Volume 2, No 8, Sep 2012 ISSN 2225-7217
ARPN Journal of Science and Technology
©2011-2012. All rights reserved.

http://www.ejournalofscience.org

Study of the Presence of Chicken Anemia Virus in Liver Homogenate Used as a Vaccine Against Hydro Pericardium Syndrome in Broile

[1]Shahid Khan, **[2]Faiz Ul Hassan, [3] S.Rahimullah Shah, [4]Farhan Anwar, [5]Swat Kalim Ullah, [6]Insan Uddin**
[1]Research Officer VR&DIC Swat, [2, 3] Senior Research officer VR&DIC Swat,
[4] Research Officer VRI Peshawar, [5, 6] Research Officer VR&DIC Swat
Email: {[1]wazirkhattak@yahoo.com}

ABSTRACT

A research study was conducted in Veterinary Research & Disease Investigation center (VR&DIC) Swat to study the presence of Chicken Anemia Virus (CAV) in liver homogenates of birds infected with Hydro pericardium Syndrome (HPS). Sixty-four 64 birds of 15-days age and free from CAV were selected and divided into five groups. One group was vaccinated with inactivated liver homogenate vaccine prepared in our laboratory from the liver of HPS infected birds. Two groups were vaccinated with similar autogenous vaccine procured from two commercial laboratories. One group was vaccinated with cell cultured DNA based vaccine while one group was kept as a control. Multiple shots of each vaccine were used in its specific group along with boosting doses. The hyper immune sera thus raised were tested for the presence of HPS and CAV antibodies using Agar Gel Precipitation Test (AGPT) and Enzyme Linked Immuno-Sorbant Assay (ELISA) techniques. The results demonstrated that antibodies against HPS were present in hyper immune sera of all the vaccinated birds. No antibodies against CAV were detected in the tested sera. It was concluded that CAV is not associated as a contributing agent in causing hydro pericardium syndrome in broilers.

Keywords: *Broiler chicken, Chicken Anemia virus, hydro-pericardium syndrome.*

1. INTROUDUCTION

The CAV was first described in Japan in 1979. This agent has since been shown to be present in poultry flocks (layer and broiler breeds) worldwide. The CAV is very small and rather resistant to chemical and physical treatment. For example, it can resist a pH of 3.0 and heat at 176°F for 30 minutes Chandra *et al*. (2000). The chicken appears to be the only known host for the CAV. That is, the virus does not infect humans or other animals. It can be transmitted horizontally, from infected to susceptible birds, and vertically, through the eggs of seronegative, infected breeders to chicks Balamurugan ,Kataria (2004). The CAV is shed through the egg until the breeder develops antibodies. Chickens of all ages are susceptible to infection with the virus; however, only young chickens without maternal antibody protection develop the disease. Chickens younger than 2 weeks of age are particularly susceptible. Although chickens become increasingly resistant to the disease with age, they may still become infected and shed the virus at any age. Following infection, vertical transmission has been observed to occur for 14 days and horizontal transmission for 6 weeks **Chandra *et al*. (2000), Kumar *et al* (2003**) Chicken Anemia Virus infection in young, susceptible chicken's results in increased mortality of chickens 12 to 28 days of age. Affected birds generally have a depressed, pale (anemic) and anorexic appearance. Lesions can lead to atrophy of the bone marrow and thymus. The bone marrow becomes fatty and yellowish. Atrophy and aplasia of the blood-forming tissues can be seen microscopically, as well as replacement by adipose tissue and nonfunctional supportive tissue. Microscopically, severe lymphoid depletion is visible throughout the gland. Gross lesions in the bursa of Fabricius are minimal and may be difficult to detect. In many cases, the liver may be swollen and mottled, and massive hemorrhages may be observed in the proventricular mucosa and in subcutaneous tissue and muscle, particularly in the wing (Chandra *et al*. (2000), Toro *et al*. (2002)

Concerns have centered on the effects of CAV on chickens' immune system and on its relationship to other poultry diseases e.g Infectious Bursal Disease and HPS. Hydropericardium syndrome, an emerging disease of poultry, has recently been detected in some countries of Asia and America, (McFerran,J.B.,and Smyth,J.(2000). particularly in broiler birds aged 3-6 weeks. The disease is characterized by its sudden occurrence with high mortality (80%) in broilers and low mortality (<10%) in layers. Its course is of 7-15 days under natural conditions. The causative agent is probably fowl adenovirus serotype 4, belonging to group I aviadenovirus genus of the family adenoviridae, which can be cultivated in primary cell cultures of chicken kidney and embryo liver cells Cheema, *et al*. (1989). The transmission of disease occurs laterally by the oral-faecal route. The livers of effected birds show necrotic foci, and basophilic intranuclear inclusion bodies.Thus booth CAV and HPS effect liver. The present study was carried out to see the presence of Chicken Anemia Virus in liver homogenate of HPS infected broilers.

2. MATERIALS AND METHODS

Vaccine Preparation: Inactivated liver homogenate vaccine were prepared in microbiology laboratory from the liver of HPS infected birds .Thirty grams of infected liver was homogenized in 100 ml normal saline by mean of Homogenizer. The homogenate was then filtered through muslin cloth into a sterilized beaker. The filtered suspension was then inactivated by using

©2011-2012. All rights reserved.

0.5%formaline. Moreover, 10,000 units of Penicillin was added per ml of suspension and incubated for 24 hours in incubator

Ravi *et al*. (1997)**,** The liver homogenate vaccine was also procured from two commercial laboratories. Similarly, Cell Cultured Vaccine was also obtained from a commercial laboratory.

Immunization of birds: Immunization was performed according to the method performed by (**Toro *et al.* (1999)** A total of 64 birds were selected for the study. These birds were checked for antibodies against CAV and all were found negative. At the age of 15 days these were divided into 5-groups. Each bird was numbered by using shoulder tag and each group was marked on wings and neck region by using distinct color for each group. Various groups were vaccinated by using autogenous and cell cultured vaccine as given in Table-1

Table-1. Grouping of experimental birds

Group	# of Birds	Vaccine used
1	1-13	Self prepared (Liver homogenate)
2	14-26	Commercial laboratory (Liver homogenate)
3	27-39	Commercial laboratory (Liver homogenate)
4	40-52	Commercial laboratory (Cell cultured)
5	53-64	Control

The first immunization was carried out at the age of 20 days by using separate vaccine for each group. Similarly, 2nd and 3rd vaccination was done on day 27 and day 34 respectively according to the following schedule (Table-2).

Table-2. Immunization schedule of experimental birds

S#	Vaccine	Age (in days)	Dose ml/bird	Day of blood collection	Sampling
1	1st immunization	20 days	0.5 I/M	27	Sample#1
2	1st boosting	27 days	0.7 I/M	34	Sample#2
3	2nd boosting	34 days	0.8 I/M	41	Sample#3

Sampling: Blood was taken from wing vein of each bird 7-days after each immunization according to the above schedule, using separate disposable syringe (23 gauge) of 5ml capacity without using any anticoagulant. Each syringe was numbered according to the bird tag number. The syringes were kept at slanted position at room temperature for one hour and then transferred to a refrigerator. After 18 hours serum from each syringe was collected in an Eppendorf tube of 1ml capacity. Each Eppendorf tube was numbered according to syringe number. Now each serum sample was centrifuged at 300 rpm for 5 minutes. The clear serum was isolated in appendorf tubes (1 ml) and stored in freezer as Sample 1, Sample 2, and Sample 3 containing all the groups.

3. SEROLOGICAL TESTS

1.Agar Gel Precipitation Test (AGPT) and Counter Immunoelectrophoresis (CIE) AGPT and CIE was performed according to the method of **(Chandra *et al.* (2000), Balamurugan , Kataria** (2004)The AGPT (Cullen and Wythe, 1975) and CIE (Berg, 1982) were performed using hyper immune sera collected 7-days after each shot of vaccine to check the antibodies against HPS and CAV. The infected liver homogenate extract was prepared from field cases and experimentally infected birds. This was filtered through muslin cloth and the filtrate was then used as an antigen in AGPT. One gram Agar was melted in 50ml normal saline in a microwave oven and then poured sterile petri dish. On setting, the gel was punched using a gel punch to cut about 5-wells. The agar plugs were removed and the center well was filled with antigen and the side four wells with serum samples. The Petri plates were then placed in incubator overnight. Results were recorded by observing the presence or absence of precipitation line.

Enzyme Linked Immunosorbant Assay (ELISA) was performed according to the method of **Balamurugan , Kataria** (2004} to detect the antibodies against CAV in serum samples #3 containing all the groups by using CAV coated ELISA plate. At start 50 microlitre of PBS was pipetted in each well. Then negative control serum was pipetted in well A1,A3,H11, and positive control serum in well A2,H10,H12.The test samples in a sample#2 and sample#3 were pipetted in well A4 to well H9. Now the plate was incubated for 45 minutes at room temperature and then washed 3 times with washing solution at interval of 3 minutes. After washing conjugated antibodies were pipetted into all wells and incubated for 45 minutes at room temperature and then washed 3 times as above. After washing, substrate was added and incubated the plate for 30 minutes at room temperature and read by ELISA reader at WTO Quality Control Laboratory and UDL. According to kit information paper, the sample testing with the sP value of less than or equal to 0.410 were received zero titer value and considered negative for CAV antibodies.

4. RESULTS

Agar Gel Precipitation Test revealed that a single precipitation line was observed in samples #3 of all the vaccinated birds after incubating for 24 hours. However, Sample#1 and Sample#2 did not give any precipitation line. This confirmed the hyper immune sera against HPS. The serum samples #3 containing all the groups were checked for the presence of antibodies against Chicken Anemia Virus using ELISA technique. Results of this test are summarized in tale-3. The average positive and negative control serum absorbance

©2011-2012. All rights reserved.

were calculated as 0.278 and 0.221, respectively. None of the test sera gave an SP value equal to 0.410 or higher. The test demonstrated that samples # 3 (hyper immune sera against HPS) were all negative for antibodies against Chicken Anemia Virus.

Table-3. ELISA Readings

Measurement count: 1 Filter: 405

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.209	0.291	0.217	0.071	0.207	0.327	0.358	0.133	0.064	0.403	0.31	0.211
B	0.232	0.074	0.133	0.064	0.172	0.172	0.215	0.067	0.218	0.347	0.105	0.269
C	0.323	0.216	0.244	0.31	0.278	0.059	0.18	0.134	0.223	0.173	0.186	0.226
D	0.215	0.156	0.23	0.195	0.065	0.111	0.135	0.246	0.166	0.327	0.143	0.175
E	0.209	0.316	0.227	0.156	0.184	0.237	0.211	0.061	0.151	0.207	0.202	0.213
F	0.038	0.041	0.037	0.04	0.052	0.052	0.036	0.046	0.038	0.033	0.037	0.036
G	0.036	0.055	0.038	0.04	0.034	0.038	0.038	0.038	0.037	0.036	0.038	0.035
H	0.045	0.041	0.038	0.032	0.039	0.036	0.039	0.04	0.038	0.273	0.237	0.271

Average positive control serum absorbance=0.291+0.273+0.271/3=0.278

Average negative control serum absorbance=0.209+0.217+0.237/3=0.221

5. DISCUSSION

Previous studies show that adeno virus is not the only cause of Hydropericardium in young susceptible birds. It is rather a syndrome in which multiple etiological agents are involved. Researchers are trying find out these associated etiological agents. The present study was carried out in Veterinary Research Institute Peshawar to check the presence of Chicken Anemia Virus in liver homogenate of HPS infected broilers. Five groups of 15 days broiler chicks were selected and vaccinated by using inactivated liver homogenate. The boosting doses of vaccine were also used at suitable intervals to raise hyper immune sera against HPS .The sera collected 7 days after final shot were checked for the presence of high titer of antibodies against HPS by using Agar Gel Precipitation test. The result was found positive in the form of precipitation line. These HPS antibodies positive ser were then tested for the presence of antibodies against CAV. ELISA was performed on these sera using antigen (CAV) coated plate purchased from a commercial source. The result was found negative indicating that CAV was not present in the liver homogenate obtained from HPS infected birds. Further studies are suggested to find out other viruses involved in Hydro Pericardium Syndrome.

REFERENCES

1.Adair, B. M. and Fitzgerald, S. D. (2008): Group I adenovirus infections. In: Saif, Y. A. (ed.) Diseases of Poultry. 12th edition. Blackwell Publishing. pp. 252–266.

2.Ahmad, K. (1999): *In vivo* pathogenicity of hydropericardium-hepatitis syndrome (Angara disease) and efficacy of vaccines. Pakistan Vet. J. 4 , 200–203.

3.Afzal,M. and I.Ahmad (1990) Efficacy of an inactivated vaccine against HPS in broilers.Vet.Rec.,126(3):59-60.

4.Balamurugan V, Kataria JM. (2004) The hydropericardium syndrome in poultry--a current scenario. Vet Res Commun. ;28(2):127-48

5.Chandra, R., S.K. Shukla and M. Kumar (2000). The Hydropericardium Syndrome and Inclusion Body Hepatitis in Domestic Fowl.321(2): 99-111.

6.Cheema.A.H.,M.Afzal and J.Ahmad (1989).An Adenovirus Infection of PoultryinPakistan.Rev.Sci.Tech.Off.Int.Epiz.,8(3):789-795

7.Kumar.R.,Chandra,R.,Shukla(2003).Isolation of etiological agent of HPS in chisken embryoliver cell culture and its serological characterization.Indian J.Exp.Biol.41(8):821-826.

8.McFerran,.J.B.,andSmyth,.J.(2002)AvianAdeno viruses. Arevive,Sci. Tech.Int. Epiz. 19:589-601

9.Natesan S, Kataria JM, Dhama K, Rahul S, Baradhwaj N. (2006)Biological and molecular characterization of chicken anaemia virus isolates of Indian origin. Virus Res. ;118(1-2):78-86.

10.Rabbani and Muneer (1997). Observation on the causative agents of hydropericardium syndrome (AngaraDisease) in chickens.Ph.D.Thesis, Microbiology Deptt.,Univ.of Agric.Faisalabad

11,Ravi Kumar,Rajesh Chandra,S. K. Shukla,D. K. Agra wal &Mahesh Kumar (1997) A preliminary study on the causative agent and control of the disease by inactivated autogenous vaccine, **Comparative Immunology, Microbiology and Infectious Diseases** 29(3) 55-60.

12.Toro H, Prusas C, Raue R, Cerda L, Geisse C, González C, Hess M. 1999 Characterization of fowl adenoviruses from outbreaks of inclusion body

©2011-2012. All rights reserved.

hepatitis/hydropericardium syndrome in ile. Avian Dis. Apr-Jun;43(2):262-70.

13.Toro H, González C, Cerda L, Morales MA, Dooner P, Salamero M. (2002 Prevention of inclusion body hepatitis/hydropericardium syndrome in progeny chickens by vaccination of breeders with fowl adenovirus and chicken anemia virus. Avian Dis. Jul-Sep;46(3):547-54