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Research Note—

Experimental and Field Results Regarding Immunity Induced by a Recombinant Turkey Herpesvirus H5 Vector Vaccine Against H5N1 and Other H5 Highly Pathogenic Avian Influenza Virus Challenges

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SUMMARY: Vaccination against H5N1 highly pathogenic avian influenza (AI) virus (HPAIV) is one of the possible complementary means available for affected countries to control AI when the disease has become, or with a high risk of becoming, endemic. Efficacy of the vaccination against AI relies essentially, but not exclusively, on the capacity of the vaccine to induce immunity against the targeted virus (which is prone to undergo antigenic variations), as well as its capacity to overcome interference with maternal immunity transmitted by immunized breeding hens to their progeny. This property of the vaccine is a prerequisite for its administration at the hatchery, which assures higher and more reliable vaccine coverage of the populations than vaccination at the farm. A recombinant vector vaccine (Vectormune® AI), based on turkey herpesvirus expressing the hemagglutinin gene of an H5N1 HPAIV as an insert, has been used in several experiments conducted in different research laboratories, as well as in controlled field trials. The results have demonstrated a high degree of homologous and cross protection against different genetic clades of the H5N1 HPAIV. Furthermore, vaccine-induced immunity was not impaired by the presence of passive immunity, but on the contrary, cumulated with it for improved early protection. The demonstrated levels of protection against the different challenge viruses exhibited variations in terms of postchallenge mortality, as well as challenge virus shedding. The data presented here highlight the advantages of this vaccine as a useful and reliable tool to complement biosecurity and sanitary policies for better controlling the disease due to HPAIV of H5 subtypes, when the vaccination is applied as a control measure.

RESUMEN. *Nota de investigación*- Resultados experimentales y de campo sobre la inmunidad inducida por una vacuna recombinante con el virus HVT como vector con el gene H5 contra el desafío con virus de la influenza altamente patógeno subtipo H5N1 y con otros virus H5.

La vacunación contra el virus H5N1 de la influenza aviar altamente patógena es uno de los medios complementarios posibles disponibles para controlar a la influenza aviar en los países afectados donde la enfermedad se ha convertido, o con alto riesgo de convertirse endémica. La eficacia de la vacunación contra la influenza aviar se basa esencialmente, pero no exclusivamente, en la capacidad de la vacuna para inducir inmunidad contra el virus al que está dirigida (el cual es posible que pueda sufrir variaciones antigénicas), así como su capacidad para superar la interferencia con la inmunidad materna transmitida por las gallinas reproductoras inmunizadas a su progenie. Esta propiedad de la vacuna es un requisito previo para su administración en la incubadora, lo que asegura la cobertura de vacunación superior y más confiable en las poblaciones en comparación con la vacunación en la granja. Una vacuna recombinante vector (Vectormune® AI), que está basada en un virus herpes de pavo que expresa un gene de la hemaglutinina de un virus de influenza altamente patógeno H5N1, se ha utilizado en varios experimentos realizados en diferentes laboratorios de investigación, así como en ensayos controlados de campo. Los resultados han demostrado un alto grado de protección homóloga y cruzada contra diferentes clados genéticos del virus de influenza aviar altamente patógeno subtipo H5N1. Por otra parte, la inmunidad inducida por la vacuna no resultó afectada por la presencia de inmunidad pasiva, sino por el contrario, se complementaron mejorando la protección temprana. Los niveles demostrados de protección contra los diferentes virus de desafío exhibieron variaciones en términos de mortalidad después del desafío, así como su diseminación. Los datos aquí presentados ponen de relieve las ventajas de esta vacuna como una herramienta útil y confiable para complementar las políticas de bioseguridad y sanitarias para un mejor control de la enfermedad ocasionadas por virus de influenza aviar altamente patógenos subtipos H5, cuando la vacunación se aplica como medida de control.

Key words: avian influenza, vaccination, rHVT-H5 vector vaccine, hatcheries, field trials

Abbreviations: AI= avian influenza; AIV = avian influenza virus; BR = broilers; BSL = biosecurity level; CODA - CERVA = Centrum voor Onderzoek in Diergeneeskunde en Agrochemie – Centre d'Etude et de Recherches Vétérinaires et Agrochimiques; COM= commercial; DIVA = differentiating infected from vaccinated animals; DOI = duration of immunity; dpc= days postchallenge; FAO = Food and Agriculture Organization of the United Nations; HA = hemagglutinin; HI=hemagglutination inhibition;

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HIT=hemagglutination inhibition test; HPAI = highly pathogenic avian influenza; HPAIV=highly pathogenic avian influenza virus; HVT= turkey herpesvirus; IN= intranasal; IZSVe = Istituto Zooprofilattico Sperimentale delle Venezie; LY = layers; MDA = maternally derived antibodies; OOI = onset of immunity; ON = oculonasal; rHVT-H5 = recombinant turkey herpesvirus vector vaccine against AI containing a clade 2.2 H5 insert; RLQP = National Reference Laboratory for Veterinary Quality Control on Poultry Production; SEPRL = Southeast Poultry Research Laboratory; SPF = specific pathogen free; VACC= vaccinated; WL= white layers; woa = weeks of age; wpv = weeks postvaccination

Vaccination of poultry against avian influenza (AI) requires consideration of many different factors, including the fast spreading of the disease, the difficulties to detect and depopulate affected farms in a timely manner, and the availability to implement surveillance and monitoring programs.

The five main issues associated with vaccination against AI are 1) the antigenic variability of the challenging virus affecting efficacy of classical inactivated vaccines (7,16,18); 2) the interference of maternally derived antibodies (MDA) with classical inactivated vaccines, preventing early vaccination at the hatchery (1,4); 3) the absolute necessity to reach a high vaccine coverage at the flock level; 4) the nonsterilizing immunity induced by inactivated AI vaccines; and 5) the difficulty to conduct a differentiating infected from vaccinated animals (DIVA) monitoring strategy when classical inactivated vaccines are used.

A recombinant AI vaccine using the turkey herpesvirus (HVT) as a vector to express the hemagglutinin (HA) gene of a clade 2.2 H5N1 highly pathogenic avian influenza virus (HPAIV) has been developed to address the MDA interference issue and offer the possibility of vaccinating in the hatchery, as commonly done with the HVT vaccine to prevent Marek's disease.

This vaccine has been officially licensed by the U.S. Department of Agriculture in the United States in 2012, and since then, in three other countries (Egypt, Bangladesh, and Mexico), and made commercially available. Complementary information included in the original registration file (Ceva Animal Health. Unpubl. data, 2011), many scientific investigations (Ceva Animal Health. Unpubl. data, 2011) (13), controlled trials (2,3,5,6,16,17,18,19,20), and field studies (8,9,14) have been conducted to increase knowledge regarding its characteristics and performances so that prescription and decision for usage can rely on broader information. Field experience from large commercial (COM) use has also enriched our experience and knowledge.

The objective of this article is to present a brief overview of the data from different experiments conducted so far, and some of the conclusions to be drawn regarding the practical usage and monitoring of vaccine performance.

MATERIALS AND METHODS

Sources of information. Data used for this summary are cited in the list of references. These references belong to the following categories: 1) peer-reviewed published studies (6,8,16,18,19); 2) unpublished trials or study performed by reference laboratories (2,3,5,9,17,20) or independent organizations (14) (J. J. De Wit. Unpubl. data, 2014); and 3) in-house private company reports (Ceva Animal Health. Unpubl. data, 2011) (12).

Locations. Corresponding experiments and studies were conducted by different research teams, in different research centers or reference laboratories, including the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro, Padova, Italy (2), University of Maryland, College Park, MD, (Ceva Animal Health. Unpubl. data, 2012), Southeast Poultry Research Laboratory (SEPRL), U.S. Department of Agriculture, Athens, GA (5,6), National Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP), Animal Health Research Institute, Giza, Egypt (8,9), Avian Virology and Immunology Unit, Veterinary and Agrochemical Research Centre (CODA CERVA), Ukkel, Brussels, Belgium (3,4,16,17,18,20), Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, Indonesia (15), and Gezondheidsdienst voor Dieren (GD) Animal Health, Deventer, the Netherlands (J. J. De Wit. Unpubl. data, 2014), as well as different locations of Ceva Animal Health (Libourne, France, Budapest, Hungary, and Lenexa, KS) (Ceva Animal Health. Unpubl. data, 2011; Ceva Animal Health. Unpubl. data, 2012). One study (14) was conducted under field conditions by independent non-for-profit organizations, including Centre de Coopération Internationale en Recherche Agronomique pour le Développement - Animal et Gestion Intégrée des Risques (Unité de Recherche, France; CIRAD-AGIRS), Institut de Recherche pour le Développement - Maladies Infectieuses et Vecteurs: Ecologie, Génétique, Evolution et Contrôle, France, IRD MIVEGEC), Oxford University Clinical Research Unit in Vietnam (OUCRU), Food and Agriculture Organization of the United Nations (FAO), Emergency Center for Transboundary Animal Diseases ECTAD (Egypt), RLQP (Cairo, Egypt), Food and Agricultural Organization (Rome, Italy, FAO), General Organization Veterinary Services (Cairo, Egypt, GOVs).

Animals. The avian species used in the studies were generally chickens (*Gallus gallus*) (2,3,5,8,9,16,17,18,19,20), but other bird species have also been used, including turkeys (*Meleagridis gallopavo*) (9), different waterfowl species, including ducks (Muscovy, [*Meleagridis gallopavo*], Pekin, [*Anas platyrhynchos domesticus*], mallard) and geese (*Anser anser domesticus*) (13), as well as pheasants, (*Phasianus colchicus*), quail (*Coturnix coturnix*), and pigeons (*Columba livia*) (Ceva Animal Health. Unpubl. data, 2012). Chickens used in these experiments were of different types, including specific-pathogen free (SPF; 2,5,17,18,20; Ceva Animal Health. Unpubl. data, 2011), COM, broilers (BR; 3,9,16,19), or layers (LY; 8). Commercial chickens were always provided with MDA against HVT and sometimes against AIV (3,8,9,16,19). MDA against AIV were directed either against H5N2 (3) or H5N1 (8,9,16,19) AIV subtypes (because of vaccination of breeders with COM H5N2 or H5N1 inactivated AI vaccines).

Housing. The experiments were conducted under either fully isolated conditions (2,3,5,6,16,17,18,19,20) (Ceva Animal Health. Unpubl. data, 2012; J. J. De Wit. Unpubl. data, 2014), a biosafety level 2 (BSL2) facility (13), controlled conditions (Ceva Animal Health. Unpubl. data, 2011), or with animals vaccinated (VACC) and raised under field conditions and then transferred to isolation units for challenge (8,9). One study was performed entirely under field conditions (14). HPAI challenge experiments were always conducted in BSL3 facilities.

Vaccination. The recombinant turkey herpesvirus vector vaccine against AI containing a clade 2.2 H5 insert (rHVT-H5) vector vaccine (rHVT-H5) is constructed from the FC126 strain of HVT. In the genome of this virus, in a region recognized as nonessential for the growth, a cDNA sequence encoding for the HA of a clade 2.2 H5N1 HPAIV (A/swan/Hungary/4999/2006) has been inserted. For safety reasons, the HP cleavage site of the HA gene has been modified for a low pathogenic motif. A *cytomegalovirus* used as a promoter has also been inserted to control the expression of the HA insert. The vaccine is produced in chicken embryo fibroblasts, presented as cell associated, deep frozen, and transported in liquid nitrogen (Ceva Animal Health. Unpubl. data, 2012). Vaccination was administered at full COM dose per bird injected at 1 day of age by the subcutaneous route, per manufacturer's recommendations.

Challenges. HPAI challenges were applied at different ages, varying between 2 and 19 wk of age (woa; i.e., 2 to 19 wk postvaccination [wpv]), by using either the intranasal (IN) or oculonasal (ON) routes of inoculation. The challenge dose was 10^6 50% egg infective dose (EID_{50}) per chicken, except in challenge experiments conducted with the H5N1 HPAIV from Vietnam (Ceva Animal Health. Unpubl. data, 2012) and the H5N8 HPAIV from Germany (20), where the dose of $10^5 EID_{50}$ was also used. Monitoring of VACC and control birds following challenge was conducted over a 14-day period for the recording of clinical signs and mortality.

Viruses. The H5 AIV used for the different challenges were all the HP type, belonging to different subtypes (H5N1, H5N2, and H5N8) or different subclades (1, 2.2, 2.2.1, 2.2.1.1, 2.1.3, and 2.3.2.1) of the Asian

Table 1. Compilation of protection results collected through various experiments conducted with the rHVT-H5 vaccine.

		Vaccination			Challenge					Protection against					
			MDA against		HPAIV					Mortality (%)		% Oropharyngeal shedders			
Location	Country	Type of chickens	HVT	AIV	Subtype	Strain	Clade	Route	Age	VACC	Control	When	VACC (%)	Control (%)	Reference
University of Maryland	United States	SPF	No	No	H5N1	A/CK/Vietnam/1203/2004	1	IN	4 woa	85	0	2 dpc	30	100	(Ceva Animal Health. Unpubl. data, 2012)
CODA CERVA	Belgium	COM BR	Yes	No	H5N1	A/Duck/Hungary/11804/2006	2.2	IN	2 woa	90	0	2 dpc	20	38	(3)
CODA CERVA	Belgium	COM BR	Yes	Yes (H5N2)	H5N1	A/Duck/Hungary/11804/2006	2.2	IN	2 woa	100	20	2 dpc	0	60	(3)
CODA CERVA	Belgium	COM BR	Yes	Yes (H5N2)	H5N1	A/Duck/Hungary/11804/2006	2.2	IN	3 woa	90	0	2 dpc	0	80	(3)
CODA CERVA	Belgium	SPF	No	No	H5N1	A/CK/Egypt/1709-1 VIR08/2007	2.2.1	ON	3 woa	100	0	3 dpc	80	100	(18)
CODA CERVA	Belgium	SPF	No	No	H5N1	A/CK/Egypt/1709-6/2008	2.2.1.1	ON	3 woa	100	0	3 dpc	100	100	(18)
CODA CERVA	Belgium	COM BR	Yes	No	H5N1	A/CK/Egypt/1709-6/2008	2.2.1.1	ON	4 woa	90	0	2 dpc	10	100	(16)
CODA CERVA	Belgium	COM BR	Yes	Yes (H5N1)	H5N1	A/CK/Egypt/1709-6/2008	2.2.1.1	ON	4 woa	100	0	2 dpc	10	90	(16)
CODA CERVA	Belgium	COM BR	Yes	Yes (H5N1)	H5N1	A/CK/Egypt/1709-1 VIR08/2007	2.2.1	ON	4 woa	90	0	2 dpc	100	100	(16)
CODA CERVA	Belgium	COM BR	Yes	Yes (H5N1)	H5N1	A/CK/Egypt/1709-6/2008	2.2.1.1	ON	4 woa	70	0	2 dpc	90	100	(16)
Bogor Agricultural University	Indonesia	COM BR	Yes	Yes (H5N1)	H5N1	A/CK/West Java Subang/29/2007	2.1.3	ON	4 woa	80	0	2 dpc	60–80	100	(19)
Bogor Agricultural University	Indonesia	COM BR	Yes	Yes (H5N1)	H5N1	A/CK/Puwakarta-Cilingga/142/2010	2.1.3	ON	4 woa	95	0	2 dpc	60–80	100	(19)
SEPRL	United States	SPF WL	No	No	H5N1	A/Whooper Swan/Mongolia/3/2005	2.2	IN	6 woa	100	0	2 dpc	13	100	(5)
SEPRL	United States	SPF BR	No	No	H5N1	A/CK/West Java Subang/29/2007	2.1.3	IN	4 woa	80	0	NT[A]	NT	NT	(5)
SEPRL	United States	SPF WL	No	No	H5N2	A/CK/Queretaro/14588/1995	—	IN	4 woa	95	0	NT	NT	NT	(5)
CODA CERVA	Belgium	SPF	No	No	H5N1	A/CK/Egypt/1709-6/2008	2.2.1.1	ON	4 woa	100	0	2 dpc	100	100	(17)
CODA CERVA	Belgium	SPF	No	No	H5N1	A/CK/Egypt/1709-6/2008	2.2.1.1	ON	8 woa	100	0	2 dpc	100	100	(17)
RLQP	Egypt	COM BR	Yes	Yes (H5N1)	H5N1	A/CK/Egypt/1709-6/2008	2.2.1.1	IN	4 woa	93	0	2 dpc	100	100	(9)
RLQP	Egypt	COM BR	Yes	Yes (H5N1)	H5N1	A/CK/Egypt-63/2010 "variant"	2.2.1.1	IN	5 woa	80	0	2 dpc	100	100	(9)
CODA CERVA	Belgium	SPF	No	No	H5N8	A/CK/Germany/2014	2.3.4.4	ON	4 woa	100	0	2 dpc	90	100	(20)

Table 1. Continued.

Location	Country	Vaccination			Challenge					Protection against					Reference
		Type of chickens	MDA against		HPAIV					Mortality (%)		% Oropharyngeal shedders			
			HVT	AIV	Subtype	Strain	Clade	Route	Age	VACC	Control	When	VACC (%)	Control (%)	
IZSVe	Italy	SPF	No	No	H5N1	A/CK/Bangladesh/11RS1 984-33/2011	2.3.2.1	ON	4 woa	100	0	2 dpc	10	All dead	(2)
RLQP	Egypt	COM LY WB	Yes	Yes (H5N1)	H5N1	A/CK/Egypt/128s/2012	2.2.1	ON	19 woa	73	0	3 dpc	0	100	(8)
RLQP	Egypt	COM LY BS	Yes	Yes (H5N1)	H5N1	A/CK/Egypt/128s/2012	2.2.1	ON	19 woa	60	0	3 dpc	7	100	(8)

[A]NT = Not Tested.

H5N1-lineage, and isolated from different countries (Vietnam, Hungary, Egypt, Mongolia, Indonesia, Bangladesh, Mexico, and Germany). The H5N8 HPAIV was isolated in Germany in November of 2014 and belonged to the 2.3.4.4 clade.

Serology. Blood samples were collected from VACC and control birds at different time points, including day of age (in case of MDA-positive chickens), the time of challenge (to assess antibody response to vaccination), and at 2 wk postchallenge to assess seroconversion. Testing was conducted by using different methods, including 1) the hemagglutination inhibition (HI) test (HIT) using, an antigen homologous to the vaccine (homology between HA) and an antigen homologous to the challenge virus, 2) an NP (nucleoprotein) ELISA (ID Vet, Montpellier, France), 3) an H5 ELISA (influenza A antibody competition ELISA; ID Vet), and 4) an M2e ELISA developed in-house at CODA CERVA (10).

Virus shedding. Swab samples were also collected at the oropharyngeal and cloacal levels at different time points after challenge, including, generally, an early sampling at 2 or 3 days postchallenge (dpc) and later samplings at 4 to 7 dpc. Detection and quantification of the challenge virus in the swabs was done using either 1) different real-time reverse transcription-PCR (2,3,6,8,9,13,16,17,18,19,20) (Ceva Animal Health. Unpubl. data, 2012), or 2) virus isolation and quantification (5,6).

RESULTS

Because this article is at the same time a compilation and a summary, the most relevant information and data are presented in the following paragraphs corresponding to the most critical points.

Antibody responses. After vaccination of SPF (18) or COM layer pullets without MDA against AIV (Fig. 1) (Ceva Animal Health. Unpubl. data, 2011), the rHVT-H5 vaccine induces detectable antibodies by HIT, as soon as 3 wpv if a homologous antigen to the vaccine is used. The majority of the tested chickens are then positive, with 8 out of 10 (18) or 19 out of 20 (Fig. 1) (Ceva Animal Health. Unpubl. data, 2011), with titers reaching 4 to 6 $\log_2$, if a homologous antigen is used. This antibody response continued to increase until 9 woa and then reached a plateau with titer values of 9 to 11 $\log_2$ (Fig. 1) (Ceva Animal Health. Unpubl. data, 2011).

Production of a rHVT-H5 reference positive serum was generated in 94-day-old SPF chickens with one dose of vaccine on the first day of age by the subcutaneous route and bled 4 wpv. HI testing was conducted by using an antigen homologous to the vaccine and titrated at 8 HA units. One hundred percent of SPF chickens demonstrated positive titers varying between 3 $\log_2$ and ≥ 7 $\log_2$ (Fig. 2) (J. J. De Wit. Unpubl. data, 2014). In COM layer pullets with passive immunity against AIV (H5N1), antibody response was slower and reached lower HI titers at all time points (Fig. 1) (Ceva Animal Health. Unpubl. data, 2011).

Most of the experiments (2,3,9,16,19,20) have demonstrated that a high level of protection is reached before HI antibodies homologous to the vaccine are detected in all chickens. Thus, unlike inactivated AI vaccines, protection with rHVT-H5 vaccine does not necessarily correlate with antibody titers prior to challenge (11).

Likewise, when testing the heterologous antigen to the vaccine, the detected level of antibody before challenge is generally low. However, this also has no predictive value regarding protection. It is thus concluded that other components of the immune response are triggered by this vaccine, and, in particular, the cellular and mucosal ones, likely play an important role in protection of the birds (6,16,18).

After homologous challenge, an increase in HI antibody titers is observed. Following the heterologous challenge, the HI titer is more limited and variable. Following challenge early after vaccination, increases in HI antibody titer homologous to the vaccine cannot necessarily be attributed to challenge virus replication because a rise of similar intensity is also observed in VACC, nonchallenged chickens, as a consequence of antibody response to vaccination (Fig. 1) (Ceva Animal Health. Unpubl.

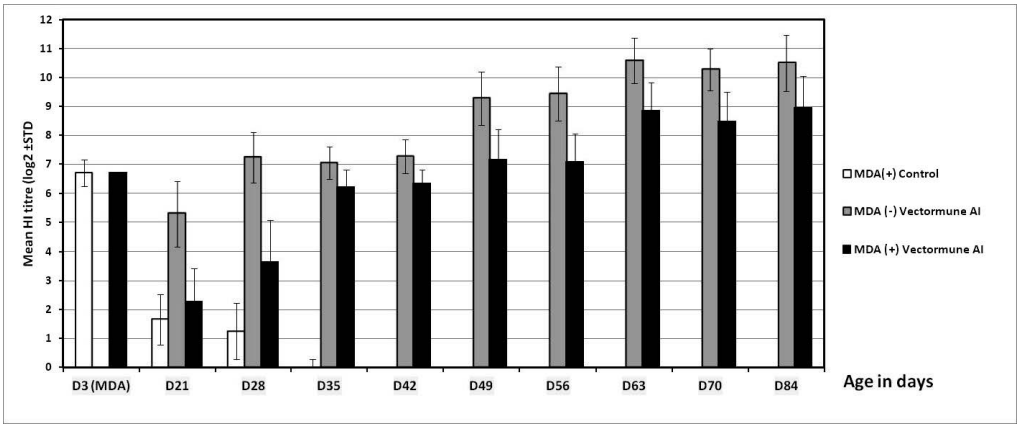

Fig. 1. Monitoring of antibody response to vaccination with a rHVT-H5 vaccine in commercial layer pullets provided (MDA+) or not (MDA-) with MDA against H5N1 AIV (Ceva Animal Health. Unpubl. data, 2011).

data, 2011). The use of other serologic tests to detect anti-NP or anti-M2 antibodies, induced by the replication of challenge virus, confirm that in some experiments, only a few VACC and challenged birds turn positive when these assays were used. It cannot be excluded that vaccination could delay infection, as well as antibody response detectable by NP or M2 ELISAs, therefore explaining negative results. This is under further investigation. The use of NP or M2 ELISAs in rHVT-HA VACC flocks may allow for a DIVA monitoring strategy with detection of VACC *vs*. infected flocks. However, a significant number of serum samples would need to be collected and assessed. It is likely that further screening for possible field infection by the use of molecular techniques would be employed.

Protective efficacy. Two criteria are generally considered as important regarding vaccine protection. They include the clinical protection (mortality and morbidity), as well as the reduction of virus shed after challenge. Table 1 gives a simple overview of the protection results collected through different experiments conducted with rHVT-H5.

In these experiments, chickens received a single dose of vaccine injected subcutaneously at day old and challenge between 2 to 8 woa (except one experiment at 19 wk [8]). The dose used in a majority of challenge studies was 10^6 EID_{50} (2,3,5,8,9,16,17,18,20), and on two occasions, a dose of 10^5 EID_{50} was used (20) (Ceva Animal Health. Unpubl. data, 2012).

Protection against mortality was variable, ranging from 60% to 100%, which included challenge with HPAIV strains of different clades of H5N1 and different subtypes (H5N2 and H5N8). The data clearly demonstrate protection against multiple clades of H5 HPAIV (5).

A reduction of virus shedding, both in terms of the percentage of shedders, as well as the amount of virus shed, was always observed following challenge. It is believed that the different experimental conditions, as well as the different techniques used to detect and quantify the virus, can explain these variations (Table 1).

Onset of immunity (OOI). The OOI is a measure of the time necessary for a vaccine to induce clinical protection against given conditions of a challenge. OOI of the rHVT-H5 vaccine varies according to the type of chicken (i.e., BR *vs.* LY) and the presence of MDA against AIV. In chickens free of MDA against AIV, early protection comes exclusively from vaccine replication. Ninety percent protection was observed in COM BR challenged 2 wk after vaccination and challenge with a H5N1 HPAIV of the same 2.2 clade (3), and 100% protection was demonstrated in SPF chicks challenged at 3 wk with Egyptian H5N1 HPAIV isolates of the 2.2.1 and 2.2.1.1 clades (18). Protection against challenge earlier than 2 wpv has not been tested.

In chickens with MDA against AIV, protection from challenge also comes from passive immunity (4). It has been observed that vaccine-induced immunity cumulates with passive protection and increases overall protection (Ceva Animal Health. Unpubl. data, 2011). For this reason, in AI vaccinating countries, vaccination of breeders against AI complements and does not contradict vaccination of BR and pullets at day of age.

Duration of immunity (DOI). DOI is a critical property of vaccine-induced immunity, especially for long-lived birds, such as LY and breeders. Data from controlled studies regarding the efficacy of rHVT-H5 to protect long-living chickens against very late challenge are not yet available. However, HVT is known to persist in infected poultry, so the expression of HA is likely to persist as well.

In addition, antibody responses up to 12 woa have been demonstrated (Fig. 1) (Ceva Animal Health. Unpubl. data, 2011). Finally, layer pullets VACC at the hatchery and challenged at 19 woa still demonstrated increased protection (8).

Target species. Experiments have demonstrated replication of rHVT-H5 and expression of the HA antigen and induction of protection in chickens of the SPF and COM BR and layer types, as well as turkeys (6) and some waterfowls species (geese and Muscovy and mallard ducks) but very reduced replication in Pekin ducks (13). Replication was also observed in quails and pheasants but not in pigeons (Ceva Animal Health. Unpubl. data, 2012).

DISCUSSION

In most experiments conducted with this rHVT-H5 vaccine, protection against mortality and clinical signs was generally very high.

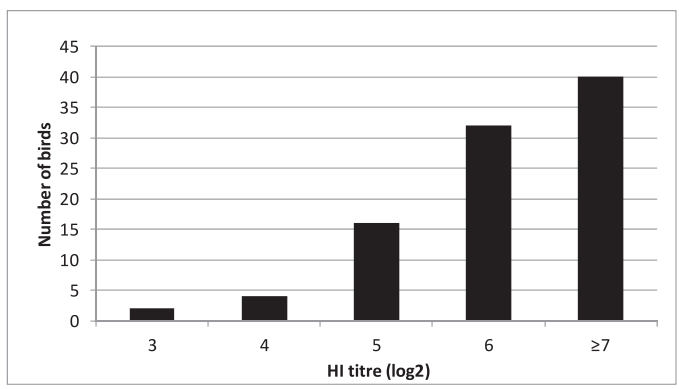

Fig. 2. HI antibody titers recorded at 4 woa for 94 SPF chickens VACC with rHVT-H5 vaccine injected at day old by the subcutaneous route (J. J. De Wit. Unpubl. data, 2014).

Reduction in the shedding of the challenging virus has also been observed such that implementation of vaccination by using the rHVT-H5 vaccine would aid in limiting transmission of the virus to susceptible populations and reducing or slowing the spreading of the disease. Replication and onset of vaccine-induced immunity have been demonstrated in multiple types of chickens and in the presence of different levels of MDA against AIV. This ability to overcome MDA is critical when it comes to vaccinating against AI in endemic countries, where the vaccination of parent stocks is largely applied and is preferred over classical inactivated vaccines because it overcomes the need of vaccination on the farm.

Due to its subunit antigenic makeup, rHVT-H5 does not interfere with a serologic differentiation based on a DIVA eradication strategy with tests targeting non-HA antibodies. However, testing under field conditions still requires further investigation and validation.

As with all vaccines, many factors contribute to successful implementation, including the quality of application, the quality of the birds, management, and control of other infectious agents. In addition, immunosuppressive factors or diseases can also have a significant negative impact when present.

The use of vaccination to control HPAI outbreaks needs to be coordinated with clear goals and expectations and joined as a piece of an eradication program that includes enhanced biosecurity and control policies. Unfortunately, vaccination for HPAI carries a negative trade status for the poultry industry in the country in which the outbreak is occurring and thus cannot be considered lightly by poultry-exporting countries. If vaccination against AI is decided, we know from a historical perspective based on classical inactivated vaccines that the best vaccines are those matched to the challenge virus. In these studies, the rHVT-H5 vaccine demonstrated broad cross-reactive protection against challenge from multiple H5 clades and subtypes, reductions in shedding and transmission potential, and protection of multiple poultry types and is specifically designed for a DIVA strategy. Taken together, these components make this type of vaccine a powerful complementary tool for the control of HPAI outbreaks.

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