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DIVA strategies in avian influenza virus; present scenario

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Abstract
For the successful eradication campaign of AIV two parameters are to be considered, which are the successful and complete vaccination complemented with the highly specific monitoring programme that can differentiate well between infected and vaccinated animals (DIVA). In all 6 DIVA strategies are applied for monitoring which are (1) Sentinel birds (2) Hetrologus NA strategy (3) Use of subunit vaccine (4) NS1 serology (5) M2e (Transmembrane protein) (6) HA2 glycoprotein based.

Keywords: avian influenza, vaccination, DIVA strategies, NS1, hemagglutinin, neuraminidase, antigenic drift

Introduction
The term 'DIVA' (Differentiating infected from vaccinated individuals) was coined in 1999 by J. T. van Oirschot of the Central Veterinary Institute, in the Netherlands. It is now generally used as an acronym for 'differentiating infected from vaccinated animals'. The term was originally applied to the use of marker vaccines, which are based on deletion mutants of wild-type microbes, in conjunction with a differentiating diagnostic test. The DIVA strategy has been extended to include subunit and killed whole-virus vaccines. This system makes possible the mass vaccination of a susceptible animal population without compromising the serological identification of convalescent individuals. The DIVA approach has been applied successfully to pseudorabies and avian influenza eradication, and has been proposed for use in foot-and-mouth disease and classical swine fever eradication campaigns.
The vaccinated birds may get infected and shed some amount of virus in the environment since 'sterile immunity' is not achievable with presently available influenza vaccine. The infected among vaccinated birds may not show clinical signs which would mean that HPAI or LPAI viruses of H5 or H7 subtypes could infect a flock and circulate for some time in that flock unnoticed. Therefore, there is need to identify vaccinated birds that have subsequently become infected with field virus so that other bio-security measures can be adopted to control the infection. This approach is popularly referred as DIVA (differentiation of infected from vaccinated animals) and is advocated by many international agencies including OIE. Six different DIVA strategies have been proposed by [1] as outlined below-

1. Use of sentinel birds
These are non-vaccinated birds that are introduced in the vaccinated flocks and routinely tested for AI infection. This method has been used in Italy [2]. Routinely the sentinels are tested by standard serologic test, and direct virus detection methods. The main disadvantage is related with the management of the sentinel birds.

2. Heterologous neuraminidase strategy
This strategy is based on providing heterologus NA protein and homologous HA protein in vaccine formulation so that vaccinated and infected birds can be differentiated based on detection of antibodies to heterologous NA. The introduction of heterologous NA vaccination application in Italy [3] various combinations of HA and NA proteins have been tested and recommended, including the use of rare NA subtypes for vaccine development such as N5 and [2]. Some of the commercially available vaccines for AI are based on this strategy and contain a NA DIVA marker. The AIV has 10 different proteins, including Hemagglutinin (HA) and neuraminidase (NA). Therefore, standard killed vaccine with heterologous NA subtype can be use effectively, and this allows the possibility of a DIVA strategy based on serologic test target

to the NA protein. Introduction of the eight plasmid reverse genetics system, which allows rapid de novo generation of reassortant live virus, has made it possible for the rapid availability of a heterologous vaccine once the NA subtype of the wild-type circulating virus is known [4].

3. Use of subunit vaccine

The subunit vaccines provide the most flexibility to work with existing type A serologic surveillance tests, specifically the AGID and commercial ELISA tests that target the matrix (MA) or nucleoprotein (NP) structural proteins. Because antibodies to the Haemagglutinin and neuraminidase proteins provide the primary protection, it is possible to protect birds by having only these proteins in a vaccine. Many different types of subunit vaccine, including virus vectored vaccines and vaccines using protein expressed in different culture system. Vaccinated birds will not develop antibodies to the internal proteins, providing a clear distinction between infected (has antibodies to matrix or nucleoprotein proteins) and vaccinated bird (has antibodies to Haemagglutinin only). HA gene is a structural virus protein with important functions for immunity and is one of the key determinants of AIV antigenic properties. Although optimum protection is achieved through the use of vaccination with whole inactivated virus homologous to the circulating strain, studies have indicated that the presence of HA alone in vaccine elicits protective immune response against viral infection [5]. In the subunit vaccine strategy, the AIV HA gene is expressed in bacteria, viruses, or yeast system before being purified and prepared for use as a vaccine. The biggest drawback; particularly for the live viral vectors is regulatory, for to be genetically modified organisms.

4. NS1 based serology

An alternative DIVA strategy can be based on differential immune response to the influenza nonstructural protein 1 (NS1). The NS1 protein is produced in large amounts in infected cells, but it is not packaged into the infectious viral particle. Hence, killed vaccines made up from whole virus should not have NS1 and should not induce an antibody response to the NS1 protein while a natural infection with influenza virus induces antibody response to NS1. This DIVA approach was demonstrated previously with equine influenza viruses in horses using an ELISA format. Development in ELISA using a recombinant non-structural protein NS1 as the diagnostic antigen; which was cloned from an AIV H9N2 subtype strain to differentiate Avian influenza virus infected chickens versus chickens immunized with inactivated avian influenza virus. This finds application in serological diagnosis [6]. Harnessed NS1 as a differential diagnostic marker for influenza virus infection. Poultry were checked for anti-NS1 Abs against recombinant NS1 or chemically synthesized NS1 peptides used as antigen and results were compared from immune sera of chickens inoculated with live AIV, inactivated purified vaccines or inactivated commercial vaccines or to positivity for Abs to NS1 was achieved in experimentally infected but not in vaccinated birds.

The primary advantage of the NS1 DIVA strategy particularly compared to the heterologous NA DIVA strategy is the ability to work with any killed vaccine using a single diagnostic test as compared to needing multiple diagnostic tests as with the heterologous NA strategy [1].

5. M2e protein in DIVA strategy

M2e is a protein present in trans membrane & its function is form ion channel on the AIV surface that are crucial for the release of viral genome in to the host cell cytoplasm during virus entry [7] and also serves as a pH regulator for the golgi apparatus, which is essential for HA glycoprotein maturation [8]. M2e is suitable as DIVA antigen because of its relatively invariable nature across AIV strains [9] & small size & low abundance in comparison to the other two surface glycoproteins (HA & NA) which allows easy escape to immune selection pressure. Though both NSI & M2e are produced following viral replication & hence not found in vaccinated birds because most AIV vaccines are produced from inactivated whole virus. Studies revealed that antibody response to NSI protein after natural injection can be consistent, short lived & so may be difficult to use as a practical DIVA strategy where as the M2e protein elicited an immune response within two weeks after injection which declined rapidly. So to enhance its tetrameric protein (tM2e) with improved antigencity & enhanced test efficiency was produced for early detection ofH5N1 injection. It was concluded that tM2e ELISA could be a useful DIVA test for serological monitoring of poultry farm that practice vaccination in H5N1- endemic region.

6. Haemagglutinin subunit 2 (HA2) glycoprotein

HA2 glycoprotein (gp) is the c-terminus fragment of the cleavage form HA protein [10]. It is highly conserved across 16 HA subtypes with only two epitope variants corresponding to the classical phylogenetic grouping of AIV HA proteins [11]. As HA2gp is highly conserved hence enables universal detection to all subtype & conserved region is only accessible to immune recognition following virus infection. HA0 cleavability is essential for virus infectivity [12] where the cleavage of HA0 to form HA1& HA2 subunits is required for membrane binding & virus entry to the host cell [10] HA2gp is not accessible in native form of HA2gp & is exposed only after cleavage of HA0 to allow the conformational change which leads to membrane fusion & virus entry. Hence presence of antibody for epitopes on HAgp directs to presence of virus infection.

Conclusion

Although the sentinel bird strategy is simple to employ, there are concerns that the native birds may increase the infection risk for the vaccinated flock following repeated and lengthy exposure to the high load shedding of the virus by the sentinels Acquiring a new infection is still possible in the vaccinated flock due to the continuously evolving nature of AIV, and technical vaccination issues, such as ineffective application or insufficient coverage, with poor antigenic match of the vaccine with the field strains.

DIVA test based on NSI, M2e & HA2 protein are more favorable & applicable on practical ground. DIVA test strategy suggests working in complement to inactivated vaccine administration but studies have shown shedding of virus in feces of infected birds though in low amount but the silent spread is possible due to the generation of escape mutants in response to vaccination pressure. NS1 affectivity is less & accuracy is questionable because of its truncated form in nature & its short term presence is to be taken into consideration. It also suffers from decreasing specificity and

immunogenicity though recombinant M2e works well but is not cost effective for large scale use. HA2gp DIVA strategy suffers as HA2 is a weak natural immunogenic which may also lead to false negative results due to low seroconversion in infected hosts. It can be concluded that for successful monitoring programme, DIVA vaccines to be used which are specific, cost effective & readily distinguishable from wild – type virus. This should be completed by DIVA strategy for mass, sensitive and specific, which makes it suitable. Each strategy has its own advantages and disadvantages so to have most effective DIVA strategy which depends on the choice of researchers which is optimally suitable to the prevailing situations.

References

1. Suarez DL, Perdue ML. Multiple alignment comparison of the non-structural genes of influenza A viruses. Virus Research. 1998; 54:59-69.
2. Capua I, Marangon S. DIVA-a vaccination strategy enabling the detection of field exposure to avian influenza. Developmental Biology (Basel). 2004; 119:229-233.
3. Capua I, Terregino C, Cattoli G *et al*. Development of a DIVA strategy using a vaccine containing a heterologous neuraminidase for the control of avian influenza. Avian Pathology. 2002; 32:47-55.
4. Lee CW, Senne DA, Suarez DL. Effect of vaccine use in the evolution of Mexican lineage H5N2 avian influenza virus. Journal of. Virology. 2004; 78:8372-8381.
5. Robinson HL, Hunt LA, Webster RG. Protection against a lethal influenza virus challenge by immunization with a haemagglutinin-expressing plasmid DNA. Vaccine, 1993; 11:957-960.
6. Tumpey TM, Alwarez R, Swayne DE, Suarez DL. Diagnostic Approach for Diffrentiating Infected from Vaccinated on the basis of Antibodies to NS1, the Nonstructural protein of Influenza virus. Journal of Clinical Microbiology. 2007; 43:676-683.
7. Lamb RA, Zabedee SL, Richardson CD. Influenza virus M2 protein is an integral membrane protein expressed on the expressed-cell surface. *Cell*, 1984; 540:627-633.
8. Sugrue RJ, Hay AJ. Structural characteristics of the M2 protein of Influenza A viruses: evidence that it forms a tetrameric channel. Virology, 1991; 180:617-624.
9. Khurana S, Suguitan AL, Rivera Y *et al*. Antigenic fingerprinting of H5N1 avian influenza using convalescent sera and monoclonal antibodies reveals potential vaccine and diagnostic targets. PLoS Medicine, 2009.
10. Skehel JJ, Bayley PM, Brown EB. Changes in theconformation of influenza virus hemagglutinin at the pH optimum of virus-mediated membrane fusion. Proc Natl Acad Sci USA. 1982; 79:968-972.
11. Sui J, Hwang WC, Perez S *et al*. Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. Natural Structure of Molecular Biology. 2009; 162:65-273.
12. Klenk HD, Rott R, Orlich M *et al*. Activation of Influenza A viruses by trypsin treatment. Virology. 1975; 68:426-439.