



Immunological Studies on the Inactivated Duck Virus Hepatitis Vaccines in Ducks

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Abstract | Duck virus hepatitis (DVH) disease had several outbreaks in Egypt during 2015 leading to mortality and nervous manifestations among 27 duck farms in Sharkia Province. Vaccination and biosecurity measures are the most important tools for protection against duck hepatitis virus which is one of the most economically important diseases to all duck-growing farms because of its high potential mortality if the infection is not controlled. Egypt used live attenuated vaccine, which is considered a potential risk for non-vaccinated birds during shedding. A total of 175, one day old Pekin ducklings were randomly clustered into 6 groups which were vaccinated with local live attenuated, local live attenuated boosted by imported commercial inactivated, local live attenuated boosted by locally commercial inactivated, imported commercial inactivated and locally prepared inactivated DHV vaccines, control group, respectively. The response to vaccination was measured by VNT; locally inactivated prepared DVH vaccine with higher NI in comparison to other DVH vaccines at 8 weeks of age. On the other hand, the vaccinated group primed with live attenuated and boosted with locally inactivated prepared DVH vaccine, the NI results at 4, 8 and 12 weeks post booster 5.5, 6.5 and 6.8, respectively. Meanwhile the vaccinated group primed with live attenuated and boosted with inactivated commercial DVH vaccine the NI results at 4, 8 and 12 weeks post booster were 5.7, 7 and 7.3, respectively. It was noticed that the vaccinated groups were primed with live attenuated and boosted with inactivated vaccines are excellent according to the neutralizing index. This study tried to develop a new and first local inactivated vaccine that provides prevention of circulating DVH, evaluated its protection efficacy and safety.

Keywords | Duck, Duck hepatitis virus (DVH), Neutralization Index (NI)

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INTRODUCTION

Duck virus hepatitis (DVH) is a highly fatal viral infection of young ducklings usually less than 3 weeks of age. characterized by rapid onset of high morbidity and mortality (up to 95%) associated with hepatitis and enlarged hemorrhagic liver (Tseng and Tsai, 2007). DVH, is a non-enveloped, small RNA virus and has icosahedral symmetry which is categorized into three types

I, II and III. According to (OIE, 2017), DVH type I can be caused by at least three different genotypes of duck hepatitis A virus (DHAV) which belongs to family Picornaviridae. The most pathogenic and widespread one is DHAV type 1 (DHAV-1) which was originally discovered in 1945 in the United States (Levine and Hofstad, 1945) and now it is globally distributed among duck-raising countries with a devastating economic impact. DVH accounts for more than 80% of mortality in ducklings less than 21 days old

(Li et al., 2013). Recently, DVH-1 had only been associated with disease in mallard and Pekin ducklings, but it has now been reported to cause pancreatitis and encephalitis in Muscovy ducks (Guèrin et al., 2007).

In Egypt, the virus was first described in the late 1970s (Shalaby et al., 1978) then continues to cause massive mortalities in both vaccinated and non-vaccinated duck flocks. In Sharkia Province, 2015, the disease outbreaks occurred in 27 duck farms, and the affected birds showed characteristic features of DHAV-1 infection (Kamomae et al., 2017; Kozdru, et al., 2014).

The incubation period lasts from 1 to 2 days and clinical signs include lethargy, anorexia and sudden death with opisthotonos within a few days. The liver is enlarged with hemorrhagic lesions (petechiae, ecchymosis) and discoloration (OIE, 2017). In vitro propagation and isolation of (DVH-1) can be done through inoculation of serial dilutions of the liver homogenate into the allantoic sac of embryonated duck eggs (10–14 days) or chicken eggs (8–10 days). Inoculated duck embryos die between 24 and 72 hours post-inoculation, whereas chicken embryos die within 5–8 days post-inoculation. Gross pathological changes in the embryos include stunting and subcutaneous hemorrhages over the whole body, with edema, particularly of the abdominal and hind limb regions. The embryo liver may be red and yellowish, swollen and may show some necrotic foci. In embryos that take longer to die, a greenish-yellow color of the allantois is more pronounced (OIE, 2017).

There is no specific treatment for duck viral hepatitis infection. Prevention and control are based on strict biosecurity and implementation of vaccination protocols. The use of DVH live attenuated and inactivated vaccines around the world has provided reliable protection of vaccinated ducklings (Rispen, 1969; Kim et al., 2009; Wen et al., 2017; Kang et al., 2018).

In Egypt, vaccination of breeder ducks depends mainly on attenuated vaccines produced from E52 Rispen strain 78 (Ellakany et al., 2002; Abd-Elhakim et al., 2009) and these attenuated vaccines were applied in 77 commercial sectors. However, upon shedding from vaccinated birds, live attenuated vaccines are considered a potential risk for non-vaccinated ones and rapid reversion to virulence of the virus following duck to duck passage (Woolcock, 1991; Shima et al., 2019). The unavailability of local DVH inactivated vaccine is considered a great obstacle and major challenge faced the duck industry in Egypt despite, many of these imported DVH vaccine batches are submitted to The Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Cairo, Egypt for reviewing their quality.

In this study, efforts were taken to evaluate protective efficacy of locally prepared inactivated DVH vaccine in comparison to local commercial live attenuated and imported commercial inactivated DVH vaccines with different vaccine schedules.

MATERIALS AND METHODS

DUCKS

One hundred seventy-five (175), one day old, Pekin ducklings were used for conducting potency (immunogenicity) of the tested DVH vaccines.

EMBRYONATED CHICKEN EGGS

Specific Pathogen Free (SPF) Embryonated Chicken Eggs (ECEs), 9–11 days old, purchased from Kom Oshim farm for SPF-eggs, El-Fayoum, Egypt were used for propagation and titration of DVH antigens.

VACCINES

LOCAL COMMERCIAL LIVE ATTENUATED DVH VACCINE

It was prepared from DVH E52 vaccinal strain. The vaccine was used for vaccination of one day old duckling subcutaneously with one recommended field dose.

IMPORTED COMMERCIAL INACTIVATED DVH VACCINE

The vaccinal strain was DVH “VGNKI-K”. The vaccine was used for vaccination of 4 weeks old ducks intramuscularly at a dose of 0.5 ml/bird.

DUCK VIRAL HEPATITIS STRAINS

LOCAL DVH STRAIN E52

It was isolated and identified by Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo. The vaccinal attenuated strain of DVH (E52) was propagated and titrated in chicken embryos (9–10 days old) through inoculating into the allantoic cavity (Lee, et al., 2010; Ou, et al., 2017; He et al., 2019).

DUCK-HEPATITIS-A-VIRUS-BH3

The strain was a local Egyptian isolate belonging to serotype I and kindly supplied from National Laboratory for Veterinary Quality Control of Poultry Production (NLQP), Animal Health Research Institute, Giza. It was designated at a gene bank under accession number of MN873051. The virus was used as antigen in Virus Neutralization Test (VNT).

PREPARATION OF THE LOCAL INACTIVATED DVH VACCINE

The local inactivated DVH vaccine was prepared according to OIE (2017). The local E52 strain was propagated in SPF ECE. The inoculated eggs were incubated at 37°C for 5 days and examined daily. At the end of the

examination period, all the live and dead embryos were collected and homogenized. The purified viral homogenate was titrated then inactivated by freshly prepared binary ethylenimine (BEI) with a final concentration of 1% at 37°C. The inactivated viral suspension was adjuvanted with Montanide ISA 70 oil (Woolcock, 1991). The vaccine dose was adjusted to be 10^6 EID₅₀/0.5 ml/ duck and inoculated subcutaneously (S/C).

EVALUATION OF DVH VACCINES

The locally prepared inactivated DVH vaccine was examined for its safety and efficacy in comparison to that of local live attenuated and imported inactivated DVH vaccines (OIE, 2017), as follows:

ASSESSMENT ON COMPLETION OF VIRUS INACTIVATION

Ten SPF 9-11-day old ECEs were inoculated with 0.2ml of each vaccine. Embryos dying within 24 hrs after inoculation are discarded. After 5 days of incubation the embryo mortality was scored and the allantoic fluid was harvested separately and tested for the presence of haemagglutinins to chicken RBCs. If the embryo mortality less than 20% and no HA occurs, the vaccine is considered inactivated.

STERILITY TEST

It was carried out to determine if the vaccine samples are free of bacterial, mycoplasmal and fungal contaminants.

SAFETY TEST

Concerning safety testing, the three tested vaccines were inoculated into ducks with twice the normal recommended dosage and observed for clinical symptom appearance.

POTENCY

It was done to demonstrate the antigenic capacity for each tested DVH vaccine. Twenty ducks are vaccinated S/C with the recommended dose of each vaccine type at the corresponding duck age as cleared in the experimental design Table 1. Serum samples were collected from vaccinated and control groups at weeks post vaccination as explained in experimental design. The serological analysis of DVH antibodies against local DVH serotype I isolate was determined by virus neutralization test and expressed as neutralization index.

VIRUS NEUTRALIZATION TEST (VNT)

The test was carried out according to (Gough and Spackman, 1981; OIE, 2017).

EXPERIMENTAL DESIGN

One hundred seventy-five white Pekin ducklings 1-day-old, ducklings were divided into 6 groups as described in Table 1. Serum samples (10 /each group) were collected at

weeks post vaccination as shown in experimental design.

RESULTS AND DISCUSSION

VACCINE STRAIN TITER

The vaccine strain titer was $10^{10.5}$ EID₅₀/ml. It was adjusted to be 10^6 EID₅₀/dose for vaccine preparation, while a titer of local DVH virus serotype I was $10^{7.3}$ EID₅₀/ml.

EVALUATION OF INACTIVATED PREPARED DVH VACCINES

ASSESSMENT ON COMPLETION OF VIRUS INACTIVATION

It was found that the final product of the 3 tested DVH vaccines was completely inactivated when propagated in SPS ECE for 2 blind passages.

STERILITY TEST

The sterility test showed that the three tested vaccines are free from any bacterial, mycoplasmal and fungal contamination.

SAFETY TEST

Concerning safety testing, the three tested vaccines were found safe and no clinical symptoms appeared when inoculated into susceptible ducks.

POTENCY

VIRUS NEUTRALIZATION TEST

The test was approved on serum samples collected from vaccinated and control ducks at weeks of age according to the experimental designs. The Neutralization Indices were estimated against field isolated DVH antigen serotype (I).

Infection with DVH leads to an acute lethal disease that results in massive losses in intensive duck-raising farms since its outbreak detection in 1969 in Egypt (Refaie, 1969). Despite the vaccination of breeder ducks, DVH continues to cause massive mortalities in both vaccinated and non-vaccinated duck flocks (Abd-Elhakim et al., 2009).

The aim of this study was to develop an inactivated local vaccine against DVH serotype I to be used with the present live vaccine for protection of ducks at all ages. Thus, the live attenuated vaccine is able to induce both humoral and cellular immune response and can be used for mass vaccination (Belshe, 2004), while the inactivated vaccines were the most practical option to produce a good maternal immunity in ducklings for controlling duck hepatitis (Gough and Spackman, 1981).

So, the use of live attenuated vaccine as primary vaccination in duckling followed by inactivated vaccine is very effective and can produce a good long lasting immune response and delivered a good maternal antibodies to the newly hatched

ducklings (Gough, et al., 1977; Wyeth and Cullen, 1979).

In Egypt, only live attenuated DVH vaccines (serotype I), were used for protection of ducks against DVH virus.

The virus titer of the propagated vaccinal DVH virus was $10^{10.5}$ EID₅₀/ml. For preparation of an effective inactivated DVH1 vaccine, the vaccinal dose was adjusted to be 10^6 EID₅₀/dose. Then the collected virus was inactivated using BEI (Woolcock, 1991).

After that the inactivated virus was examined for completion of inactivation by inoculating it into embryonated chicken eggs for 2 passages. Eventually, it was found that no deaths or clinical lesions occurred in egg embryos. These results provide strong evidence that the vaccinal strain is completely inactivated (OIE, 2017).

The inactivated DVH virus was emulsified by Montanide ISA70 to develop an effective inactivated vaccine, which is agreed with the results obtained by Dungu et al. (2009) who said that the Montanide ISA70 is a safe adjuvant and has no adverse local reaction. The safety results in this study illustrated that all the inactivated DVH vaccines are completely inactivated and have no residual of live virus.

Also, all the tested vaccines are free from any bacterial, mycoplasmal and fungal contaminants and safe even when administered with higher dose than the normal under the field condition.

The prepared inactivated DVH vaccine was evaluated and compared its efficacy with local live and imported commercial vaccines in ducks.

As described in the experimental design, the live DVH vaccine was delivered to duck at 3-day-old while the locally prepared and imported inactivated vaccines were administered at 4 weeks old through the S/C route (Sung et al., 2000; Kim et al., 2009).

Evaluation of the potency of all DVH vaccines in ducks has been based on serological study performed a few weeks after vaccination as described in experimental design (Table 1).

It was known that the DVH is age resistant and causes severe mortality and morbidity in the young duckling (OIE, 2017). So, application of challenge test for evaluation the DVH vaccines only applied to measure the maternally derived immunity in progeny while the evaluation of DVH vaccines strategy in breeders depend on the VNT (Jae-Hee and Min Kang, 2018).

In our study, the evaluation of DVH vaccine strategy

depends on detection of ability of the produced antibodies due to DVH vaccines to neutralize the infectivity of DVH virus that expresses as the neutralization index (Hwang, 1969; Gough and Spackman, 1981; Woolcock et al., 1982). Also, it was shown that if a neutralizing antibody could neutralize ≥ 100 LD₅₀ of DVH infectively, it was considered protective (OIE, 2017).

The results of this study indicate that vaccination with all DVH vaccines were protective (> 1.5) for ducks against the local strain serotype (I) where the live DVH vaccine produced neutralizing antibodies indices (NIs) as 2.1, 2.5, 3.5 and 4.5 at 2, 4, 8 and 12 weeks post vaccination, meanwhile, the imported commercial inactivated produced 2.8, 5 and 5.6 at 4, 8 and 12 weeks post vaccination, respectively. On the other hand, the neutralization indices resulting in locally prepared inactivated DVH vaccines were 3, 5.5 and 6 at 4, 8 and 12 weeks post vaccination (Table 2).

Also, the results of neutralization indices of vaccinated groups primed with live attenuated and boosted with inactivated commercial DVH vaccine were 2.1, 2.5 at 2 and 4 weeks post vaccination and 5.5, 6.5 and 6.8 at 4, 8 and 12 weeks post boosting. Meanwhile the value of neutralization indices of the vaccinated group primed with live attenuated and boosted with the inactivated locally prepared DVH vaccine was 2.1, 2.5 at 2 and 4 weeks post vaccination 5.7, 7 and 7.3 at 4, 8 and 12 weeks post boosting (Table 3).

So, the neutralization index of the produced antibodies due to DVH vaccination were higher than 10^2 in all vaccinated ducks and suggested that they were sufficient to confer protection in their progeny. While the serum of the control non vaccinated group could not neutralize the DVH used in the VN test. This means that this group had not any neutralizing antibody against DVH (NI = 0 at all weeks as in Tables 2 and 3). These results are in parallel to those reported in previous studies (Gough and Spackman, 1981; Woolcock, 1991). who indicated that the vaccinated ducks with DVH vaccines could be completely protected against viral serotype (I) when that vaccine gives a NI greater than 1.5.

So, the results of this study indicate that imported and locally inactivated DVH vaccines are more potent as measured by the neutralization index than the live DVH vaccine. On the other hand, duck groups primed with live attenuated then boosted with inactivated either commercial and prepared DVH vaccine were protected effectively against DVH serotype (I) disease. This is matched with Jae-Hee and Min (2018) and Woolcock (1991) who reported that a prior exposure to live DVH (priming) is required to promote a strong immune response to inactivated vaccines.

Table 1: The experimental design for evaluation methods of different types of DVH vaccines.

Groups code	Treatment	Evaluation methods					
		Potency				Safety	
		No. of ducks	Age of 1 st dose	Age of booster dose	Time of blood sample collection	Age	No.
1	Vaccinated with local commercial live attenuated DVH	20	at 3 days old	Nd*	2, 4, 8 and 12 WPV	at 3 days old	15
2	Vaccinated with local commercial live attenuated DVH boosted by imported commercial inactivated DVH vaccine	20	at 3 days old	at 28 th day old	2 and 4 WPV 4,8 and 12 WPB	Nd	Nd
3	Vaccinated with local commercial live attenuated DVH then boosted by Inactivated locally prepared DVH vaccine	20	at 3 days old	at 28 th day old	2 and 4 WPV 4,8 and 12 WPB	Nd	Nd
4	Vaccinated with imported commercial inactivated DVH vaccine	20	at 28 th day old	Nd	2,4,8 and 12 WPV	at 28 th day old	15
5	Vaccinated with locally prepared inactivated DVH vaccine	20	at 28 th day old	Nd	2,4,8 and 12 WPV	at 28 th day old	15
6	Control	20	Non	Non	2,4,8 and 12	Non	10

* Nd, not done; * WPV, weeks post 1st dose of each vaccine; ** WPB, weeks post booster dose.

Table 2: Mean neutralization indices of duck groups that were vaccinated with single dose of tested DVH vaccines against locally isolated virus serotype (I) virus.

Group code	Treatment	Neutralizing index			
		2 nd WPV*	4 th WPV	8 th WPV	12 th WPV
1	Local live attenuated commercial DVH vaccine	2.1	2.5	3.5	4.5
4	Imported inactivated commercial DVH vaccine	Not done	2.8	5	5.6
5	Locally inactivated prepared DVH vaccine	Not done	3	5.5	6
6	Control	0	0	0	0

* WPV, weeks post 1st dose of each vaccine. Neutralizing Index (NI)= A value of <1.5 was considered to be negative. This means that this group had not any neutralizing antibody against DVH.

Table 3: Mean neutralization indices of duck groups that are prime-boosted with live then inactivated DVH vaccines against locally isolated virus serotype (I) virus.

Group code	Treatment	Neutralizing index				
		2 nd WPV*	4 th WPV	4 th WPB**	8 th WPB	12 th WPB
2	Live attenuated commercial DVH vaccine boosted by inactivated commercial DVH vaccine	2.1	2.5	5.5	6.5	6.8
3	Live attenuated commercial DVH vaccine boosted by inactivated locally prepared DVH vaccine	2.1	2.5	5.7	7	7.3
6	Control	0	0	0	0	0

* WPV, weeks post 1st dose of each vaccine; ** WPB, weeks post booster dose; Neutralizing Index (NI)= A value of <1.5 was considered to be negative. This means that this group had not any neutralizing antibody against DVH.

Finally, the obtained findings showed that the locally prepared DVH vaccine is effective in giving protective antibody titers with a high level permit to protect the ducks from DVH virus serotype (I) infection.

In conclusion, the current study is expected to be helpful for further studies on DVH in Egypt to develop protective vaccines against its other serotypes infections.

NOVELTY STATEMENT

New and first local inactivated DVH vaccine in Egypt.

AUTHOR'S CONTRIBUTION

All authors contributed equally.

The authors have declared no conflict of interest.

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