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HYDROPERICARDIUM IN BROILER CHICKENS IN IRAQ : ISOLATION AND IDENTIFICATION OF THE CAUSATIVE VIRUS

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SUMMARY

An adenovirus was successfully isolated from infected liver, spleen and pericardial fluid from infected broiler chicks in primary cell cultures prepared from chicken embryo liver and kidneys. The isolated virus induced cytopathogenic effect (CPE) in infected cell cultures which consisted of cell granulation, aggregation and rounding of infectedd cells. An adenovirus like morphology was identified by Electron microscope examination. In addition the isolated virus was diagnosed, by agar gel diffusion test (AGD). Indirect immune fluorescenct test (IFA) and serum neutralization (SN). Using reference hyperimmune serum and sera from recovered chicks.

INTRODUCTION

Hydropericardium syndrome or angara disease an apparently new disease, has caused large economic losses to the broiler industry (1). The disease was first observed in broiler growing area near Angara Goth, Karachi, Pakistan in August 1987 (2), and it spread rapidly to broiler production units throughout the country within a year period (3).

The disease has typically been seen in 3-6 week old growing broiler chickens and result in up to 60 percent mortality (4).

The disease can be experimentally reproduced by inoculation of a bacteria-free liver homogenate from infected birds (5).

By Electron microscopy an adenovirus has been detected from infected liver (6).

The disease was first recognized in Iraq in 1989 and results of clinical manifestation and pathological finding were reported on experimentally infected chickens (7). The purpose of the present report was to isolate, propagate and identify the causative agent from infected chicks samples.

MATERIALS AND METHODS

Specimens for virus isolation :-

Samples of liver, spleen and pericardial fluid were collected from sick and dead chicken. The infected chicks characterized by accumulation of clear-straw-coloured fluid in the pericardial sac. and swollon, discoloured liver and enlarged kidneys. A 10 % tissue suspension was made in phosphate buffer saline of pH 7.2. The suspension was centrifuged at 2000 r.p.m. For 20 minutes. The supernatant was treated with a mixture of penicillin (500 I.U) and streptomycin (500 µg/ml) for 1 hour. The prepared samples were used for virus isolation and in AGD test.

Cell culture :

Different types of cell cultures were used for virus isolation, primary chicken embryo kidney, chicken embryo liver, sheep testis, calf kidney and vero cell line. The primary cell cultures were prepared by using 0.25 % trypsin and grown in minimum essential media (MEM), with 10 % feotal calf serum. When monolayer was

completed, the cells were inoculated with the prepared samples and adsorbed for 1 hour at 37 °C, then maintenance media containing 2 % calf serum was added. The cell cultures were examined daily for cytopathic effect (CPE).

Electron microscopic examination :

For EM examination the isolated virus third passage in chicken kidney were centrifugation 2 times at 1500 r.p.m for 1/2 hour at 4 °C to remove the cell debris. Then equal volume of the supernatant (10 ml) was treated with sera from recovered chick (Hydro pericardium syndrome sera) the mixture incubated at 4 °C for 24 hours. The virus - serum mixture was centrifuged at 25,000 r.p.m in cold centrifuged, for 1 hour the pellet were suspended in 0.1 ml of PBS. This material was mounted on carbon-formvar grids, stained with 4 percent sodium phosphotungstate pH 7.1 and examined in E.M.

Serology :

Reference avian adenovirus type 2,3, and type 5, antisera were supplied by spafas company CT.U.S.A. Also : sera collected from naturally infected and recovered chicks with hydropercardium syndrome were used in these serological studies.

1. Agar gel diffusion (AGD) test :

Suspension of liver from infected birds and the cell culture passage of the isolated virus were used as virus antigens against the reference adenovirus antisera and sera collected from recovered chicks. Nobel agar 1 % was prepared in 4 % sodium chloride was used in the test.

2. Virus neutralization test :

Microtiter technique was carried out in embryonic chicken kidney by applying two fold dilution of the antiserum against 100 TCD50 of the isolated virus.

3. Indirect immunoflourescent test :

Embryonic chicken kidney cell culture in leighton tube was inoculated with the 3rd passage of the isolated virus. At 24 hours and 48 hours post inoculation period, the cells were fixed with cold acetone (-10 °C) and were treated with reference avian adenovirus antisera and sera from recovered chicks, and then stained with rabbit antichickene gammaglobulin FITC conjugate (9).

Virus characterization:

1. Hemagglutination test :

One percent of readily washed chicken, sheep, horse, rabbits, rate, and human type O. red blood cells (RBCs) were used.

The test was done at 25 °C and using PBS (pH 7.2) for washing and dilution of the isolated virus.

2. Sensitivity to lipid solvent :

By following the methods described (10,11). Samples of the isolated virus was treated with 20 % ether for 18 hours and 40 % chloroform for 1 hours. Followed by titration of treated virus and untreated virus (control samples). In chicken embryonic kidney cell cultures.

Experimental infection of chicks :

Fifty broilers chicks of 3 weeks old were inoculated with 3rd passage of isolated virus (in chicken embryonic kidney cells). These chicks were inoculated intravenously by 0.5 ml or 100 TCD50 of the isolated virus. Samples from sick and died chicks (heart, pericardial fluid, spleen, liver, bursa of fabricus and blood) were collected at different times post infection from infected birds.

RESULTS

Virus isolation :

Cell cultures inoculated with suspension of the samples of liver, spleen and pericardial showed a foci of rounded cells with a number of degenerated flooting cells in tissue cultures media on 2nd day post infection (PI), the cytopathic effect was more clear, on 4th day PI in which a clusters of rounded cells were evident in addition to some large giant cells which resulted from fusion of infected cells.

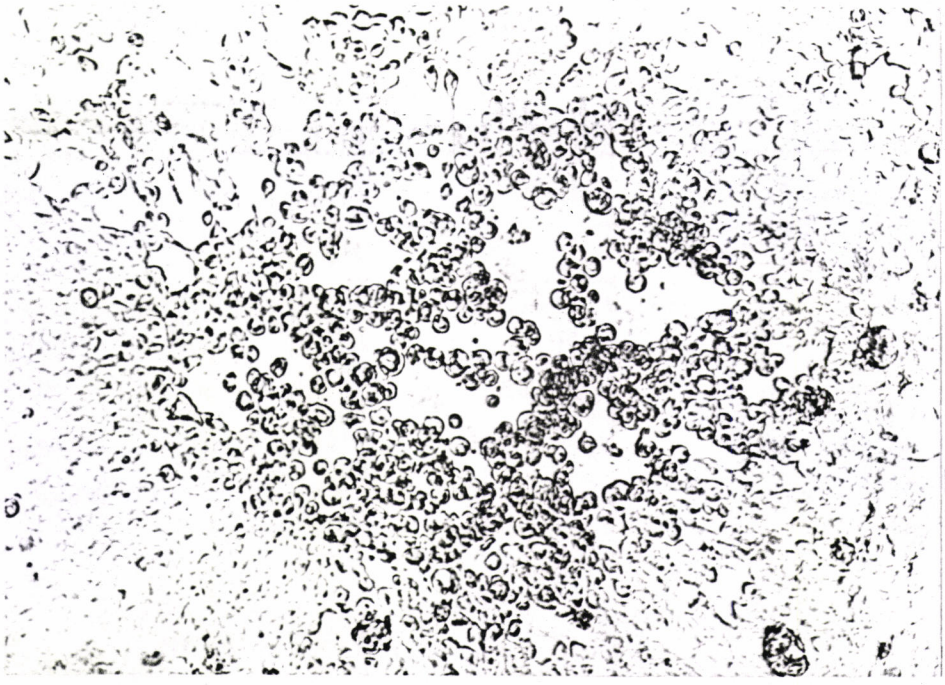

Fig. 1 : CPE adenovirus in Vero cell line after 48 hrs post inoculated.

The cytopathic effect were more clear and generalized in high percentage of cells in chicken embryo kidney and liver cell culture than other types of cell culture used in this study - (Table - 1).

Table-1:Isolation of adenovirus from hydropericardium infected chicks in different types cell cultures.

Cell culture	Growth of Virus
1- Chicken embryonic kidney cells	++++
2- Chicken embryonic liver cells	++++
3- Chicken embryonic fibroblast cells	+
4- Sheep testis cells	+
5- Secondary calf kidney cells	+
6- Vero cell line cells	+

(+) Positive result is less than 25 % CPE.
(++++) Positive result 100 % CPE.

Electron Microscopic examination :

Negative staining of pelleted virus from infected chicken kidney cells revealed raxed naked virus particles with cupical shape measuring 80 nm in diameter. A clumped adenovirus particles were also seen in infected tissue.

Fig 2 : Clumps of adenovirus particle in section of infected tissue by E.M.

Serological identification :-

1- Agar gel diffusion :

A clear two precipitin lines were detected between suspension of liver from infected chicks and tissue culture propagated viral antigen by using sera collected from recovered chicks after 24 hours period of incubation (Figure - 3).

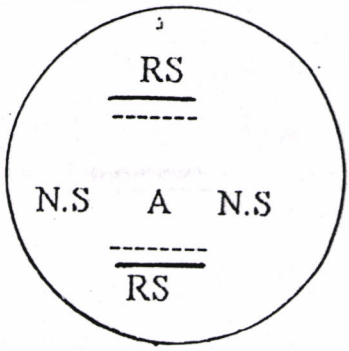

Fig.3 : Agar gel immunodiffusion reaction

A : Liver suspension (central well).
RS.: Sera from recovered birds with hydropericardium syndrom
N.S : Sera from noninfected birds.

Fine common precipitation line was seen with reference antisera type (2, 3, 5) when reacted with similar previous antigens (Figure - 4).

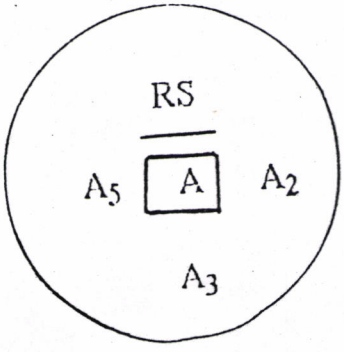

Fig. 4 : Agar gel immunodiffusion reaction

A : Tissue culture viral antigen or liver suspension (central well).
RS.: Sera from recovered birds with hydropericardium syndrom
A(2,3,5) : Reference Avian adenovirus sera type 2, 3, 5.

2- Virus neutralization :

The sera from recovered chicks neutralized the isolated virus with a titer 32. The reference sera (Avian adeno type A_2, A_3, A_5) could not neutralized the isolated virus.

3- Indirect immunoflourescent test :

A clear cytoplasmic and nuclear flourescine were observed in infected embryonic chicken kidney cells after 24 and 48 hours. (PI) by using recovered sera and reference antiserum. No specific immuno flourescence was detected by using normal chicken sera and non virus infected cell.

Virus characterization :

1- Haemagglutination activity :

The isolated virus agglutinated RBCs of rate only with titer 16. RBCs of other animal species and human type O were negative.

2- Sensitivity of lipid solvent :

The isolated virus was resistant to both ether and chloroform treatment no reduction in titer of the treated isolated virus with ether and chloroform were seen.

3- Experimental infection :

Bird inoculated with 3rd passage of the isolated virus in embryonic chicken kidney cell culture show typical lesion of hydropericardium syndrome after 2-5 days (PI). The clinical findings characterized by accumulation of clear - straw coloured fluid in the pericardial sac approximately 5 ml), swollen pale or discoloured liver, and pale enlarged kidneys. Other pathogenic changes in lung, intestine, spleen could be seen. The virus was

reisolated from samples collected from organs of infected and dead birds (Table - 2).

Table - 2 : Results of experimental infection in broiler chikcs and reisolation of the virus from infected chicks.

Organ	Virus isolation in chicken kidney cell culture
Pericardial fluid	+
Heart	+
Spleen	+
Liver	+
Kidney	+
Bursa	-
Blood	-

(+) Positive result.
(-) Negative result.

DISCUSSION

The hydropericardium syndrome was initially believed to be a nutritional disorder (4). However the reproduction of the disease by the inoculation of a liver homogenate and development of successful vaccine have demonstrated that the disease is infectious in nature (5).

In our study is considered in country the first isolation and propagation of causative agent of hydropericardium syndrome disease in cell culture and also identification of isolated virus by electron microscopy and serological tests using reference sera and sera from recovered birds.

Also it is proved that the isolated virus from hydropericardium syndrome share a common antigen with avian adenovirus by using reference adenovirus antisera (types 2, 3, and 5) in AGD test.

However another precipitin antigen detected in the isolated virus or in liver extract of infected bird by using sera collected from hydropericardium recovered chicks.

This indicates that our isolated virus differ antigenically from avian adenoviruses as was more distinct by serum neutralization test in which the references avian adenoviruses antisera did not neutralize the hydropericardium virus in contact to the sera collected from chicks recovered from hydropericardim syndrome.

The present study also indicates the causative of hydropericardium syndrome in broiler chicks in Iraq is an adenovirus as it is indicated by E.M. examination, as other worker (5,6).

Beside that the isolated virus was the only agent responsible for reproduction of the disease upon experimental inoculation of susceptible broiler chicks, as in other study (7). Ether and chloroform treatment, which does not reduce infectivity of the virus which prove the agent is naked virus beside haemagglutinate the RBCs only.

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عزل وتشخيص الفايروس المسبب لمتلازمة موه التامور في دجاج اللحم في العراق

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الخلاصة

عزل فايروس الادينو من الكبد ، الطحال وسائل الشغاف لطيور مصابة بمتلازمة موه التامور في خلايا الكبد والكلية لأجنة الدجاج.

وقد أظهر الفايروس المعزول تأثيرات مرضية في الخلايا المحقونة تميزت باستدارة وتحبب الخلايا مع تجمعها وتكوين ما يشبه الخلية العملاقة ، ولوحظ شكل فايروس الادينو بوسطة المجهر الالكتروني وتم تشخيص الفايروس بوسطة فحص الانتشار في الهلام والاستشعاع المناعي غير المباشر والتعادل المصلي باستعمال امصال ممنعة معروفة ومصل طيور مصابة في طور النقاهة.