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التهاب الكلى الفيروسي الناتج عن عترة تنتمي لفيروس التهاب الشعب المعدي

١ ـ عزل وتصنيف الفيروس

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لوحظ عدة أوبئة من التهاب الكلى في مزارع البياض في مصر في الفترة الاخيرة ، وقد سجل هذا المرض في قطيع عمره ٧٠ يوم يعاني من هزال شديد واسهال شديد مائي وشلل غير كامل ونسب نافق عاليه وكانت أهم التغيرات التشريحية في معظم الحالات التي فحصت كالتالي :

- التهاب الكلى الشديد ، اتساع الحالبين ، ضمور في الكليتين
- التهاب في القصبة الهوائية لبعض الحالات ٠

وقد عزل المسبب في أجنة بيض الدجاج ونتج عنها تكور وصغر الحجم بعد عدة تمريرات٠

- عند دراسة الصفات الطبيعية والكيميائية للفيروس المعزول وجد أنه حساس للكلورفورم (٢٥٪) كذلك حساسية لدرجة حرارة ٥٦م^{٥} لمدة ساعة ، كما أعطى نتيجة سلبية مع اختبار التلازن مع كرات الدم الحمراء للدجاج ، الحصان، الاغنام ، الفئران البيضاء ٠
- أما عن صفاته السيرولوجيه باستخدام اختبار الترسب في الاجار أثبت الفيروس المعزول علاقة وفيروس التهاب الشعب المعدي ، ولكن عند استخدام اختبار التعادل العكسي أثبت اختلافه عن فيروس التهاب الشعب المعدي عند اجراء العدوى الصناعية بالحقن داخل الغشاء البريتوني لكتاكيت سن يوم ، ٣٠ يوم ، ٦٠ يوم نتجت أعراض وصفات تشريحية مشابهه تماما لتلك التي لوحظت على الحالات الاصلية والتي عزل منها الفيروس
- وكذا تم اعادة عزل الفيروس من كلى الكتاكيت المعدية صناعيا من هذه النتائج يمكن تصنيف هذا الفيروس المعزول على أنه ينتمي لفيروس التهاب الشعب المعدي ولكنه عترة مختلفه ذات قابلية لخلايا الكلى ٠

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VIRAL NEPHRITIS INDUCED BY AN ISOLANT RELATED TO INFECTIOUS BRONCHITIS VIRUS. I:ISOLATION AND IDENTIFICATION OF THE ISOLANT

(With 3 tables & 3 Figs.)

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SUMMARY

Several outbreaks of nephritis were noticed in laying flocks in Egypt. The disease reported in a flock aging 70 days old suffering from severe emaciation, profuse whitich diarrhoea, paresis and high mortality. The predominant gross lesions in the most examined cases were severe nephritis, dilitation of ureters and atrophy of the kidneys, some cases showed catarrhal trachitis.

The causal agent was isolated, produced curling, dwarfing and deaths of infected chicken embryos after several passages.

The physico-chemical proparaties of the isolant revealed that the isolant viral agent sensitive to chloroform (25%) and sensitive to 56°C for one hour. The isolant failed to agglutinate chicken, horse, sheep and rat erythrocytes. Serological studies by agar gel preaipitation test revealed its relationship to infectious bronchitis virus, by cross neutralization test revealed its variation than infectious bronchitis virus.

Intraoperitanial infection of one day, 30 days and 60 days old chicks with the isolant gave the typical symptoms, lesions and also the reisolation of the isolant from the affected kidneys. From the above properaties of the isolant suggested its tentative grouping as infectious bronchitis (variant strain of renal tropism).

INTRODUCTION

Some strains of infectious bronchitis virus are the cause of a wide spread disease syndrome known as "Infectious uremia", viral nephritis and nephritis nephrosis syndrome. A number of strains of virus including the Australian "T" strain, Holte, Gray and GM_2 were subsequently isolated from infected fowls (CUMING, 1962; 1963; WINTERFIELD and HITCHNER, 1962 and RINALDI, et al. 1966).

Severity of renal lesions induced by infectious bronchitis virus varied according to the strain (MACDONALD and MACMARTIN, 1976; ALEXANDER, et al. 1978 and MCDONALD, 1980).

The nephropathogenic strains of infectious bronchitis virus are sensitive to ether, chloroform and inactivated after heating at 56°C for 45 minutes (SHINAKURA and HIRAI, 1970). CHUBB,

et al. (1976) showed that the nephropathogenic strains were serologically distincit from infectious bronchitis virus.

In Egypt, the existance of infectious bronchitis was first reported by AHMED (1954) and later confirned by findings of EISSA, et al. (1963) as well as AHMED (1964). In serological studies on respiratory affections of poultry AHMED, et al. (1968) screened 11 adult chicken flocks in various localities for infectious bronchitis precipitating antibodies. Evidence of infectious bronchitis infection was found in all flocks, and the incidence of precipitating antibodies varied between 2.0 and 35% and averaged 11.0%, SALAMA (1976) reported 11.8% positive reactors in using agar gel precipitation test for serological testing of chicken sera in Sharkia province. Moreover, AMER (1984) used the AGP-test for screening sera from 27 chicken flocks for infectious bronchitis virus infection and reported that positive reactors were varied between 12.5% and 64.3% and total incidence fo 25.9%.

Trials to isolate the virus were unsuccessfull, till the first successfull virus isolation by AMIN and MOOUSTAGEER (1977) who isolated a strain of infectious bronchitis virus "Dokki strain" which was involved in an outbreak of uremia in broilers.

The situation of viral nephritis infection of chickens in Egypt is still in need for several investigations and our present trial is one in this way.

MATERIAL and METHODS

1- Embryonated chicken eggs:Commercial fertile chicken eggs were used in this study.

2- Virus strains:One isolant recovered from the kidneys of morbid chickens, was used in this stuyd. In addition to the infectious bronchitis virus (Beadutte strain).

3- Antisera: Antisera against the isolant were prepared by initial intra-peritonial inoculation of infective allanto-ammiotic fluids and membranes (1ml/rabbit) into 4 adult rabbits followed on the 7th. day and 10th. day by 2 injections indoses of ml and 4 ml. respectively. Four and eleven days after the last injection, the rabbits were bled and sera were separated. Antisera against infectious bronchitis virus was used.

4- Heat stability: Samples of the isolant was subjected to a temperature 56°C for one hour in a water bath. An additional sample left at room temperature and served as control. Both the treated and control samples were checked by titeration in embryonated chicken eggs, (HESS and DARDIRI, 1968).

5- Chloroform resistance: Sample of the isolant was treated with chloroform (25%) at the ratio fo 3:1. Treated and untreated isolant were titerated in embryonated chicken eggs, as described by FELDMAN and WANG (1961).

6- Hemagglutination (HA) activity: Samples of the isolated virus were tested for HA activity against chicken, horse, sheep and rat erythrocytes by plate method except rat erythrocytes in tubes according to ANON, 1971.

7- Agar gel precepitation test: The isolant was tested against homologus precipitatin antisera and antisera against infectious bronchitis virus using the method ofWOERNLE (1959).

8- Cross neutralization test: Serial ten fold dilutions of the isolant and infectious bronchitis virus were mixed with constant amount of the homologus and heterologus antisera, incubated for one hour, then titerated in embryonsted eggs.

The EID_{50} was calculated according to REED and MUENCH (1938).

9- Pathogenecity to Embryonated chicken eggs: The isolant was inoculated into the allantoic sac of 9-10 days old embryonated chicken eggs for serial passages and studing the mortality and pattern of deaths as well as the pathological changes.

10- Pathogenecity of the isolant for chicks: Ninety chicks were inoculated intraperitonially with 0.2 ml of infective allantoamniotic fluids and membranes containing the isolant at the 1st, 30th, and 60th days old; 30 chicks for each age. Symptoms and/or deaths were recorded daily for 4 weeks. At 3,5,7,10,14,21 and 28 days postinoculation, two birds were sacrified from each group, sera were collected for the agar gel precipitation teasting and the internal organs were examined for gross lesions Samples from the Kidneys also taken for virus reisolation. Moreover, 60 chicks were kept as control; 20 chicks for each age; from which 2 birds were sacrified at intervals simillar to the infected ones.

RESULTS

1- A viral agent was isolated from the kidneys of 70 days old replacement laying chicken suffering from severe emaciation, profuse whitich diarrhoea, paresis and mortality rate reach 30%. The Kidneys of diseased birds from which the virus was isolated showed severe nephritis, dilitation of ureters with urates and atrophy of the kidneys as shown in Fig. 1.

2- The isolated strain was found to be thermolabile to 56°C for one hour, sensitive to chloroform as shown in table 1, and failed to agglutinate chicken, horse, sheep and rat erythrocytes.

3- The isolated strain gave positive precipitation reaction with both locally prepared and standard infectious bronchitis antisera. The isolated strain was neutralized by locally prepared (Homologus) antisera and not by infectious bronchtis (Heterologus) antisera, as shown in table 2.

4- The embryonic mortality increased parallel to the increase in the number of viral passage in embryonated chicken eggs, till reach 70% in the 7th. passage, (Table 3). The dead infected embryos showed curling and dwarfing from the 3rd passage, as shown in fig.2.

5- Intraperitonial inoculation of the isolant into one day-old chichs resulted in neither symptoms nor mortality. Infection of 30 and 60 days old chicks resulted in typical symptoms and lesions as observed on naturally infected birds and from the affected kidneys the viral agent was reisolated, (Fig. 3). The Control non infected group was negative for virus isolation, symptoms, lesions and serological examination.

DISCUSSION

The kidney lesion that has tentatively been named "Urolithiasis" or viral nephritis increased among laying flocks in the last years (RANDALL, et al. 1977; BLAXAND, et al. 1980 and SILLER, 1981).

In prescent investigation, an viral agent was isolated from the kidneys of a flock suffering from high mortality, severe emaciation, profuse whitich diarrhoea and paralysis. The isolated

strain gave the typical symptoms and lesions in experimentally infected chicks. The age of the affected flock was 70 days, this agree with MACDONALD (1980) and disagreed with CUMING (1962) and WINTERFIELD and ALBAASSAM (1983) which reported the age resistance of chickens to this virus infection more than 4 or 5 weeks.

The physico-chemical and biological properaties of the isolated suggested its grouping as infectious bronchitis virus, but the serological studies proved its variation and this agreed with SHIMAKURA and HIRAI (1970) and CHUBB, et al. (1976).

Further studies on the pathogenecity of the isolated virus to chickens of different ages and by different routs and the histopathological changes of naturally and experimentally infected chickens kidneys will be needed. Our study can be considered as one of the first trial to study this viral infection in Egypt.

REFERENCES

Ahmed, N.N. (1954): Incidence and treatment of some infectious viral respiratory diseases of poultry in Egypt. D.V. Thesis, Facult. of Vet. Med., Cairo Univ.

Ahmed, A.A.S. (1964): Infektiose Bronchitis des Huhnes in Agypten. BMTW., 77, 487.

Ahmed, A.A.S.; El-Sisi, M.A.; Abbasi, K.H.; Saber, M. and Reda, I.M. (1968): Further studies on respiratory infections of poultry in Egypt. J. Vet. Sci. U.A.R. 5, 85.

Alexander, D.J.; Gough, R.E. and Pattison, M. (1978): A long-term study of the pathogenesis of infection of fowls with three strains of infectious bronchitis virus. Res. Vet. Sci. 24, 228.

Amer, M.M. (1984): Evaluation of emergency vaccination in case of outbreaks of Newcastle disease. Ph.D. Thesis, Poult. Dis., Facult. Vet. Med., Cairo Univ.

Amin, A.A. and Moustageer, M. (1977): A preliminary report on an avian infectious bronchitis virus strain associated with nephritis nephrosis syndrom in chickens. J. Egypt. Vet. Med. Ass.

Anon. (1971): Methods of examining poultry biologics and for identifying and quantifying avian pathgens. National Academy of Science, Washington, D.C.

Blaxland, T.D.; Borland, E.D.; Siller, W.G. and Martindale, L. (1980): An investigation of urolithiasis in two flocks of laying fowls. Av. Pathol. 9,5.

Chubb, R.C.; Wells, B.A. and Cuming, R.B. (1976): Some immunological aspects of a recent Australian isolate of infectious bronchitis virus. Australian Vet. J. 52 (8) 378.

Cuming, R.B. (1962): The aetiology of "Uremia" of chickens. Australian Vet. J. 38 (11) 554.

Cumining, R.B. (1963): The isolation of a virus from "Uremia" infected chickens. Australian J. Sci. 25 (7) 314.

Cunningham, H.C. (1966): A laboratory guide in virology. 6th. ed. Burdess Publishing Co., Minneapolis.

Eissa, Y.M.; Zaher, A. and Nafai, E. (1963): Studies on respiratory diseases. Isolation of infectious bronchitis virus. J. Arab. Vet. Med. Ass. 23, 381.

Feldman, W.A. and Wang, S.S. (1961): Sensitivity of various viruses to chloroform. Proc. Soc. Exp. Biol. (N.Y.) 106, 736.

Hess, W.R. and Dardiri, A.H. (1968): Some properaties of the virus of duck plaque. Archiv für die Gasamte Virus Furschung, 24, 148.

Macdonald, J.W. and McMartin, D.A. (1976): Observations on the effect of the H52 and H120 vaccine straines of infectious bronchitis virus in the domestic fowl. Av. Pathol. 5, 157.

Macdonald, J.W.; Randall, C.J. and MacMartin, D.A. (1980): An inverse age resistance of chicken kidneys to infectious bronchitis virus. Av. Pathol. 9, 245.

Randall, C.J.; Blandford, T.B.; Borland, E.D.; Broksbank, N.H.; Hall, S.A.; Herbert, G.N. and Richards, S.R. (1977): A survey of mortality in 15 caged laying flocks. Av. Pathol. 6, 149.

Reed, L.J. and Muench, H. (1938): A simple method for estimating fifty percent end point. Amer. J. Hyg. 27, 493.

Rinaldi, A.; Crepi, A.; Cervio, E. and Mondelli, G. (1966): Isolamento di un cepps nefropathogens del verus della bronchite infettiva del pollo. Selezione Veterinaria 7, 284.

Salama, A.B.El-R. (1976): Incidence of infectious bronchitis in chickens in Sharkia province. M.V.Sc. Thesis, Facult. Vet. Med., Zagazig Univ.

Shimakura, S. and Hirai, K. (1971): Incidence of avian nephrosis in Japan with special reference to isolation and transmssion tests of infective agent. Jap. J. Vet. Sci., 33, 206.

Siller, W.G. (1981): Renal pathology of the fowl. A review. Av. Pathol. 10, 187.

Winterfield, R.W. and Albassam, M.A. (1984): Nephropathogenecity of infectious bronchitis virus. Poult. Sci., 63 (12) 2358.

Winterfield, R.W. and Hitchner, S.B. (1962): Etiology of an infectious nephritis-nephrosis syndrome of chickens. Amer. J. Vet. Res., 97, 1273.

Woernle, H. (1959): Diagnose der Infektiosen Bronchitis der Huhner mit Hilfe der Prazipitationsreaktion in festen Agarmedium. Mh. Tierheilk, 11, 154.

Table (1)

Effect of heat and chloroform on the isolated virus strain

Treatment	Virus infectivity (EID_{50}) Log_{10}
Heat 56°C for 1 hour	1.4
Chloroform 25%	1.8
Untrated control	5.2

Table (2)

Cross Neutralization test between homologus and heterologus reaction

Virus	antisera against	EID_{50} (Log_{10})	index
Isolated strain	-	4.8	0
Isolated strain	isolated strain	2.6	2.2
Isolated strain	IB virus	4.1	0.7
IB virus	-	5.4	0
IB virus	isolated strain	5.2	0.2
IB virus	IB virus	2.7	2.5

Table (3)

Pattern of embryonic deaths and mortality rate of chicken embryos during the serial passage of the isolated nephritis virus

Passage No.	No. of chicken embryos	Death Pattern Days post inoculation 1	2	3	4	5	6	7	8	9	10	Total deaths	Mortality Rate
1	30	-	-	-	-	-	-	-	-	-	-	0	0.0
2	30	-	-	-	1	-	-	-	1	-	-	2	6.6
3	30	-	-	1	1	-	-	2	-	1	-	5	16.6
4	30	-	-	1	2	3	-	1	1	-	-	8	26.6
5	30	-	-	2	4	-	1	1	2	1	1	12	40.0
6	30	-	2	1	3	3	1	2	3	-	-	15	50.0
7	30	-	4	4	5	3	4	1	-	-	-	21	70.0

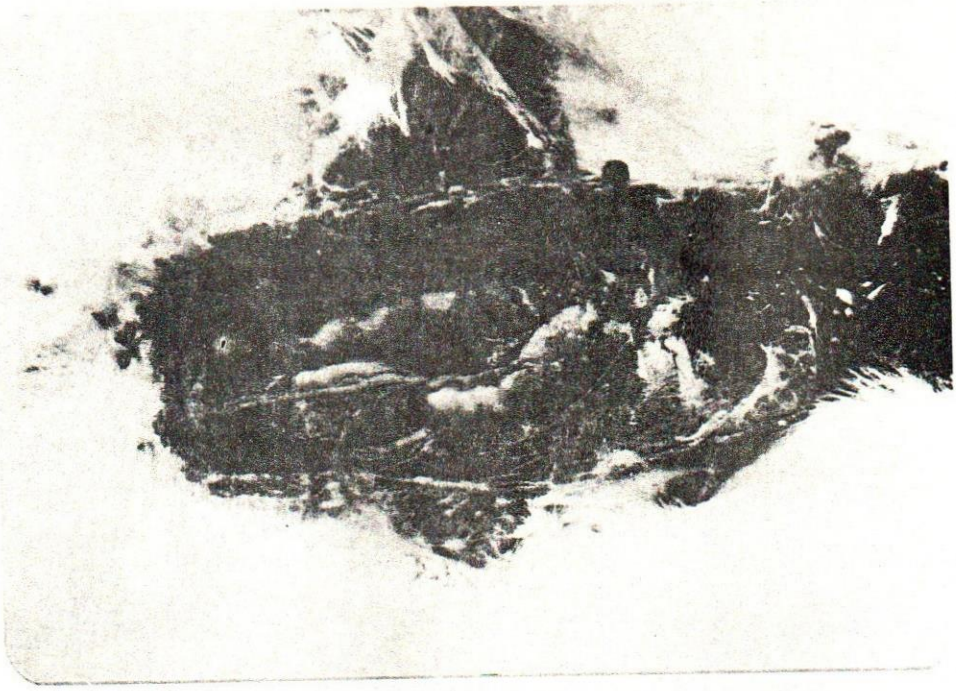

Fig. (1): Severe nephritis, dilatation of ureters and atrophy of kidneys of naturally infected birds

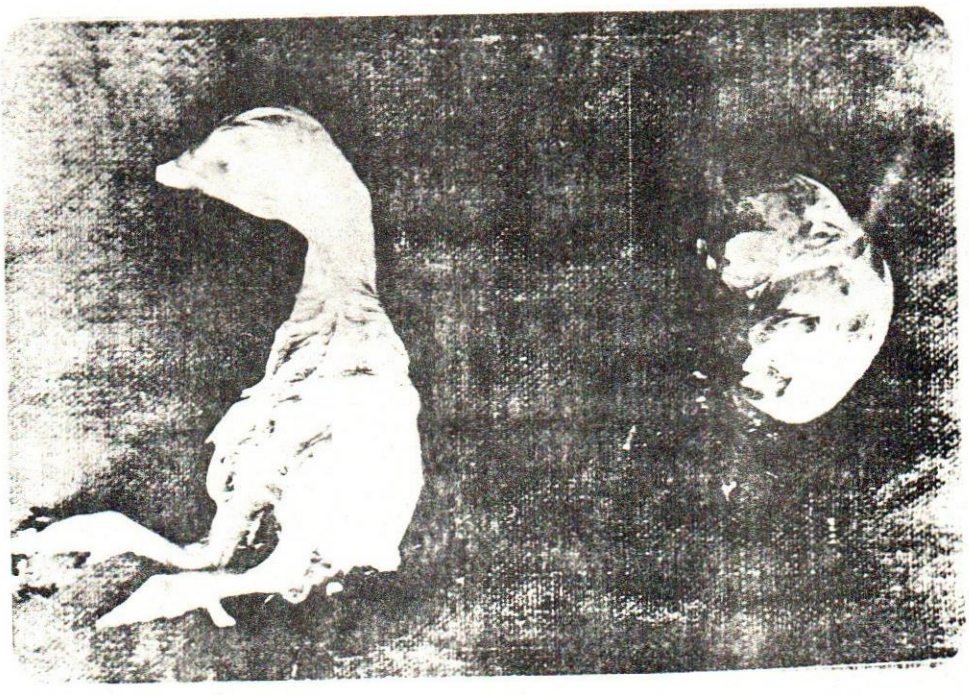

Fig. (2): Curling and dwarfing of infected chicken embryo (right) as compared with non-infected one of the same age (left)

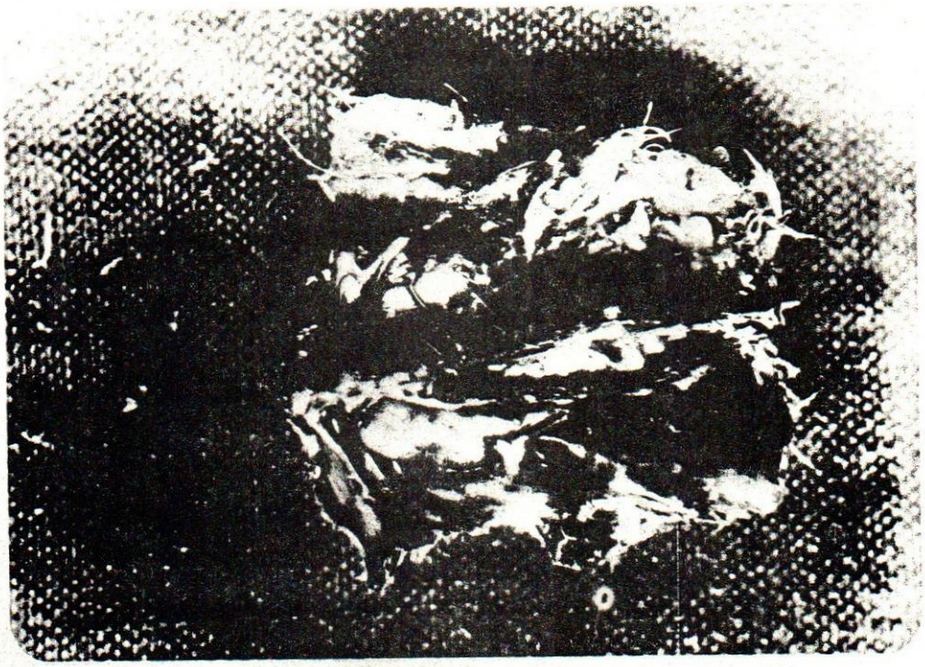

Fig. (3): Nephritis of experimentally infected birds with the isolated viral agent