as hemagglutinin and neuraminidase. About this genetic mutation, it is necessary to prove the truth, therefore, there is a jumping theory, that the AI virus from poultry can infect humans without the need to go through the role of receptor (2.6 sialic acid), it usually takes the very greater number of viruses that can do this mechanism. One important thing is to prepare a suitable AI vaccine, safe and easily prepared in accordance

with the nature of AI virus vaccines that are easily changed, therefore, that AI vaccination can succeed or unfailed in chickens (Tizzard, 2000).

False-positive rate / level of natural immunity. False-positive rate is the probability of the protective existence of AI vaccine protection to chicken that is not vaccinated, it is used to determine the level of protective natural immunity. In this study the results showed zero percent(0%), it meant that no formation of native chickens were naturally immune to the level of protective immunity that was able to prevent the AI disease, therefore it is absolutely necessary for AI vaccination in chickens, although it was found that antibody titers could be measured (8 of 20 samples measured the antibody AI). According to Prijono (2004) low levels of antibodies can not protect chickens in the event of disease. Aamir et al. (2005) says that zero titer is very susceptible to disease, because chickens are able to protect themselves from the attack of AI challenge if the test shows a minimum score of 10, while the immune HI titer is needed to protect poultry immunity if the titer test indicates a geometric HI of 15 or 2^4, a good immune titer if HI is greater than or equal to 2^4.

The chickens that are not AI vaccinated is most likely to protect themselves from disease through the mechanism of resistance nonimmunologic resistance. The factors that play roles among other things are lysozyme, bile, liver, bone marrow, thymus gland and the main thing is the interference factor and interferon. According to Tizzard (2000), nonimmunologic antiviral defense mechanism interference is the term name due to the inhibition of viral replication of other viruses, because these other viruses trigger the release of interferon, and the released interferon were released by infected cells or infected virus, within a few hours after the invasion of the virus then interferon is produced in significant amounts.

Natural immunity can be formed by possible exposure to low doses of virus and a less virulent strain, when the chicken is in good condition. This phenomenon can not describe the venture selection of chicken in response to AI virus attacks in the field, and may also explain the persistence of the AI activities in an environment that is a potential outbreak of chickens and humans. In accordance with the indication expressed by Dharmayanti and Darminto (2009) that the AI virus in the past five years have infected different types of birds. Clearer statement by Widiasih et al. (2006) that birds that are kept at home (chicken) is one risk factor in these cases of AI and Sim et al. (2005) suggest that the migratory bird would have had limited potential for carrying the viruses over long distances unless subclinical infection were prevalent, so they have played a more significant role in spreading the disease.

Specificity of vaccination. The specificity of AI vaccines in chicken percentage was calculated based on the ability to make chicken immunity after vaccination was carried out, while the results showed a value of 100% with a record of all the chickens are only capable of making immunity levels varying from low to high. It showed all the vaccinated chickens showed specific immune responses that vary from 2^1 to 2^9, and it could explain that the treatment of vaccination was very important or necessary in order to increase the immune status of chickens because of the specific immune response. According to Tizzard(2000), specific immune response is a reaction against foreign objects (virus vaccines) that include a series of cellular interactions were excreted and spreadered of specific cell products.

Specificity is the ability to choose an immune response with high sensitivity, the products of the immune response (antibodies) will react completely with foreign objects and

can distinguish between substances that are closely related, this is the nature of specific immune responses that distinguish one gene from the other antigens. Tizzard (2000) also explained that the specific immune response is antibody memory, this memory is able to accelerate and enhance the response by the proliferation and differentiation of cells that have been sensitized in the event of subsequent exposure to the immunogen. Clearer statement of Suarez and Scultz-Cherry (2000), the cytotoxic T lymphocyte response can reduce viral shedding in mild pathogenic avian influenza viruses, but provides questionable protection against HPAI. Influenza viruses can directly affect the immune response of infected birds, and the role of the *Mx* gene, interferons, and other cytokines in the protection from a certain disease remains unknown.

Accuracy/effectiveness of vaccines. Accuracy is the proportion of vaccines that are formed, protective of its existence as a result of vaccination compared to chickens that are not vaccinated. In this study, the accuracy rate of the vaccine was 71.20 %, this figure could illustrate the effectiveness of the vaccine. These figures indicated that the implementation of the vaccination was to give more protection (to stimulate the formation of immunity) in a group, relative to non vaccinated chickens. Inactivated vaccine that is prepared from virulent virus with the process of chemical or physical agents is targetted to destroy the infectivity or imunogenicity temporary defense, therefore, good preparation is necessary to guarantee an appropriate and safe, the amount of virus that is needed, is able to make a substantial immune antibodies response. In addition, the inactivated vaccine can not cause disease or residual virulence (Tizzard, 2000).

The accuracy/effectiveness of AI vaccines could not reach 100 percent due to various factors, including factors in this method of vaccine production. According to Setijanto (2005), the manufacture of vaccines can be made by reassortment process or manufacture of conventional vaccines. Other researchers suggest (Nidom, 2010) that the vaccine is made with different virus sub-type due to mutation, therefore a higher level of protection can be achieved. Subbarao et al. (2003), explains that the AI vaccine should be made based on reversible plasmid genetic and the results are evaluated by hemagglutination assay.

Thus 2 x 2 contingency table could describe a more complete on the management of the vaccination in the field. In the standard analysis, the data obtained in the application of the vaccine, was only concerned with the percentage (%) a protective antibody titers in chicken samples that got vaccination (sensitivity) and vaccination failure (false negative), while this analysis obtained some features i.e, the specificity of the vaccine, natural immunity of the chicken (false positive), and the accuracy of the vaccine. This calculation method could enhance the efforts of observation (surveillance) of epidemic or endemic disease because it could reveal the natural immune status such as the efforts undertaken by Azhar et al. (2010) and Robyn et al. (2012) in surveillance of HPAI in Indonesia, due to the influence of immunity (natural) of local poultry in commercial farms.

Conclusion

The conclusion obtained in this study is that the level of vaccine protection measured by calculating 2 x 2 contingency table is 68.92%, 31.31% for vaccine failure rate, the level of natural protective immunity in samples is 0%, a specificity is 100% and the effectiveness of the vaccine is 71.20%.

References

Aamir G, Shaamoon N, Mohammed Y dan Jawad N. 2005. Immunomodullatory Effects of Multistrain Probiotics (Protexin) on Broiler Chicken Vaccinated Againts Avian Influenza Virus (H9). International Journal of Poultry Science. 4(10): 777-780.

Azhar M, AS Lubis, ES Siregar, RG Adler, E Brum, J McGrane, I Morgan, and P Roeder. 2010. Participatory Disease Surveillance and Response in Indonesia: Strengthening Veterinary Services and Empowering Communities to Prevent and Control Highly Pathogenic Avian Influenza. J Avian Disease, (54): 749-753.

Dharmayanti NLPI, R Indriani, A Wiyono and RMA Adjid. 2005. Field of effication of avian infuenza vaccine using local isolate on local chicken in Pandeglang and Tangerang. J. Biol. Indon. 3(10) : 466-473.

Dharmayanti NLPI and Darminto. 2009. Mutations AI Virus in Indonesia: Antigenic Drift Protein hemagglutinin (HA) of H5N1 Influenza Virus 2003-2006. J. Media Kedokteran Hewan, 25(1). Airlangga University, Surabaya.

Doan, CN, Timothy M, US Jadhao and T Maines. 2005. Isolation and Characterization of Avian Inluenza Viruses Including Higly Pathogenic H5N1 From Poultry in Live Bird Market in Hanoy Vietnam in 2001. J of Virology. 79 : 4201-4212.

Gary MI, 2010. Analysis of 2x2 Contingency Tables in Educational Research and Evaluation. International Journal for Research in Education.27: 1-14.

Khan OA, Shuaib MA, Rahman SS, Ismail MM, Hammad YA, Baky MH, Fusaro A, Salviato A, Cattoli G. 2009. Isolation and identification of highly pathogenic avian influenza H5N1 virus from Houbara bustards (Chlamydotis undulata macqueenii) and contact falcons. J. Avian Pathologi. 38(1):35-9.

Nidom CA, R Takano, S Yamada, Y Sakai-Tagawa, Syafril D, D Aswadi, T Suzuki, Y Suzuki, K Shinya, K Iwatsuki-Horimoto, Y Muramoto, and Y Kawaoka.

2010. Influenza A (H5N1) Viruses from Pigs, Indonesia. Emerging Infectious Disease J.16(10):1515-1523.

Priyono, WB, 2004. Avian Influenza. Clinical symptoms. Anatomical Pathological Changes and Handling. Department of Health. Yogyakarta.

Robyn M, WB Priyono, LM Kim and E. Brum. 2012. Diagnostic Sensitivity and Specificity of a Participatory Disease Surveillance Method for Highly Pathogenic Avian Influenza in Household Chicken Flocks in Indonesia.J. Avian Disease, 56(2) : 377-380.

Setijanto H. 2005. Discussion of Implementation Research. Avian Influenza: Epidemiology and Disease Prevention Strategy. Faculty of Veterinary Medicine. IPB. Bogor.

Sims LD, Domenech J, Benigno C, Kahn S, Kamata A, Lubroth J, Martin V, Roeder P. 2005. Origin and evolution of highly pathogenic H5N1 avian influenza in Asia. J. Vet Rec.;157(6):159-64.

Suardana, IBK, NMRK Dewi and IGNK Mahardika. 2009. Immune Response of Bali Duck to Various Doses of Avian Influenza H5N1 Vaccine. J. Veteriner. 10(3) : 150-155.

Suarez DL and S. Schultz-Cherry (2000). Immunology of Avian Influenza: a review. J. Developmental and Comparative Immunology. (24):269-283.

Suarez DL, A Das, and E Ellis. 2007. Review of Rapid Molecular Diagnostic Tools for Avian Influenza Virus. J Avian Disease. 51:201–208, 2007

Subbarao, K, Chen, H Swayne, D Mingay, L Fodor, E Bromnlee,G Xu, X Lu, X Katz, J Cox and Y Matsuoka. 2003. Evaluation of a genetically modified reasortant H5N1 influenza A virus vaccine candidate generated by plsmid based reverse genetics. J.Virology 305 : 192-200.

Tizzard I. 2000. Veterinary Immunology An Introduction. W.B Sounders Co., Philadelphia. P. 143-275.

Widiasih DA, H Susetya, B Sumiarto, CR Tabbu and S Budiharta. 2006. Case Control Studies of Avian Influenza in Poultry in East Java, Central Java and Yogyakarta. J. Sain Vet 24(1).