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التهاب الكلى الفيروسي الناتج عن مرض غدي في فئة الفيروس التهاب الشعب المعدى

1- عزل وتصنيف الفيروس

مصطفى البستاني، محرس عام، أحمد حمودة

لملاحظة أوبئة من التهاب الكلى في زراع البيض في مصر في الفترة الأخيرة، وقد
سجل هذا المرض في قطيع صغير 70 يوم بعثاني من زوايا جديرة واسع شديد مائي وخلل غير
كامل ونسب ناتج عليها وكانت أهم الفترات التشريحي في معظم الحالات التي فحصت

كالتالي:

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

ونقد عزل المسبب في أجنحة بيج الدجاج ونتيجة تكوري وصغر الحجم بعد عدة تمريرات.

- عند دراسة الصفات الطبيعية والكيميائية للفيروس المعزول، وجد أنه حساس للكلورفوم
  (54%) وذلك حساسية لدرجة حرارة 60 مئوية سنة، كما أعطى نتيجة سلبية مسع
  اختبار التلوية مع كرات الدم الحمراء للدجاج، الحمام، الأغنام، النعوم البيضاء.

- أما عن صفاته البيولوجية باستخدام اختبار التربس في الأجار أثبت الفيروس المعزول
  علاقة فيروس التهاب الشعب المعدى، ولكن عند استخدام اختبار التعادل العكسي
  أثبت اختلافه عن فيروس التهاب الشعب المعدى عند إجراء العدوى الصناعية بالحقن
  داخل الغشاء البريتوني للكانكسيت سن يوم، 30 يوم ونرجع أعراض وصفات
  تشريحي مشابه تما مع تلك التي لوحظت على الحالات الأصلية، والتي عزل منها الفيروس

- وكذا تم إعادة عزل الفيروس من كل الكاتكسيت المعزول، من هذه النتائج يمكن
  تصنيف هذا الفيروس المعزول على أنه ينتمي لفيروس التهاب الشعب المعدى ولكنه
  عرض مختلف ذات قابلية لخلايا الكلى.
VIRAL NEPHRITIS INDUCED BY AN ISOLANT RELATED TO INFECTIOUS BRONCHITIS VIRUS. ISOLATION AND IDENTIFICATION OF THE ISOLANT
(With 3 tables & 3 Figs.)

By
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(Received at 5/4/1987)

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 whitish diarrhoea, paresis and high mortality. The predominant gross lesions in the most examined cases were severe nephritis, dilatation of ureters and atrophy of the kidneys, some cases showed catarhal trachitis.

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

The physico-chemical properties 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 preajitation test revealed its relationship to infectious bronchitis virus, by cross neutralization test revealed its variation than infectious bronchitis virus.

Intraocular 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 widespread disease syndrome known as "infectious uremia", viral nephritis and nephritis nephrosis syndrome. A number of strains of virus including the Australian "7" strain, Holte, Gray and GM, were subsequently isolated from infected fowls (CUMING, 1962; 1963; WINTERFIELD and HITCHNER, 1962 and RINALDI, et al. 1966).


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 distinct from infectious bronchitis virus.

In Egypt, the existence of infectious bronchitis was first reported by AHMED (1954) and later confirmed by findings of EISSERT, 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 successful virus isolation by AMIN and MOUSTAGEER (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 study. In addition to the infectious bronchitis virus (Beaudette strain).

3- Antisera: Antisera against the isolant were prepared by initial intra-peritoneal inoculation of infective allanto-amniotic 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 titration 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 titered 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 precipitation test: The isolant was tested against homologus precipitin antisera and antisera against infectious bronchitis virus using the method of WOERNLE (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 embryonated eggs.

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The EID was calculated according to REED and MUENCH (1938).

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

10. Pathogenicity of the isolate for chicks: Ninety chicks were inoculated intraperitoneally with 0.2 ml of infective allantoamniotic fluids and membranes containing the isolate 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 sacrificed from each group, sera were collected for the agar gel precipitation testing 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 sacrificed at intervals similar 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 whitish diarrhoea, paralysis and mortality rate reach 30%. The kidneys of diseased birds from which the virus was isolated showed severe nephritis, dilatation of uriters with urates and atrophy of the kidneys as shown in Fig. 1.

2. The isolated strain was found to be thermalabile 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 (Heterologous) antisera and not by infectious bronchitis (Heterologous) 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 dwarfish from the 3rd passage, as shown in Fig. 2.

5. Intraperitoneal inoculation of the isolate into one day-old chicks resulted in neither symptoms for 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, al. 1977; BLAXAND, al. 1980 and SILLER, 1981).

In present investigation, an viral agent was isolated from the kidneys of a flock suffering from high mortality, severe emaciation, profuse whitish 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 properties 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 pathogeneity of the isolated virus to chickens of different ages and by different routes 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


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Table (1)

Effect of heat and chloroform on the isolated virus strain

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Virus Infectivity (EID_{50}^\circ{F} \cdot \log_{10})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat 56°C for 1 hour</td>
<td>1.4</td>
</tr>
<tr>
<td>Chloroform 25%</td>
<td>1.8</td>
</tr>
<tr>
<td>Untreated control</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Table (2)

Cross Neutralization test between homologus and heterologus reaction

<table>
<thead>
<tr>
<th>Virus</th>
<th>antiserum against</th>
<th>EID_{50} \cdot (\log_{10})</th>
<th>Index</th>
</tr>
</thead>
<tbody>
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<td>-</td>
<td>4.8</td>
<td>0</td>
</tr>
<tr>
<td>Isolated</td>
<td>Isolated strain</td>
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<td>2.2</td>
</tr>
<tr>
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<td>IB virus</td>
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<td>0.7</td>
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<tr>
<td>IB virus</td>
<td>-</td>
<td>5.4</td>
<td>0</td>
</tr>
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<td>IB virus</td>
<td>Isolated strain</td>
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</tr>
<tr>
<td>IB virus</td>
<td>IB virus</td>
<td>2.7</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Table (3)
Pattern of embryonic deaths and mortality rate of chicken embryos during the serial passage of the isolated nephritis virus

<table>
<thead>
<tr>
<th>Passage No.</th>
<th>No. of chicken embryos</th>
<th>Days post inoculation</th>
<th>Total deaths</th>
<th>Mortality Rate</th>
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<tbody>
<tr>
<td>1</td>
<td>30</td>
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<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>- - 1 - - - - 1 -</td>
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<td>7</td>
<td>30</td>
<td>- 4 4 5 3 4 1 - -</td>
<td>21</td>
<td>70.0</td>
</tr>
</tbody>
</table>
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