Short Communication

Transovarian Transmission of Hydropericardium Syndrome Virus in Experimentally Infected Poultry Birds

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Abstract | The primary aim of this study was to investigate the transmission pattern of avian adenovirus serotype 4 (AAV-4) causing hydropericardium syndrome (HPS) by transovarian route. For this purpose, liver and spleen samples (n=90) were collected from day-old-chicks derived from breeders at 14, 21 and 30 days of post-infection with AAV-4. The presence of AAV-4 DNA was detected through PCR. Chicks from virus-challenged breeders failed to show a clear and expected PCR positivity. The connotations derived from these findings are that vertical transmission via transovarian route is reported the field conditions. However, based on genetic detection such evidences are lacking. The molecular mechanism and establishment of latent infections warrant future investigations to elucidate these discrepancies.

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Introduction

Hydropericardium syndrome (HPS) was first reported in the areas of Karachi named Angara Goth, from where the Angara disease (Jaffrey, 1988) was derived. HPS affects 3-6 week old broiler flock with mortality up to 80% (Kumar et al. 2003). The causative agent is highly infectious virus belonging to avian adenovirus serotype 4 (Balamurugan and Kataria, 2004). These are non-enveloped viruses with diameter ranging from 70-90 nm. The capsid proteins of adenoviruses are arranged in icosahedrons having 20 triangular faces and 12 vertices (Ginsberg et al., 1966).

This disease is characterized by the accumulation of straw coloured jelly like fluid in the pericardial sac, discoloured and inflamed liver with basophilic intranuclear inclusion bodies and congested kidneys (Dahiya et al., 2002). Moreover Nakamura et al. (2002) have also observed pinpoint white foci in the pancreas and ventricular erosions in the affected birds. Purified virus or liver homogenate inoculation of HPS virus resulted in incubation period of 2-5 days (Roy et al., 2001).

Fowl adenoviruses are readily transmitted by both horizontal and vertical modes. Vertical transmission of adenovirus has been inducted in various experi-

ments; however, the vertical transmission of HPSV in the field outbreaks is contradictory. This research was planned to clarify the ambiguity about trans-ovarian transmission of the HPS virus.

Materials and Methods

Procurement and processing of challenge virus

Livers samples from bird infected with HPS virus were harvested. Collected samples were processed in the Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan. Briefly approximately 15 g liver samples were triturated in a sterilized pestle and mortar with the help of sand, phosphate buffer saline and antibiotics (penicillin @10,000 units per ml and streptomycin @10,000 microgram per ml). The homogenized suspension was subjected to centrifugation at 1500 rpm for 15 minutes. The supernatant was filtered through 0.2 micrometer filter and was used for further inoculation. Supernatant was mixed with chloroform (1:1) in centrifuge tube and centrifuged at 5000 rpm for 20 minutes. The middle layer having liver proteins, cell debris and the bottom layer of chloroform were discarded. The clear supernatant was collected in sterilized screw capped test tube and stored at -20 celsius for further use.

Animal experiment and samples collection

A total of 90 breeder birds of 18 weeks of age were obtained and were divided into three groups, each containing 30 birds. The members of group I were regularly vaccinated against HPS, members of Group II and Group III were devoid of any vaccination against HPS. Later on the members of Group I and III were challenged by the HPS virus by inoculating intramuscularly liver homogenate of HPS affected livers while the birds of group II were injected with the mixture of HPS antigen and antibodies. Then at 7-14, 15-21 and 22-30 days of post-infection, eggs were collected from breeders of each group. The collected eggs post- challenges were labeled and were allowed to hatch. After hatching the day old chicks were slaughtered and liver and spleen were taken as sample for HPS virus confirmation and were frozen until further processing.

Confirmation of virus

DNA was extracted extraction and PCR assays were performed to confirm the virus in liver and spleen of day old chicks as described by Rehman et al. (2011). Briefly, liver and spleen from infected birds was taken and subjected to DNA Mini Kit (Germany, Qiagen Gmbh, D -40724) for DNA extraction. Amplification of avian adenovirus was achieved by PCR with the help of following two primers H1, forward (5-TGGACATGGGGCGACCTA-3) and H2, Reverse (5AAGGGATTGACGTTGTCCA-3). PCR was conducted in a 25 microliter reaction mixture volume comprising of 12.5 microliter master mix, 1 microliter (10 Pico mole) each of the primer (H1, H2), and 1 microliter template DNA. In the end, 9.5 microliter nuclease free water was added to make the total reaction volume of 25 microliters. PCR was performed in an automatic thermo cycler. Optimized cycling conditions comprised of an initial denaturation at 95 Celsius for 5 minutes followed by 35 cycles at 95 Celsius for 45 sec, 55 Celsius for 45 sec and 72 Celsius for 1.5 min. In the final step, ultimate extension was carried out at 72 Celsius for 10 min (Rehman et al., 2011). Amplified genome was visualized with the help of Agarose gel electrophoresis.

Ethical Statement

This research work was approved by the Advance studies and Research board (ASRB) of the University of Veterinary and Animal Sciences Lahore, Pakistan which is responsible for all the moral and ethical issues of research.

Results and Discussion

All samples of liver and spleen of day old chicks which were taken at 7-14, 15-21, and 22-30 days post infection failed to produce a visible band after staining with ethidium bromide on 1% agarose gel (Table 1). While the infected liver homogenate (inoculated for transovarian transmission) produced visible bands when visualized through 1% Agarose gel electrophoresis. The positive band were compared with 100 base pair plus ladder to know the size of avian adenovirus type 4 causing hydropericardium syndrome which yielded the size of genome as 1219 base pair (Figure 1).

Fowl adenoviruses (FAV) are well known for their transmission from parent birds to progenies (McCracken and Adair, 1993) through either of two methods. One important route for transmission is through infected eggs (McFerran and Adair, 1977). Study of Ashraf et al. (2000) on liver, lungs, spleen and kidneys samples of 18 days old embryos taken

Table 1: *PCR detection in day old chicks of Group I and II.*

	Samples (number positive/number tested)				
Parameter	Group I (n=30)		Group II (n=30)		Control
Days p.i.	Liver	Spleen	Liver	Spleen	Infected Liver Homogenate
7-14days p.i	0/30(0)	0/30(0)	0/30(0)	0/30(0)	1/1 (100 %)
15-21 days p.i.	0/30 (0)	0/30(0)	0/30 (0)	0/30(0)	1/1 (100%)
22-30 days p.i.	0/30 (0)	0/30(0)	0/30 (0)	0/30(0)	1/1 (100%)
Total	0/90 (0)	0/90(0)	0/90 (0)	0/90(0)	1/1 (100%)

Group I: Birds challenged by HPS at age of 22 weeks; Group II: Birds challenged by mixture of HPS and Antibodies against it; P.i: post infection

from HPS recovered birds at 30, 37 and 44 weeks of age probably explains transmission pattern of adenovirus to progeny at the time of egg production. Dot ELISA, AGPT and tissue culture were the adapted methods for virus detection. AGPT failed to detect any viral load however Dot Elisa and tissue culture showed 66% positive results. Second method of disease transmission is in form of lysogeny. In the infected birds or carrier birds, the viral genome become integrated with host chromosome and adapts latent phase. Virus shedding by infected birds continues till three to six weeks, after that sufficient antibody titer against infection develops (Mazaheri et al., 2003). It is evident that reoccurrence of infections is enhanced during periods of stress, like the onset of egg production or intake of any immunosuppressant agent (Girshick et al., 1980). Philippe et al. (2007) also checked vertical transmission in day old chicks taken from HPS infected birds at 28 weeks of age via PCR. They also revealed negative results for vertical transmission via PCR in day-old-chicks.

Primers used for amplification of genome obtained from day old chick were adopted from study of Rehman et al. (2010). These primers efficiently amplified the genome obtained from infected liver (**Figure** 1). Therefore, it is unlikely that negative results were due to PCR performance. PCR has been shown to be sufficiently sensitive to detect transmission pattern and latency of FAdV associated chickens (Grigc et al., 2006). PCR is sensitive enough to detect 1 pg of viral DNA, but the possibility that the viral load in the samples was below the detection limit might be the reason for negative results of PCR.

Apart from PCR efficiency other reason may be the presence of neutralizing antibodies (Ab). A strong correlation is observed between vertical transmission and titer of neutralizing antibodies against FAdV in blood (Cowen et al., 1978; Dawson et al., 1981). Similarly pattern of virus excretion relates with development of neutralizing Ab (Adair and Fitzgerald, 2008). Contradictory results are reported about vertical transmission of FAdV in relation to neutralizing antibodies. According to Saifuddin and Wilks (1991) vertical transmission of FAdV do occur in the presence of virus neutralizing (VN) Ab, however, Philippe et al. (2007) states that VN Ab masks the vertical transmission of HPS virus. Other possibility for negative results of study would be intra variations predominant in serotype or strain in vertical transmission of FAdV (Dawson et al., 1981).

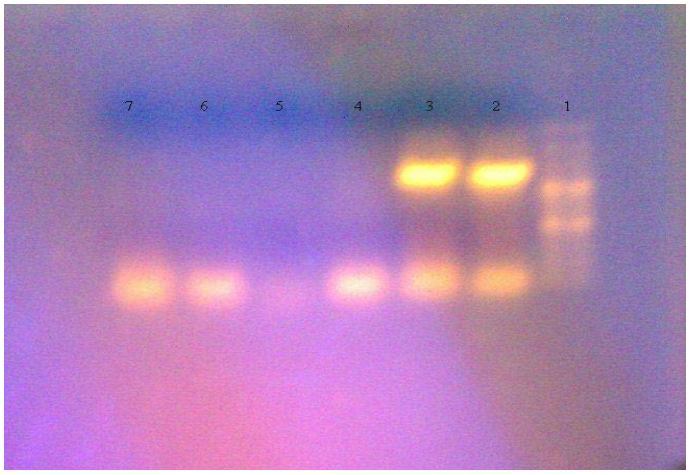

Figure 1: *Gel electrograph of samples processed for transovarian Transmission*

Lane I indicates the 100 base pair plus ladder while lane II is positive control and III is showing band of infected liver homogenate (1219 bp) used for infection. While Lane 4,5,6,7 are showing negative results of samples processed for HPS detection.

Author's Contribution

All the authors contributed equally.

References

- Adair BM, Fitzgerald SD. 2008. Group I adeno-

virus infections. In: Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK, D.E. Swayne (Ed.), Diseases of Poultry,12 edn. Blackwell Publishing Professional, Iowa. 251-266.
- Ashraf S,Malik SA, Naeem K.2000. Persistence and transmission of Avian Adeno virus-4 in broiler breeder. Pak. Journal of Biological Sciences.3 (4):633-635. https://doi.org/10.3923/pjbs.2000.633.635
- Balamurugan V, Kataria JM.2004. The hydropericardium syndrome in poultry: a current scenario. Veterinary Research Communications. 28: 127-148. https://doi.org/10.1023/B:VERC.0000012115.86894.1e
- Cowen B, Mitchell GB, Calnek BW. 1978. An adenovirus survey of poultry flocks during the growing and laying periods. Avian Dis. 22(1): 115-121. https://doi.org/10.2307/1589514
- Dawson GJ, Yates VJ, Chang PW, Wattanavijarin W.1981. Egg transmission of avian adenovirus -associated virus and CELO virus during experimental infections. Am. J. Vet. Res. 42(10): 1833-1837.
- Dahiya S, Rivastaav RN, Hess M, Gulati BR. 2002.Fowl adenovirus serotype 4 associated with outbreaks of hydropericardium syndrome in Haryana India. Avian dis. 46:230-233. https://doi.org/10.1637/0005-2086(2002)046[0230:FASAWO]2.0.CO;2
- Ginsberg HS, Pereira HG, Valentine RC, Wilcox WC. 1966. A proposed terminology for the adenovirus antigens and virion morphological subunits. Virology, 228: 782-783. https://doi.org/10.2307/1589725
- Girshick T, Crary CK, Luginbuhl RE. 1980. Serologic detection of adenovirus infections in Specific-pathogen-free chickens. Avian Dis. 24: 527-531.
- Grgic H, Philippe C, Ojkic D, Nagy É.2006. Study of vertical transmission of fowl adenoviruses. Can. J. Vet. Res. 70: 230-233.
- Jaffery MS.1988. A treatise on Angara disease (hydropericardium pulmonary oedema – hepatonephritis syndrome). Pak Vet Med Assoc, pp: 1-33.
- Kumar R, Chandra R, Shukla SK. 2003. Isolation of etiological agent of hydropericardium syndrome in chicken embryo liver cell culture and its serological characterization. Indian J. Biol., 41: 821-826.
- Khan AA. 2008. A comparative study on the production and efficacy of different vaccines against avian adenovirus associated with hydropericardium syndrome.
- McCracken RM, Adair BM. 1993. Avian adenoviruses. In: McFerran, JB, McNulty MS. Eds., Virus infections of birds Vol. 4. Elsevier Science Publishers, Amsterdam. 123 –144. McFerran JB, Adair BM. 1977. Avian adenoviruses - A review. Avian Pathology.6: 189-217.
- Mazaheri A, Prusas C, Hess M.2003. Vertical transmission of fowl adenovirus serotype 4 investigated in specified pathogen-free birds after experimental infection (German Translation). Arch. Geflugelk. 67(1): 6-10.
- Nakamura K, Mase M, Yamaguchi S, Shibahara T, Yuasa N. 1999. Pathologic study of specific-pathogen -free chicks and hens inoculated with adenovirus isolated from hydropericardium syndrome Avian Dis. 43(3): 414 -423. https://doi.org/10.2307/1592638
- Philippe C, Grgic H, Ojkic D, Nagy É.2007. Serologic monitoring of a broiler breeder flock previously affected by inclusion body hepatitis and testing of the progeny for vertical transmission of fowl adenoviruses. Can. J. Vet. Res. 71(2): 98-102.
- Roy P, Kotteeswaran A, Manickam R. 2001. Serological, cytopathological and cytochemical studies on hydropericardium syndrome virus. VeterinarskiArhiv. 71: 97-103.
- Rehman L, Sultan B, Ali I, Bhatti MA, Khan S, Zaman k, Jahangiri AT, Khan N, Iqbal A, Bakht J, Swati ZA, Rehman M. 2011. Duplex PCR assay for the detection of avian adenovirus and chicken anemia prevalent in Pakistan. Virology J. 8:440. https://doi.org/10.1186/1743-422X-8-440
- Saifuddin M, Wilks CR. 1991. Pathogenesis of an acute viral hepatitis: inclusion body hepatitis in the chicken. Arch. Virol. 116(1): 33-43. https://doi.org/10.1007/BF01319229