International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; SP-9(5): 390-393

www.veterinarypaper.com
Received: 23-09-2024
Accepted: 09-10-2024

Dr. G Kalaiselvi
Central University Laboratory, Centre for Animal Health Studies, Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamil Nadu, India

M Parthiban
Central University Laboratory, Centre for Animal Health Studies, Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamil Nadu, India

Dr. G Balakrishnan
Central University Laboratory, Centre for Animal Health Studies, Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamil Nadu, India

Dr. R Ramya
Central University Laboratory, Centre for Animal Health Studies, Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamil Nadu, India

Dr. S Jaisree
Central University Laboratory, Centre for Animal Health Studies, Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamil Nadu, India

Dr. C Soundararajan
Central University Laboratory, Centre for Animal Health Studies, Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamil Nadu, India

Corresponding Author:
Dr. G Kalaiselvi
Central University Laboratory, Centre for Animal Health Studies, Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamil Nadu, India

Physico chemical characterization of fowl adenovirus serotype 4 isolated from Tamil Nadu

Dr. G Kalaiselvi, M Parthiban, Dr. G Balakrishnan, Dr. R Ramya, Dr. S Jaisree and Dr. C Soundararajan

Abstract
Hydropericardium hepatitis syndrome is a newly emerging disease of poultry with sudden occurrence of high mortality. The CPE produced were more marked after 5-6th passages. Cytopathic effect due to the growth of CEL adopted FAV was observed from 24-76 hrs of post infection. Clumping, rounding and fusion of cells were observed during early stages followed by shrinkage of monolayer spaces and formation of inclusion bodies from the cells as reported earlier as cytopathic effect advances, multinucleated giant cells and vaculation in the cytoplasm often observed compared with normal uninfected cells. The physical characterization of FAV 4 was done at different temperatures and different ranges of p^{H} and the virus was stable in 50-70% chloroform, 50-70% ether, 0.25% trysin, heat at 50 °C but labile to 100% ethanol, acidic condition at 3p^{H} and heat at 52 °C, 1M of $Mgcl_2$ at 50 °C for 1 hour, 1:2000 formalin at 37 °C with the $TCID_{50}$ 50 ºC for 15 hours. The nonenveloped FAV 4 was not sensitive to lipolytic agents such as chloroform and ether.

Keywords: Hydropericardium hepatitis syndrome-CPE-physical characterization-Chemical

Introduction
Hydropericardium hepatitis syndrome is a newly emerging disease of poultry with sudden occurrence of high mortality. All strains of poultry are equally susceptible to fowl adenovirus infection. The disease was first noticed in Angora Kotch of Pakistan so named as Angora disease (Jaffery *et al.*, 1988) [4]. In India, the disease was first noticed in Jammu during 1994 and spread Punjab (Gowda *et al*., 1994) [6] named as leechy or litchi disease resembling the look of heart floating in pericardial fluid simulating de skinned litchi fruit (Gowda *et al.*, 1999) [7]. Death of embryo within 4-7 days after post-infection.
Transmission of virus mainly through horizontal among the broilers. The disease could be experimentally transmitted by inoculating infected liver homogenate through intramuscularly, subcutaneously and orally (Hasan *et al.*, 1995) [9]. Presence of aflatoxin in the feed at high concentration commonly associated with large number of outbreak among the broilers (Singh *et al.*, 1994) [10]. Simultaneous presence of infectious bursal disease virus and chicken infectious anaemia will predispose HPS outbreak and no specific clinical symptoms associated with outbreak of HPS. High mortality upto 75% sudden onset in well grown healthy broiler flock of 3-5 weeks of age.

Materials and methods
Sample collection for virus isolation
Sample collection
Suspected liver samples were collected from various outbreak areas of Tamil Nadu. All suspected samples were collected in PBS solution (pH 7.2) and stored at-20 ºC.

Electron microscopic study of infected chicken liver
Electron microscopic study of FAV4 were carried out according the method described by Cheema *et al.* (1989). The artificially infected chicken liver were trimmed to 8-10 mm ^{3}. After washed three times with 0.2 M phosphate buffered saline (PBS). The tissue were fixed with 2.5% glutaraldehyde (Sigma USA) kept at 4 ºC for 2 hours.

The tissue were gently stirred every 10 min then the tissue were poured on a petri dish and incubated at room temperature for 10 min, and stored in 0.2 M PBS at 4 ºC. These were post – fixed with osmium tetraoxide at 4 ^{O}C for 1 hr and washed in PBS. The tissue were dehydrated through a graded series of ethanol and substituted with propylene oxide. The section were cut on an ultra-microtome using diamond knife. The ultra-thin sections were stained with uranyl acetate for 20 min. the sections were examined for FAV particle under transmission electron microscope.

In vitro propagation of fowl adeno virus serotype 4

Egg inoculation technique

The 6-8 days old chicken embryonated eggs were purchased from Avian Disease Diagnostic laboratory (Nandanam) were used for primary CEL culture as well as egg inoculation. The eggs are candled and sterilized properly. The egg inoculation technique was carried out based on method described by Shane *et al.*, 1996. Eight days old specific pathogen free eggs were used for propagation of virus.0.5 ml of filtered FAV4 positive liver homogenate was inoculated through yolk sac and CAM route. Resulted in stunted growth of and death of embryo and hemorrhage was noticed after 3 day of post infection in CAM route. Then the fluid was harvested by sterile syringe.

Chicken embryo primary culture

The primary chicken embryo liver culture were prepared based on the method described by Naeem *et al.* (1995a) [3] with slight modification. Liver was isolated and washed with medium and liver tissues homogenized properly and trypsinized with 0.1% trypsin.

The tissues were filtered and centrifuged at 1200rpm for 10min.The supernatant was discarded and the pellet was resuspended in medium. Then cell suspension was equally distributed in the culture flask finally 10% serum was added in all flask and the flasks were viewed under inverted microscope. The flasks were kept in CO_2 incubator at 37 ºC until complete monolayer formation.

Isolation of FAV4 from infected culture

Once complete monolayer formation the CEL were infected with 0.5 ml of positive FAV4 samples as inoculum. The infected culture were incubated in CO_2 incubator at 37 ºC. Cytopathic changes were observed in 3-4 days of post infection.

Tissue culture infective dose 50% end point ($TCID_{50}$)

$TCID_{50}$ was carried out for the positive FAV4 and known positive samples after third passage as per the methods described by Naeem *et al.* (1995a) [3]. The CEL seeded in 96 well microtitre plate at the concentration of $2X10^6$ cells / ml. Then 100 µl of cell suspension were added to each well in the microtitre plate and incubated at 37 ºC until the formation of complete monolayer. Serial 10 fold dilution of FAV4 was prepared in maintenance medium. Each dilution of virus was added in triplicate wells. The infected and uninfected control were maintained for assessing the infectivity of the virus. The culture were examined for characteristic CPE and the 50% tissue culture infective dose were calculated as per standard procedure (Reed and Muench, 1938) [11].

Determination of physic chemical properties

Sensitivity to chloroform

1 ml of viral fluid was added with 0.5 ml of chloroform (Merck, Germany) and shaken for 10 min at room temperature. After centrifuged at 2,000 rpm for 10 min, the supernatant was ten-fold diluted with M199 and cultured in CEL cells.

Sensitivity to ether

1 ml of viral fluid was mixed with 0.2 ml ethyl ether and incubated at 4 $^{\circ}$C for 18 h. Then it was poured on to a petri dish and kept at room temperature for 20 min to evaporate the ether. It was ten-fold diluted and cultured in CEL cells.

Sensitivity to ethanol

Each 1 ml of viral fluid was mixed with 0.5 ml of 50%, 70% and 100% ethanol (Merck, Germany), respectively. Then they were incubated at room temperature for 1 h. After poured onto a Petridis and kept at room temperature for 20 min to evaporate the ether. Then the sample was ten-fold diluted and cultured in CEL cells.

Sensitivity to formalin

Formaldehyde (40%) was diluted to 1:2000 with distilled water and mixed with an equal volume of viral fluid. After incubation at 37 °C for 17 h, the mixture was ten-fold diluted and cultured in CEL cells.

Sensitivity to trypsin

0.25% trypsin was prepared by using distilled water and viral fluid was diluted to 1:10 with PBS. Equal volumes of virus and trypsin were mixed and incubated at 37 °C for 30 min, followed by cooling on ice for 5 min. The mixture was ten-fold diluted and cultured in CEL cells.

Sensitivity to heat

Viral fluid was ten-fold diluted with M1199 and incubated for 30 min at 50 °C, 52 °C, 54 °C, 56 °C, 60 °C. Each of the diluted virus was cultured in CEL cells.

Sensitivity to $MgCl_2$

Ten times diluted viral fluid was mixed with equal volume of 2M $MgCl_2$ and incubated at 50 ºC for 1 h, then cooled for 5 min. After it was cultured in CEL cells.

Analysis of polypeptides of FAV4

To analysis of polypeptides of FAV4 the purified virus was subjected to sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

Isolation of FAV4

PCR positive liver homogenates were cultured in CEL cells. Visible cytopathic effect like nuclear degeneration, rounding, ballooning and cluster notice after 48 hrs of post infection and complete detachment noticed on 3rd day of post inoculation. The viruses were harvested and further passaged, after 3rd passage, the titres of the virus isolate was $10^{6.3}$ $TCID_{50}$/ml. (Table 1).

The sensitivity of the virus to various chemical agents like ethanol, chloroform, ether, acidic condition, trypsin, $Mgcl_2$, heat were assessed in chicken embryo liver culture. The virus ($10^{6.5}$ $TCID_{50}$/ml) was stable in chloroform, ether,55-70% ethanol, acidic condition at P^H 3, 0.25% trypsin and 50 °C heat for 30 min but labile to 100% ethanol, heat at 60 ºC for 30min, 1 M $Mgcl_2$ at 55 ^{0}C to the virus titre reduced ($10^{4.6}$ to $10^{1.0}$ $TCID_{50}$) means, virus completely labile to heat at 56 ºC for 30 min, 1 M $Mgcl_2$ 50 ºC for 30 min, 1:2000 formalin 37 ºC for15 hours (Table 2).

Discussion

Fowl adenovirus were propagated in 8-11 days old specific pathogen free embryonated eggs through yolk sac and CAM route. Yolk sac route causes early death of embryo than CAM route and virus propagation also higher in yolk sac route and this antigen shows high titre 10^{-8} comparatively CEL which is showing 10^{-6} titre value. Egg inoculation is one among the best method for propagation of FAV4. Cell culture provide an useful alternative system for virus propagation and adaptation. Where in both the transmission of avian pathogens as well as interference of maternal antibodies in embryonated egg could be avoided. In the present study various FAV isolates were propagated in primary chicken embryo liver culture as it has greater sensitivity than chicken embryo kidney culture. However, care should be taken before using cell culture adopted virus strains as candidates virus for vaccine production. The present study has been aimed at adopting FAV to CEL culture (primary culture and studding its pathogenesity by biological and molecular methods. In this study, the CPE produced were more marked after 5-6th passages. Cytopathic effect due to the growth of CEL adopted FAV was observed from 24-76 hrs of post infection. Clumping, rounding and fusion of cells were observed during early stages followed by shrinkage of monolayer spaces and formation of inclusion bodies from the cells as reported earlier. As cytopathic effect advances, multinucleated giant cells and vaculation in the cytoplasm often observed compared with normal uninfected cells. In present study, physical characterization of FAV 4 was done at different temperatures and different ranges of P^H and the virus was stable in 50-70% chloroform, 50-70% ether, 0.25% trysin, heat at 50 ^oC but labile to 100% ethanol, acidic condition at $3p^H$ and heat at 52 ^oC, 1M of $Mgcl_2$ at 50 ^oC for 1 hour, 1:2000 formalin at 37 ^oC with the $TCID_{50}$ 50 ^oC for 15 hours. The no enveloped FAV 4 was not sensitive to lipolytic agents such as chloroform and ether and virus was not inactivated by them similar to the finding of Afzal *et al*. (1991) [1].

Control on viral diseases is importance than the treatment after an outbreak. The information on the physicochemical characterization of a virus can help in prevention of mechanical and horizontal spread of virus. In this present study attempts were made to know the physicochemical characters of FAV4 which may help for its proper management through biosecurity measures. Studying the biological and physicochemical properties of FAV4 will help to characterization of Different types FAV4 isolates and selection of suitable disinfectant for controlling the spread of infection.

Reed and Muench formula

Proportional distance = % above 50%-50

---------- = 85-50

(% above 50-%below 50%)

$=10^{-6.89}$

Table 1: Calculation of $TCID_{50}$ of fowl adeno virus serotype 4

Dilution	No of wells infected	No. of wells		Cumulative index		Percentage of infection
		With CPE	Without CPE	With CPE	Without CPE	
10^{-1}	7	7	0	44	0	100
10^{-2}	7	7	0	37	0	100
10^{-3}	7	7	0	30	0	100
10^{-4}	7	6	1	23	1	96
10^{-5}	7	5	2	17	3	85
10^{-6}	7	5	2	12	5	71
10^{-7}	7	4	3	7	8	47
10^{-8}	7	3	4	3	12	20

Table 2: Determination of Physio chemical properties of Fowl adeno virus serotype 4

S. No	Physicochemical treatment	Virus titer($TCID_{50}$)After treatment	Properties of isolates
1	Chloroform at 37 ^oC for 10min	$10^{6.6}$	Stable
2	50% ether at 4 ^OC for 15 hrs	$10^{6.4}$	Stable
3	70% ether at 4 ^OC for 15 hrs	$10^{6.5}$	Stable
4	100% ether at 4 ^OC for 15 hrs	$10^{4.3}$	Labile
5	Acidic condition at 3 P^H at 37 ^oC for 1 hr	$10^{6.5}$	Stable
6	Trypsin 0.25% for 37 ^oC for one hour	$10^{6.4}$	Stable
7	Heat at 50 ^oC for 30 min	$10^{6.5}$	Stable
8	Heat at 52 ^oC for 30 min.	$10^{4.0}$	Labile
9	Heat at 54 ^oC for 30 min.	$10^{3.0}$	Labile
10	Heat at 56 ^oC for 30 min.	$10^{1.0}$	Labile
11	1 M $Mgcl_2$ 50 ^oC for 30 min.	$<10^{1.0}$	Labile
12	1:2000 formalin 37 ^oC for15 hours	$<10^{1.0}$	Labile

Conclusion

Hydropericardium Hepatitis Syndrome (HPS) is a rapidly emerging, highly fatal disease in poultry, with all strains equally susceptible to fowl adenovirus infection. Initially identified in Pakistan, the disease subsequently spread to India, where it was colloquially termed “leechy” or “litchi disease” due to the appearance of a heart in pericardial fluid. HPS is mainly transmitted horizontally, and experimental infection can be induced via various inoculation methods. Outbreaks are often associated with high levels of aflatoxins in feed and may be exacerbated by co-infections with other viruses. Due to the virus's resilience to certain chemical agents and conditions, the study of its physicochemical

properties is essential for effective biosecurity and control measures in poultry management.

Conflict of interest: Not interested

References

1. Afzal M, Muneer R, Sterin G. Studies on the etiology of *hydropericardium syndromic* Angara disease in broilers. Vet Rec. 1991;128:591-593.
2. Chandra R, Shukla ST, Kumar M, Garg SK. Electron microscopic demonstration of an adenovirus in the hepatocytes of birds experimentally infected with *hydropericardium syndrome*. Vet Rec. 1997;140:70-71.
3. Naeem K, Rabbani M, Hussain M, Cheema AH. Development of cell culture vaccine against HPS in poultry. Pak Vet J. 1995;15:150-151.
4. Jaffery MS. A treatise on Angora disease (hydropericardium pulmonary edema-*hepatohephritis syndrome*). J Pak Vet Med Assoc. 1988;34:1-38.
5. Hasan SA, Afzal M, Hameed A, Khan AR. Immune response to inactivated *hydropericardium syndrome* vaccine in broilers. Pak Vet J. 1993;14:5-10.
6. Gowda RNS, Satyanarayana ML. *Hydropericardium syndrome* in poultry. Vet Pathol. 1994;18:159-161.
7. Gowda R, *et al*. Death of embryo within 4-7 days after post-infection. Shane S, *et al*. Transmission of virus mainly through horizontal spread among broilers. Avian Pathol; c1999.
8. Akhtar M, *et al*. Transmission of virus mainly through horizontal among broilers. Indian Vet J; c1992.
9. Hasan R, *et al*. Experimental transmission of the disease through intramuscular, subcutaneous, and oral inoculation of infected liver homogenate. Indian J Poult Sci; c1995.
10. Singh R, *et al*. Presence of aflatoxin in feed at high concentration associated with outbreaks among broilers. Indian J Anim Sci; c1994.
11. Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. Am J Epidemiol. 1938;27(3):493-7.

How to Cite This Article
Kalaiselvi G, Parthiban M, Balakrishnan G, Ramya R, Jaisree S, Soundararajan C. Physico chemical characterization of fowl adenovirus serotype 4 isolated from Tamil Nadu. International Journal of Veterinary Sciences and Animal Husbandry. 2024;SP-9(5):390-393.