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# In Process Quality Control Factors Affecting Efficacy of Hydropericardium Syndrome Virus Vaccine

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**Abstract.-** Hydropericardium syndrome (HPS) is common even in the commercial broilers that are vaccinated using locally prepared HPS virus infected liver homogenate (HPS-LH) vaccine. In the HPS-LH vaccine production process, some of the in process quality control factors mitigate its efficacy. The HPS-LH vaccine containing more than $10^{4.6}$ units of bird lethal dose 50 ($LD_{50}$) when injected to 14 days old broilers and 21 days post vaccination given challenge with infection dose (100 units of $LD_{50}$ of HPS-LH) showed 100 per cent protection. Moreover, less than 25 doses of the vaccine prepared from one gram of the HPS-LH induced 90 per cent protection in the vaccinated birds. Addition of adjuvant such as oil base (Montanide ISA 70) or aluminum hydroxide gel (AHG) in the vaccine showed additive effect on its efficacy. The birds vaccinated with montanide based HPS-LH vaccine ($10^{4.6}$ units of $LD_{50}$: 25 doses/gram) showed 100 percent protection to challenge infection. The montanide is more effective adjuvant as compared to AHG. Infectivity titer ($LD_{50}$) of HPS virus infected chicken embryo hepatocyte homogenate (CEHH) was 100 times less ($10^{2.5}$ units) than that of HPS-LH. Gel based HPS-CEHH vaccine ($LD_{50}$ titer $10^{2.5}$ units) showed poor response in the vaccinated birds (40 per cent protection) as compared to that of gel based HPS-LH vaccine (90 per cent). It is concluded that gel based vaccine prepared from fresh HPS-LH is more effective and economical as compared to that of HPS-CEHH vaccine.

**Keywords**: Lethal dose 50, aluminum hydroxide gel (AHG), montanide, liver homogenate vaccine

## INTRODUCTION

Hydropericardium syndrome (Angara disease) is a viral disease mostly affecting 3-5 weeks old growing broilers. It is characterized by high morbidity and mortality rate (up to 80 per cent), with fluid in pericardium, necrosed and enlarged pale looking liver, enteritis, reactive spleen, congestion of lungs, hemorrhages on heart and kidneys as major post mortem findings (Rabbani, 1997; Ganesh, 1998). The disease is caused by avian adenovirus serotype-4 (Rabbani and Naeem, 1996).

Diagnosis of the disease before appearance of symptoms is difficult since the birds do not always show specific clinical signs. Sudden death in dorsal recumbancy at third week of age and postmortem changes such as hydropericardium and demonstration of basophilic intranuclear inclusion bodies in hepatocytes are considered as pathognomonic. Indirect haemagglutination assay (IHA), enzyme linked immunosorbant assay (ELISA), agar gel precipitation test (AGPT), virus neutralization (VN) test, dot immunobinding assay (DIA) and immuno-electrophoresis using infected liver homogenate filtrate as crude antigen and specific antiserum have also been used in HPS diagnosis (Ganesh, 1998).

Standard vaccines are available against most of the poultry diseases but the literature regarding the standard protocols for development and evaluation of avian HPS virus vaccine is scanty. Therefore, the present project has been undertaken to monitor the "In Process Quality Control Factors" affecting the efficacy of HPS virus infected liver homogenate vaccine.

## MATERIALS AND METHODS

Twenty one (26-days-old) broilers were purchased from local farm and shifted to the experimental shed of Quality Operations Laboratory, University of Veterinary and Animal Sciences (UVAS), Lahore. HPS infected liver was obtained from M/S Ottoman Pharma (A Biologics Production Unit, Raiwind Road, Lahore). In

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laboratory the HPS virus in the liver homogenate was characterized by using serum neutralization test in 26-days-old susceptible birds (Khalid, 2003). On day 26$^{th}$ of age, 15 birds were challenged with virulent HPS virus and remaining 6 birds were kept as control (non challenged birds). The birds were observed for 5 days post challenge (PC) for any morbidity and mortality. The livers of HPS infected birds were removed and stored at -20$^{o}$C.

Out of this liver one portion was separately stored for 45 days at -20°C and tested for it biological titer with 15 days interval.

One gram of HPS infected liver was homogenized in electric homogenizer with 9 ml of normal saline (pH 7.2) and centrifuged at 3000 xg for 10 minutes. The clear supernatant was taken out and gentamycin @200ug/ml, penicillin @1000 i.u./ml and streptomycin @ 100ug/ml were added to the supernatant fluid (Rabbani, 1997). This 10 per cent HPS infected liver homogenate supernatant was processed for determination of bird lethal dose ($LD_{50}$) as described by Reed and Muench (1938). The supernatant was diluted to achieve virus suspension having 1x $10^{4.6}$ and 1 x $10^{3.6}$ units of $LD_{50}$ HPS virus and were inactivated by using formaldehyde (37 per cent; Merck) in ratio of 1.5:1000. The virus suspension was subjected to safety and sterility testing as described by Khalid (2003). Formalin inactivated virus suspensions 1x$10^{5.6}$ (25 doses per gram of HPS infected liver homogenate) was processed for vaccine preparation by admixing virus suspension with montanide ISA 70 (oil-adjuvant), aluminum hydroxide gel (Muhammadi *et al*., 1996) and lanolin. All the vaccines were stored at 4°C. The scheme for preparation of HPS vaccines is shown in Table I.

For evaluation of these 13 vaccines, 112 day old broiler chicks were purchased from M/s Hi-Tech Hatchery (Pvt.), Sundar, Multan Road, Lahore, reared for two weeks and were divided into 14 groups comprising 8 birds in each group. Birds of each group were marked for identification and vaccinated (through subcutaneous route with dose rate of 0.5 ml per bird) at 14$^{th}$ day of age with respective vaccines as shown in Table I. Birds of 14$^{th}$ group (Group-E) were kept as non vaccinated control. Blood was collected randomly from 4 birds of each group on zero, 14 and 28 days post vaccination (PV). Serum was separated and inactivated at 56°C for 40 minutes to inactivate the complement.

**Table I. Different vaccines and their respective groups.**

| Groups (n=8) | Vaccine | Groups (n=8) | Vaccine |
|---|---|---|---|
| A1 | F-HPSV-$10^{5.6}$ $LD_{50}$ | C2 | F-25D-HPSV-$10^{5.6}$ $LD_{50}$ |
| A2 | F-HPSV-$10^{4.6}$ $LD_{50}$ | C3 | F-30D-HPSV-$10^{5.6}$ $LD_{50}$ |
| A3 | F-HPSV-$10^{3.6}$ $LD_{50}$ | C4 | F-35D-HPSV-$10^{5.6}$ $LD_{50}$ |
| B1 | F-LB-HPSV-$10^{5.6}$ $LD_{50}$ | C5 | F-45D-HPSV-$10^{5.6}$ $LD_{50}$ |
| B2 | F-MB-HPSV-$10^{5.6}$ $LD_{50}$ | D1 | CEHCC-HPSV-$10^{3.6}$ $LD_{50}$ |
| B3 | F-AHGB-HPSV-$10^{5.6}$ $LD_{50}$ | D2 | F-S-HPSV |
| C1 | F-20D-HPSV-$10^{5.6}$ $LD_{50}$ | E* | Control non vaccinated |

E*, control non vaccinated group; F-HPSV , formalized HPS virus vaccine ; F-LB-HPSV, formalized linolin based HPS virus vaccine; F-MB-HPSV, formalized montanide based HPS virus vaccine F-AHGB-HPSV, formalized aluminum hydroxide gel based HPS virus vaccine; F-20D-HPSV, formalized inactivated HPS virus infected liver 20 doses/gram; CEHCC-HPSV, chicken embryo hepatocytes cell culture HPS virus vaccine; F-S-HPSV, formalized supernatant HPS virus vaccine; $LD_{50}$, bird lethal dose.

On day 42$^{nd}$ of age (28 days PV) all the birds were given challenge dose of virulent HPS virus (1ml of one percent w/v HPS infected liver homogenate). Mortality and morbidity of all the challenged birds were recorded up to 7 days post challenge (PC). The serum samples collected from all the groups were subjected to AGPT for monitoring sero-conversion of the vaccinates.

## RESULTS AND DISCUSSION

Broilers (3-5 weeks old) are highly susceptible to challenge infection using one percent w/v HPS infected liver homogenate. In the present study mortality started and reached to its peak 70-90 hrs post challenge (PC). Dead birds showed characteristic hydropericardium with enlarged, pale, necrosed liver, congestion of lungs, enteritis and hemorrhages on heart and kidneys. These findings are in line with Khawaja *et al*. (1988), Shah *et al*.

(1988), Irfan (1988) and Ganesh (1998).

HPS infected liver homogenate (10 percent) when mixed with specific immune serum neutralized the causative agent of HPS. Similar findings are also observed by Rabbani (1997). The liver collected from HPS infected birds showed high level of biological titer/bird lethal dose 50-$LD_{50}$. The biological titer was $10^{5.6}$ /ml units of $LD_{50}$ that is partially in agreement with that of Rabbani (1997) and Khalid (2003), who recorded $10^{4.6}$/ml units of $LD_{50.}$ The variation in the biological titer could be due to stage of death, susceptibility of the birds, virus storage conditions and concurrent problems of birds especially mycotoxicosis.

Concentration of immunogen is an important factor to induce antibody production in the vaccinated birds but in present study either of the two immunogen doses such as $10^{5.6}$ and $10^{4.6}$ units of $LD_{50}$ induced similar results while, $10^{3.6}$ units of $LD_{50}$ induced immunity in test group that was near to that of control. This finding partially corroborates with the study of Ahmad *et al*. (1989) who reported no difference in protection percentage of birds vaccinated with $10^{4.4}$ units of $LD_{50}$ of HPS virus containing vaccines. The variation could be due to *in vivo* response of the birds (humoral and non-specific resistance factors, etc.) and the sample size of the experiment.

Adjuvant plays an important role in augmenting the efficacy of inactivated vaccines. The protection percentage in vaccinated birds was found to be the highest for montanide based HPS virus vaccine (100 per cent), followed by aluminum hydroxide gel based vaccine (80 percent) and lanolin based vaccine (60 percent), whereas, the vaccine without adjuvant provided 40 per cent protection when challenged with virulent virus at $28^{th}$ day post vaccination. These findings are congruent with the observations of Hussain *et al*. (1999) and Roy *et al*. (1999). The variation in the protection percentage induced by montanide and lanolin based vaccine could be due to instability of the latter during storage.

It was recorded that the vaccine containing 20, 25, 30, 35 and 45 doses as an immunogen without the gel induced lesser immunity and such birds, therefore, showed 100, 90, 90, 50 and 20 per cent resistance to challenge infection, respectively. While, vaccines with the gel containing 20, 25, 30, 35 and 45 doses, induced higher immunity thus showing 100, 100, 100, 70 and 30 per cent protection to the challenge infection, respectively. This is some what in line with Ahmad (1994) who recorded difference in protection percentage of birds vaccinated with 1, 2.5, 5, 7.5 and 10 per cent liver organ vaccines. Therefore, it was concluded that the gel based vaccines provide better protection as compared to vaccine without gel.

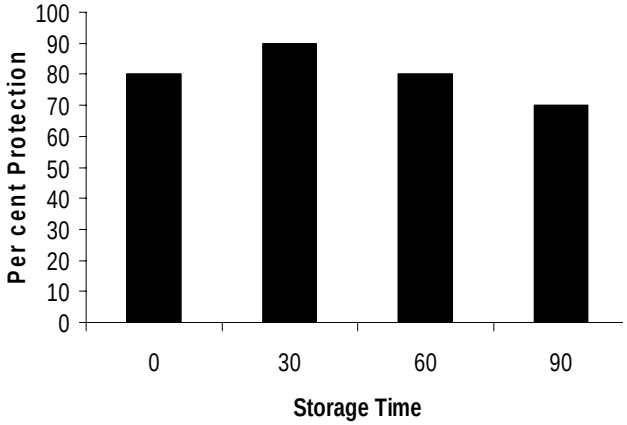


Fig. 1. Effect of vaccine storage time on its efficacy in birds.

The vaccine containing aluminum hydroxide gel as an adjuvant and thiomersal sodium as preservative (Clements *et al*., 2000; Haviid *et al*., 2003) when stored at $4^{o}$C for zero, 30, 60 and 90 days showed 80, 90, 80 and 70 per cent protection, respectively, when given challenge infection on 14 day and 28 day post vaccination (Fig. 1). It is, therefore, concluded that HPS virus infected liver homogenate vaccine can be stored for 90 days without any ill effect on its efficacy. It was also noted that group of broilers primed and boosted with either of the vaccine did not show any significant difference in weight gain. Afzal and Ahmad (1990) also reported that on the farms where disease did not appear, the weight gain of the vaccinated and unvaccinated birds was similar, whereas, in the infected flocks, the vaccinated birds gained more weight than the unvaccinated birds.

The HPS virus infected liver when stored for 45 days at $-20^{o}$C and tested for it biological titer with 15 days interval showed no change in its

infectivity virus titer. Therefore, it indicates that the HPS infected liver can be stored for 45 days for preparation of vaccine.

The HPS virus cultivated chicken embryo hepatocytes suspension showed biological titer $10^{3.6}$ per ml units of $LD_{50}$ whereas HPS virus infected liver homogenate showed biological activity $10^{5.6}$ units of $LD_{50}$ per gram of the tissue. It indicates that 100 times more virus can be harvested from the HPS infected liver homogenate. The HPS virus chicken embryo hepatocyte vaccine developed 40 percent protection in contrast to HPSV liver homogenate vaccine that has induced 80 percent protection to challenge infection on 42 days of age. Hussain *et al*. (1999) found all the birds sero-positive 14 day post vaccination, while Chishti *et al.* (1989) and Sarwar *et al.* (1995) reported in 10 to 14 days. This difference in the protection percentage could be due to composition of cell cultured vaccines, breed of chickens, managemental conditions and the immune status of the vaccinates. The results of this study are also in agreement with Rabbani *et al.* (1998) and Kumar *et al.* (2003) who propagated HPS virus on chicken embryo liver cells and calculated tissue culture infective dose ($TCID_{50}$) of virus, which were $10^4$ per 0.5ml.

It is concluded that HPS virus vaccine containing more than $10^{4.6}$ units of immunogen and either montanide or gel as an adjuvant is effective for broilers to achieve the required level of resistance to field challenge. The HPS infected liver homogenate is a good source of virus for the vaccine production. Each serum sample when monitored for anti –HPSV antibodies through agar gel precipitation test, showed undetectable titer.

Moreover, the gel based vaccine prepared from fresh HPS virus infected liver homogenate is more effective and economical as compared to HPS virus infected chicken embryo hepatocytes homogenate vaccine.

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