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Research Note—

Efficacy of a Recombinant HVT-H5 Vaccine Against Challenge with Two Genetically Divergent Indonesian HPAI H5N1 Strains

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SUMMARY. The swift evolution rate of avian influenza (AI) H5N1 virus demands constant efforts to update inactivated vaccines to match antigenically with the emerging new field virus strains. Recently, a recombinant turkey herpesvirus (rHVT)-AI vaccine, rHVT-H5, expressing the HA gene of a highly pathogenic avian influenza (HPAI) H5N1 clade 2.2 A/Swan/Hungary/499/2006 strain inserted into FC-126 strain of HVT vector, has been developed to combat current threats in poultry industry. Here, we present the results of two trials where rHVT-H5 was tested alone or in combination with inactivated H5N1 vaccines (the latter vaccines contained antigens produced by using a clade 2.1.3 HPAI H5N1 virus [A/Ck/WestJava-Nagrak/2007] in the first trial or mixture of antigen produced by strain A/Ck/WestJava-Nagrak/2007 and A/Ck/Banten-Tangerang/2010 [bivalent vaccine] for second trial) in broiler chickens (*Gallus gallus domesticus*) carrying maternally derived antibodies to H5N1 and then challenged with Indonesian HPAI H5N1 field isolates. The effectiveness of vaccination was evaluated on the basis of clinical protection (morbidity and mortality) and measurement of virus shedding after challenge. Immune response to vaccination was followed by serology. In the first experiment, chickens were vaccinated at the day of hatch with rHVT-H5 alone (Group 1) or combined with inactivated vaccine at day old (Group 2) or at 10 days of age (Group 3). The chickens along with nonvaccinated hatch-mates were challenged at 28 days of age with the HPAI H5N1 field isolate clade 2.1.3 A/Chicken/WestJava-Subang/29/2007. Eighty, 100%, and 80% clinical protection was recorded in Group 1, 2, and 3, respectively. A similar experiment was performed a second time, but the chicks in Group 3 received the inactivated vaccine earlier, at 7 days of age. Challenge was performed at 28 days of age using a different H5N1 isolate, clade 2.1.3 A/Ck/Purwakarta-Cilingga/142/10. Clinical protection achieved in the second trial was 95%, 75%, and 90% in Group 1, 2, and 3, respectively. Shedding of challenge virus was significantly lower in the vaccinated groups compared with controls in both experiments. Vaccinated birds developed hemagglutination inhibition antibody response to H5N1 by the time of challenge. These experiments confirmed that the rHVT-H5 vaccine applied alone or in combination with inactivated H5N1 vaccines could provide high level (>80%) clinical protection against divergent HPAI H5N1 field isolates after single immunization by 4 wk of age and a significant reduction in the excretion of challenge virus.

RESUMEN. *Nota de Investigación*—Eficacia de una vacuna recombinante HVT-H5 frente al desafío con dos cepas de influenza aviar de alta patogenicidad subtipo H5N1 genéticamente divergentes.

La tasa de evolución rápida del virus de la influenza aviar H5N1 exige un esfuerzo constante para la actualización de las vacunas inactivadas para que estas sean cercanas antigénicamente con los nuevos virus de campo emergentes. Se desarrolló recientemente, una vacuna contra la influenza aviar recombinante (rHVT-H5) con un herpesvirus de pavo (rHVT) que expresa el gene HA de la influenza aviar altamente patógena subtipo H5N1, de la cepa A/Cisne/Hungría/499/2006, clado 2.2; que está insertado en la cepa FC-126 como vector HVT. Esta vacuna se desarrolló para enfrentar los desafíos actuales en la industria avícola. A continuación, se presentan los resultados de dos ensayos en los que el virus rHVT-H5 ha sido probado solo o en combinación con vacunas inactivadas conteniendo virus de influenza subtipo H5N1 (estas vacunas contenían antígenos de un virus de alta patogenicidad subtipo H5N1 clado 2.1.3 [A/pollo/WestJava-Nagrak/2007] para el primer ensayo, o una mezcla de antígenos producidos de la mezcla de las cepas A/pollo/WestJava-Nagrak/2007 y la cepa A/pollo/Banten-Tangerang/2010 [vacuna bivalente] para el segundo ensayo) en pollos de engorde con anticuerpos maternos contra el subtipo H5N1 y luego desafiados con aislamientos de campo de influenza aviar de alta patogenicidad subtipo H5N1 de Indonesia. La eficacia de la vacunación se evaluó con base en la protección clínica (morbilidad y mortalidad) y la medición de la eliminación del virus después del desafío. La respuesta inmune a la vacunación fue evaluada por serología. En el primer experimento, los pollos fueron vacunados en el día de la eclosión, con la vacuna rHVT-H5 únicamente (Grupo 1) o en combinación con la vacuna inactivada al día de edad (grupo 2), o a los 10 días de edad (Grupo 3). Los pollos junto con sus compañeros de lote no vacunados se desafiaron a los 28 días de edad con un aislamiento de campo de la influenza aviar H5N1, clado 2.1.3 A/pollo/WestJava-Subang/29/2007. Se observaron porcentajes de protección del 80%, 100%, y 80% para los grupos 1, 2 y 3, respectivamente. Un experimento similar se realizó una segunda vez, pero los pollos del Grupo 3 recibieron la vacuna inactivada antes, a los 7 días de edad. El desafío se llevó a cabo a los 28 días de edad con un aislamiento subtipo H5N1 diferente, clado 2.1.3 A/pollo/Purwakarta-Cilingga/142/10. La protección clínica que se alcanzó en el segundo ensayo fue del 95%, 75% y 90% para los grupos 1, 2 y 3, respectivamente. La diseminación del virus de desafío fue significativamente menor en los grupos vacunados en comparación con los controles en ambos experimentos. Las aves vacunadas desarrollaron respuestas de anticuerpos inhibidores de la hemaglutinación al virus H5N1 en el momento de desafío. Estos experimentos confirmaron que la vacuna rHVT-H5 aplicada sola o en combinación con vacunas inactivadas H5N1 podría proporcionar un nivel alto de protección clínica (mayor al 80%), frente a aislamientos de campo de influenza aviar altamente patógena subtipo H5N1 divergentes después de una inmunización por la semana cuatro de edad con una reducción significativa en la excreción de virus de desafío.

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Abbreviations: AI = avian influenza; AIV = avian influenza virus; BSL = biosecurity level; dpch = days postchallenge; HA = hemagglutinating antigen or hemagglutinin; HPAI = highly pathogenic avian influenza; HI = hemagglutination inhibition; MDA = maternally derived antibody; rHVT = recombinant turkey herpes virus; rHVT-H5 = recombinant turkey herpes virus expressing hemagglutinin gene of H5 avian influenza virus strain; RT = reverse transcription; RRT = real-time reverse transcription

Outbreaks of H5N1 highly pathogenic avian influenza (HPAI) have been occurring in Indonesia since 2004. The Indonesian government implemented vaccination strategy to prevent H5N1 HPAI outbreaks (5,8). Although vaccination reduced the number of outbreaks significantly, sporadic clinical cases have still continuously been occurring in the field, mainly from clade 2.1.3 (4). This condition indicated that presently available vaccines against avian influenza (AI) are largely of poor quality, do not provide sterilizing immunity, and probably promote antigenic drift (7,10). The swift evolution rate of H5N1 HPAI virus demands constant efforts to develop and update inactivated vaccines against emerging new field virus strains. Recently, a recombinant HVT-H5 vaccine, expressing the HA gene of an HPAI H5N1 strain, has been developed to combat current threats of H5N1 infection in poultry industry (7). Here, we report on the efficacy of this rHVT-H5 vaccine against challenge with two divergent Indonesian HPAI H5N1 field isolates.

MATERIALS AND METHODS

Test animals. Day-old commercial broiler chicks (*Gallus gallus domesticus*) were purchased from local breeder farm. The breeders were vaccinated *in ovo* with live HVT+Rispense vaccine against Marek's disease, and six times (at 2, 6, 14, 20, 32, and 41 wk of age) against H5N1 with inactivated vaccines of PT Vaksindo, containing recent local H5N1 virus strains. At time of purchase of the eggs, no clinical signs of AI in the breeders were reported, indicating the absence of HPAI H5N1 virus at that time.

Vaccines. *Vectormune HVT AI*. Source: Ceva-Biomune Co. Lenexa, KS. Recombinant HVT-AI vaccine (rHVT-H5), expressing the HA gene of an HPAI H5N1 clade 2.2 A/Swan/Hungary/499/2006 strain inserted into FC-126 strain of HVT vector.

Vaksimune AI. Source: PT Vaksindo Satwa Nusantara, Jakarta, Indonesia. Inactivated avian influenza virus (AIV) H5N1 whole virus vaccine with oil adjuvant. The H5N1 HPAIV strains used in the production of the inactivated vaccines were A/Ck/WestJava-Nagrak/2007 for first trial or strains A/Ck/WestJava-Nagrak/2007 and A/Ck/Banten-Tangerang/2010 (bivalent vaccine) for second trial. Both strains belong to clade 2.1.3.

Genetic analysis of challenge strains. Sequences of Indonesian AIV strains were aligned by ClustalW multiple alignment method using Bioedit sequence alignment editor program (Ibis Bioscience, Carlsbad, CA). Sequence differences were calculated by Kimura's two-parameter method. Phylogenetic tree was constructed using the neighbor-joining method by TREECON for Windows program (9) (Fig. 1.)

Study design. The experiments were carried out at the biosecurity level (BSL)-3 Animal facility at PT. Vaksindo Satwa Nusantara, Bogor, Indonesia. The laboratory has been approved as one of the BSL-3 animal facilities for vaccine testing in Indonesia by the National Commission of Animal Drug, Ministry of Agriculture, Republic of Indonesia. Two trials were conducted wherein the efficacy of the rHVT-H5 vaccine against challenge was tested. The rHVT-H5 vaccine was administered at hatch by subcutaneous route at a commercial dose either alone or in combination with an autogenous inactivated H5N1 vaccine in commercial broiler chickens carrying monoclonal antibodies (MDAs) to AIV. The single application of inactivated vaccines was not tested because previous studies demonstrated that the presence of MDAs interferes with the development of immune response against various antigens (3), including AIV (1,6). The vaccinated chickens along with nonvaccinated hatch-mates were challenged with Indonesian HPAIV H5N1 field isolates. The effectiveness of vaccination was evaluated on the basis of clinical protection (morbidity and mortality) and measurement of challenge virus shedding. Immune response to vaccination was monitored by hemagglutination inhibition test, and serum samples were collected at hatch and at 28 days of age (before the challenge).

In the first experiment, chickens were vaccinated on the day of hatch with the rHVT-H5 vaccine alone (Group 1) or combined with inactivated vaccine at 1 day old (Group 2) or boosted with inactivated vaccine at 10 days of age (Group 3). Challenge was performed at 28 days of age with the HPAI H5N1 field isolate A/Ck/WestJava-Subang/29/2007. A similar experiment was performed the second time, but the chicks in Group 3 received the inactivated vaccine earlier, at 7 days of age; and for the challenge at 28 days of age, a more recent HPAIV H5N1 field isolate, A/Ck/Purwakarta-Cilingga/142/10, was used (for a trial design of the two experiments, see Table 1). Challenge was performed by inoculating each chicken with a dose of $10^{6.0}$ 50% egg lethal dose challenge virus by the oronasal route (inoculum was injected in the nares but also entered the oral cavity through the choanal opening). For reverse transcription (RT)-PCR analysis oropharyngeal and cloacal swab samples were collected at 2, 4, and 7 days postchallenge (dpch).

Serology tests. Serologic testing of serum samples was carried out by standard HI test using various HA antigens of H5N1 AIV that were homologs either with the rHVT-H5 insert (A/Ck/Egypt/D1455-B2/2006) or with antigen used in inactivated H5N1 vaccine (antigen provided by vaccine manufacturer) or with the challenge strains (A/Ck/WestJava-Subang/29/07 [trial 1] or A/Ck/Purwakarta-Cilingga/142/10 [trial 2]).

RT-PCR measurements. Monitoring of challenge virus shedding was done with the matrix gene–specific TaqMan® AIV-M one-step real-time RT (RRT)-PCR kit (Ambion® Life Technologies Inc., Grand Island, NY) according to manufacturer's instructions, with the positivity limit of Ct $<$ 35. Plasmid control was used as standard for quantification. Results were expressed as copy number/μl of eluted swab sample RNA.

Statistical analyses. All statistical analyses were performed with STATGRAPHICS Centurion XVI program (StatPoint Technologies, Inc., Warrenton, VA). Statistical analysis of RRT-PCR results was done on $\log_{10}$-transformed copy numbers using Kruskal-Wallis test with 95% confidence limit. Statistical analysis of $\log_2$-transformed HI titers in serologic tests was done with ANOVA at a 95% level of significance.

RESULTS AND DISCUSSION

In trial 1, the levels of MDAs at day old were moderate, and comparable values were obtained with the three antigens: 4.30 $\pm$ 0.65 (mean $\pm$ SD) $\log_2$ titer against the killed H5N1 vaccine strain; 4.00 $\pm$ 2.60 against the strain donating the HA gene for the rHVT-H5 vaccine; and 3.78 $\pm$ 1.09 against the challenge strain. By 4 wk of age, MDAs decayed below the positivity limit in the unvaccinated group. Humoral immune response to vaccination was detected in all vaccinated groups at the time of challenge (Table 2). The levels of HI titers induced by the different vaccination programs did not differ significantly. On the contrary, significantly different HI titers

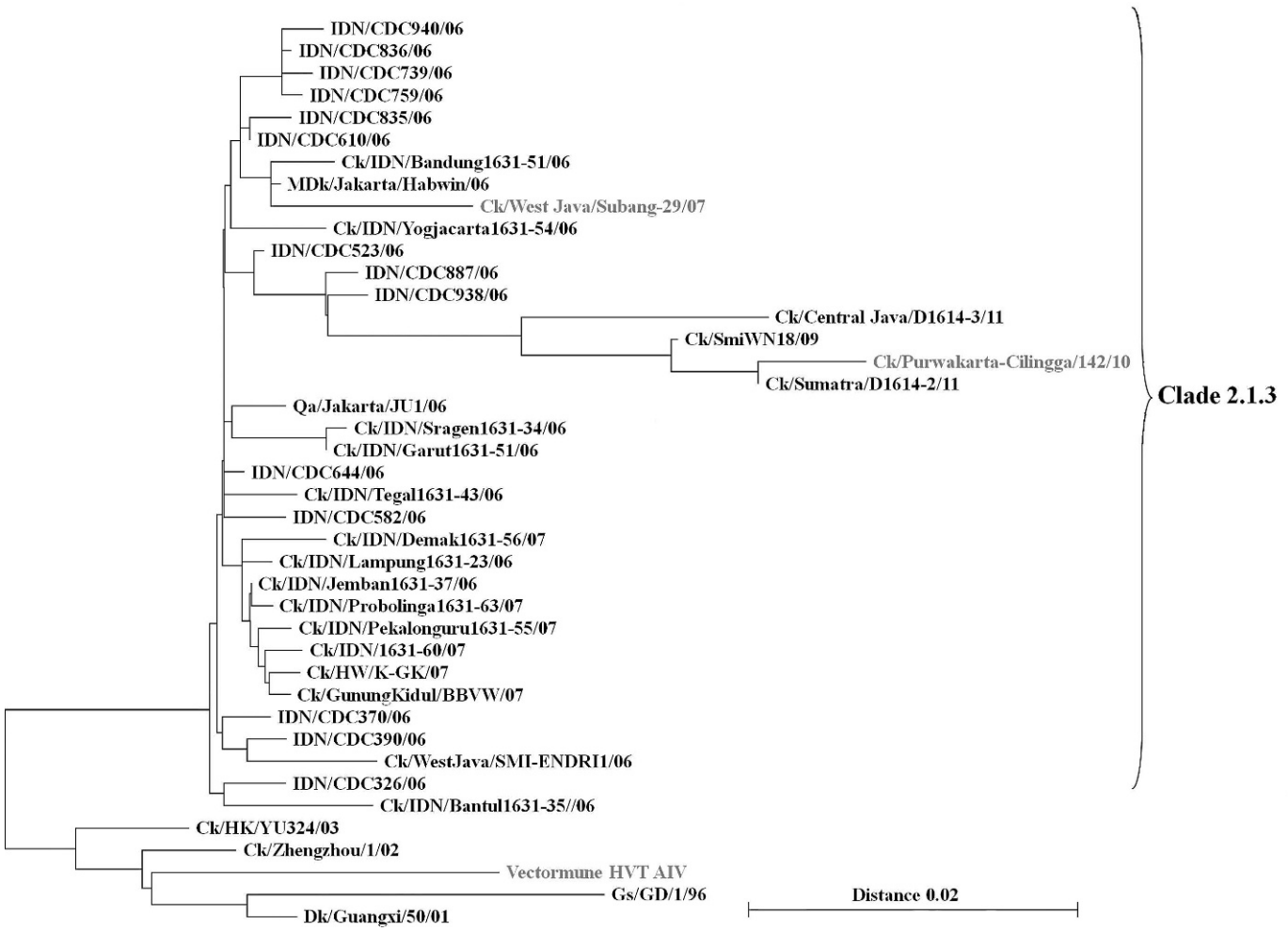

Fig. 1. Genetic analysis of tested challenge strains and their relation to other reference AIV strains and to the insert used in rHVT-H5 vaccine.

were obtained against the different HA antigens: the highest values were measured with the HA antigen homolog with the insert of the rHVT-H5 vaccine and lowest values with the HA antigen homolog with the challenge strain. In trial 2 (Table 3), a similar MDA level was found against the HA antigen homologs with the inactivated and the challenge virus, but a lower level was detected against the antigen homologs with the rHVT-H5 insert. By the time of challenge at 28 days of age, MDAs to H5N1 dropped to an undetectable level. Humoral immune response to vaccination could be detected in all immunized groups at 28 days of age, but significantly higher HI titers were obtained in rHVT-H5 and killed H5N1 prime-boost (at day 7) groups against the HA antigen homologs with the killed H5N1 vaccine and the challenge strain compared with the other vaccination programs. No significant difference was found among the vaccination programs with rHVT-H5 homologous antigen. Similarly to trial 1, the highest HI titers could be measured against the HA antigen homolog with the rHVT-H5 vaccine insert in all groups (titers were lower compared with trial 1). No significant difference was found between the HI titers obtained with the other two HA antigens. Similar results were demonstrated

Table 1. Trial designs. Both trials included the same three vaccination programs, but the application of booster vaccination and the challenge strain was different. Challenge infection was performed at 28 days of age.

Group	Vaccination program		Challenge strain
	First vaccination	Second vaccination	
Trial 1			
1	rHVT-AIV at day old	—	AIV H5N1 A/CK/WJava-Subang/029/ 2007
2	rHVT-AIV and killed H5N1 at day old	—	
3	rHVT-AIV at day old	Killed H5N1 at 10 days of age	
Trial 2			
1	rHVT-AIV at day old	—	
2	rHVT-AIV and killed H5N1 at day old	—	
3	rHVT-AIV at day old	Killed H5N1 at 7 days of age	AIV H5N1 A/CK/Purwakarta-Cilingga/ 142/2010

Table 2. Summary of serologic results obtained with different antigens in trial 1.

Vaccination program	Sampling date[A]	Log_2 HI titer (mean ± SD)[B]		
		Homologous with killed H5N1	Homologous with rHVT-H5	Homologous with challenge strain
MDA (represents all groups)	D0	4.30 ± 0.65	4.00 ± 2.60	3.78 ± 1.09
rHVT-H5 at day old	D28	3.06 ± 1.41	7.14 ± 1.12	1.92 ± 0.82
rHVT-H5 and killed H5N1 simultaneously	D28	3.50 ± 1.35	7.10 ± 1.62	2.45 ± 0.69
rHVT-H5 and killed H5N1 prime-boost	D28	3.18 ± 1.76	6.70 ± 1.81	1.69 ± 1.18

[A]D28 refers to the prechallenge sampling.
[B]Results obtained with different HA antigens: homolog with the inactivated H5N1 vaccine was provided by the manufacturer, A/Ck/Egypt/D1455-B2/2006 (homolog with rHVT-H5 insert), and A/Ck/WestJava-Subang/029/2007 (challenge strain).

Table 3. Summary of serologic results obtained with different antigens in trial 2.

Vaccination program	Sampling date[A]	Log_2 HI titer (mean ± SD)[B]		
		Homologous with killed H5N1	Homologous with rHVT-H5	Homologous with challenge strain
MDA (represents all groups)	D0	4.76 ± 1.57	2.65 ± 0.93	4.39 ± 1.46
rHVT-H5 at day old	D28	2.13 ± 1.56	4.61 ± 2.04	1.85 ± 1.62
rHVT-H5 and killed H5N1 simultaneously	D28	2.57 ± 1.97	4.92 ± 2.27	2.34 ± 2.13
rHVT-H5 and killed H5N1 prime-boost	D28	3.88 ± 1.64[C]	5.64 ± 1.52	3.62 ± 1.72[C]

[A]D28 refers to the prechallenge sampling.
[B]Results obtained with different HA antigens: homolog with the inactivated H5N1 vaccine was provided by the manufacturer, A/Ck/Egypt/D1455-B2/2006 (homologous with rHVT-H5 insert), and A/Ck/Pwt-Cilingga/142/10 (challenge strain).
[C]HI titers are statistically significantly higher in rHVT-H5 and killed H5N1 prime-boost group with HA antigen homolog with the killed H5N1 vaccine and the challenges strain compared with other vaccination groups.

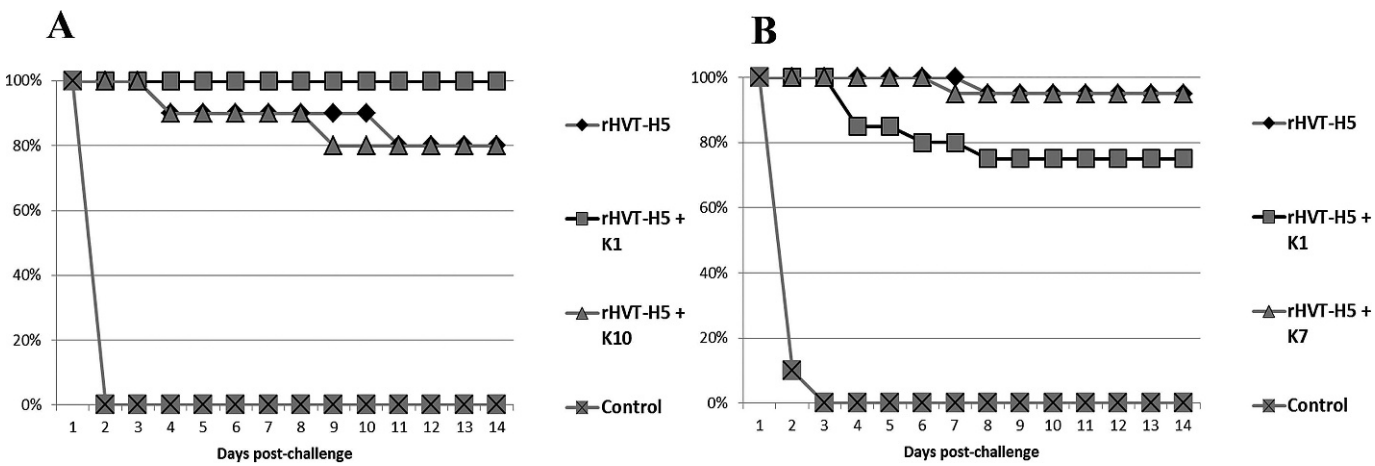

Fig. 2. Protection rate (ratio of healthy survivors). (A) Protection against A/Ck/WestJava-Subang/29/2007 strain (trial 1). (B) Protection against A/Ck/Purwakarta-Cilingga/142/10 strain (trial 2).

by Hai *et al.* (2) in their study that used recombinant influenza vaccine in mice.

To determine the efficacy of the rHVT-AIV vaccine and the possible positive effect of its combination with inactivated H5N1 vaccine, chickens receiving the different vaccination regimes were challenged, along with hatch-mate nonvaccinated control birds. Unvaccinated chickens (control group) started to show clinical signs of AI already 24 hr after challenge, and all of them died within 2–4 days. In the first trial clinical protection was 80%, 100%, and 80% in Group 1, 2, and 3, respectively, whereas in the second trial 95%, 75%, and 90% clinical protection was achieved in Group 1, 2, and 3, respectively (Fig. 2). Less than 20% of chickens vaccinated either with rHVT-AIV vaccine alone or in combination with AI-killed vaccine developed clinical signs and died because of the challenge, but the onset of clinical signs and the time of mortality were delayed significantly even in those birds that succumbed to challenge in the vaccinated groups compared with the unvaccinated group. These results confirmed that vaccination with rHVT-H5 at day old could induce strong immunity that was able to protect the majority of chickens against high-challenge dose of antigenically divergent HPAI H5N1 field isolates.

By measuring the level of challenge virus excretion at 2 dpch, it was shown that all unvaccinated chickens shed the virus through both oral and cloacal routes, whereas at the same day ≈60%–80% of the vaccinated chickens shed the virus only through oral route, and not by the cloacal route (Fig. 3). Statistical analyses showed that unvaccinated chicken (control group) excreted the challenge virus in significantly higher amount than any of the vaccinated groups at 2

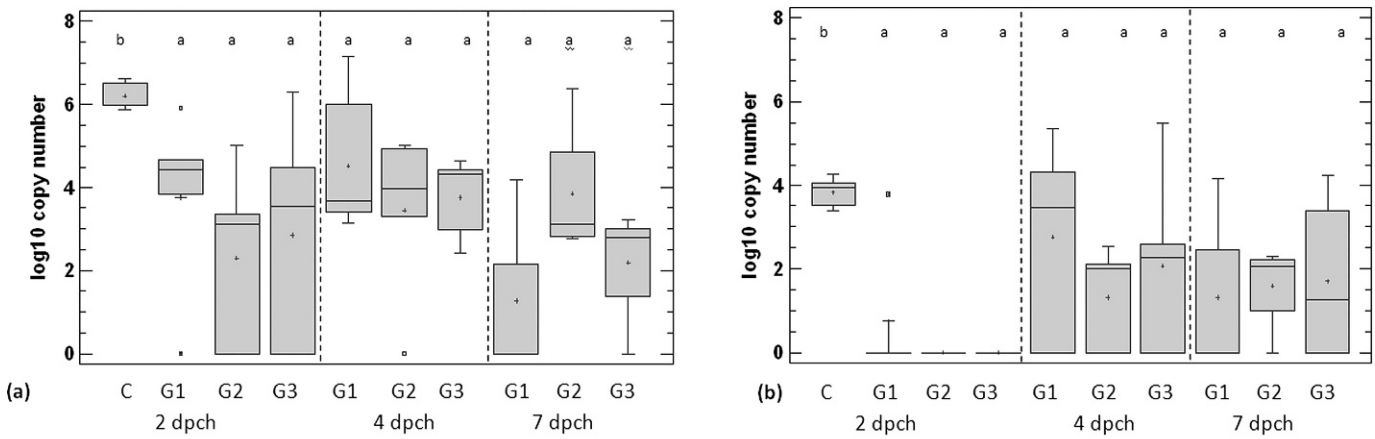

Fig. 3. Summary of RRT-PCR results for oronasal shedding (a) and cloacal shedding (b). Abbreviation of groups: C = control; G1 = rHVT-H5; G2 = rHVT-H5 and killed H5N1 simultaneously; G3 = rHVT-H5 and killed H5N1 prime-boost. Sampling dates were 2, 4, and 7 dpch. All chickens in the control group died by 4 dpch sampling (results for the control group available only at 2 dpch). Results are expressed as copy number/1 μl of eluted RNA of swab sample. Same letters indicate statistically homogenous groups among the samples collected at the same date (Kruskal-Wallis test, $P > 0.05$).

dpch, but no significant difference was observed between the groups receiving the different vaccination programs. The amount of shed challenge virus by the unvaccinated group through the oropharyngeal route was 100–1000 times higher than the amounts shed by the vaccinated groups. At later samplings, only vaccinated groups were compared because all chickens in control group died by 4 dpch sampling. At these samplings, the vaccinated chickens shed the challenge virus in moderate amount through the oropharyngeal route, with a peak at 4 dpch, whereas the cloacal shedding was minimal and only a small portion of the vaccinated birds shed detectable amount of challenge virus. No significant difference among the vaccinated groups at any time point, regardless the swab type was found. Similar results were obtained in trials 1 and 2 (only trial 1 data shown). In summary, quantitative measurement of challenge virus shedding showed that vaccination with rHVT-H5 vaccine alone or in combination with inactivated H5N1 vaccine delayed and significantly reduced the challenge virus shedding both by the oropharyngeal and cloacal route compared with controls in both experiments.

These experiments confirmed that the rHVT-H5 vaccine expressing the HA gene of a clade 2.2 H5N1 HPAI virus could provide a high level of clinical protection (80% or 95%) by 4 wk of age against challenge with two genetically divergent HPAI H5N1 field isolates.

REFERENCES

1. De Vriese, J., M. Steensels, V. Palya, Y. Gardin, M. Dorsey, B. Lambrecht, S. Van Borm, and T. van den Berg. Passive protection afforded by maternally-derived antibodies in chickens and the antibodies' interference with the protection elicited by avian influenza-inactivated vaccines in progeny. Avian Dis. 54(Suppl. 1):246–252. 2010.

2. Hai, R., A. García-Sastre, D. E. Swayne, and P. Palese. A reassortment-incompetent live attenuated influenza virus vaccine for protection against pandemic virus strains. J. Virol. 85:6832–6843. 2011.

3. Klipper, E., D. Sklan, and A. Friedman. Maternal antibodies block induction of oral tolerance in newly hatched chicks. Vaccine 22:493–502. 2004.

4. Nidom, C. A., S. Yamada, R. V. Nidom, K. Rahmawati, M. Y. Alamudi, Kholik, S. Indrasari, R. S. Hayati, K. Iwatsuki Horimoto, and Y. Kawaoka. Genetic characterization of H5N1 influenza viruses isolated from chickens in Indonesia in 2010. Virus Genes 44:459–465. 2012.

5. Peyre, M., G. Fusheng, S. Desvaux, and F. Roger. Avian influenza vaccines: a practical review in relation to their application in the field with a focus on the Asian experience. Epidemiol. Infect. 137:1–21. 2009.

6. Poetri, O., A. Bouma, I. Claassen, G. Koch, R. Soejoedono, A. Stegeman, and M. van Boven. A single vaccination of commercial broilers does not reduce transmission of H5N1 highly pathogenic avian influenza. Vet. Res. 42:74. 2011.

7. Rauw, F., V. Palya, S. Van Borm, S. Welby, T. Tatar-Kis, Y. Gardin, K. M. Dorsey, M. M. Aly, M. K. Hassan, M. A. Soliman, B. Lambrecht, and T. van den Berg. Further evidence of antigenic drift and protective efficacy afforded by a recombinant HVT-H5 vaccine against challenge with two antigenically divergent Egyptian clade 2.2.1 HPAI H5N1 strains. Vaccine 29:2590–2600. 2011.

8. Sawitri, S. E., Darminto, J. Weaver, and A. Bouma. The vaccination programme in Indonesia. Dev. Biol. (Basel) 130:149–156. 2006.

9. van de Peer, Y., and R. De Wacher. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the MS Windows environment. Comput. Appl. Biosci. 10:569–570. 1994.

10. Webster, R. G., and E. A. Govorkova. H5N1 influenza—continuing evolution and spread. N. Engl. J. Med. 355:2174–2177. 2006.

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