AN OUTBREAK OF AVIAN REOVIRUS IN NATIVE BREED BROILER CHICKS AT EL FAYOUM GOVERNORATE

BY

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ABSTRACT

Avian reovirus was isolated from native breed broiler chicks located at El- Fayoum Governorate. The virus was identified by AGPT, Dot- ELISA using specific reference hyperimmune serum. Reovirus antibodies were detected in serum samples of breeder chickens by solid phase ELISA. Confirmatory diagnosis was applied using experimental infection to one-day-old native chicks.

INTRODUCTION

The avian reoviruses are actually an important infectious agents of chicks affecting mainly synovial membrane, tendon sheaths, liver and myocardium of infected chicks (**Sahu & Olson 1975**). It has a significant economic value in poultry industry. Economic losses caused by reovirus infections are frequently the result of crippling (viral arthritis/tenosynovitis) and general lack of performance including loss of weight gains, poor feed conversion and a reduced marketability of affected birds (**Dobson & Gilsson, 1992**).

Reovirus have been isolated from a variety of tissues in chickens affected by assorted disease conditions including viral arthritis/ tenosynovitis, stunting syndrome, respiratory disease, enteric disease and the so-called malabsorption syndrome. Moreover, they have frequently been found in chickens that were clinically normal (**Robertson *et al.*, 1984**).

The clinical findings of the malabsorption syndrome were varying and characterized by stunting growth, poor feathering, leg weakness, enteritis, proventriculitis and pancreatic degeneration (**Kouwenhoven *et al.*, 1978; Page *et al.*, 1982 & Van der Heide, 1982**). Due to the variety of clinical and pathological changes observed, the syndrome has been described under various names as helicopter disease, brittle bone disease, femoral head necrosis, pale bird syndrome (**Van der Heide *et al.*, 1981**)

malabsorption syndrome (**Page *et al.*, 1982**), Infectious proventriculitis (**Kouwenhoven *et al.*,** 1978) and infectious stunting and runting syndrome (**Braceulell & Wyeth 1981& Pass *et al.*, 1982**).

In Egypt **Kheir El- Din & El- Sanousi (1986)** described Runting syndrome in broiler parent flocks and succeeded in isolating Reovirus from the runted birds.

The aim of this study lies in rapid diagnosis of the disease by using the recent technique of ELISA for surveying Reovirus antibodies beside isolation of the Reovirus from native breeds and rapid identification of the virus antigen in infected tissues using the most sensitive and accurate technique (Dot-ELISA) in addition to Experimental studies that considered the most confirmatory diagnostic procedure for detecting Reovirus affections.

MATERIAL AND METHODS

Material:

1- Samples for virus isolation:

Pooled internal organs {liver, spleen, heart, gizzard, proventriculus and intestine} of one-week old chicks showed signs of Reovirus infection were prepared as 10% suspension and used for virus isolation.

2- Antiserum:

Reference chicken antireovirus S1133 serum, SPAFAS Comp. USA, was obtained from Immunology Dept. Animal Health Research Institute, Dokki, Egypt and was used for virus identification.

3- Sample for antigen detection:

50% of the pooled internal organs suspension were used as antigen in AGPT and Dot-ELISA.

4- Embryonated Chicken Eggs (ECE):

Commercial ECE 7 and 10 days old were used for virus isolation by Y/S and CAM routes of inoculation respectively.

5- Sera :

Tested sera: 450 serum samples were collected from five farms at El- Azab Project for improvement of native breeds at El- Fayoum Governorate. These serum samples were subjected for detecting antibodies by Solid- Phase ELISA.

6- ELISA Kit:

Reovirus antibody test kit was purchased from Kirkegaard & Perry Laboratories (KPL) USA.

7- Experimental birds:

A total of 50, one- day old balady chicks were obtained from a private hatchery receiving its fertile eggs from reovirus- free and non-vaccinated parent flock.

8- Agar gel medium for precipitation test:

This was prepared as described by (**Crawle, 1961**).

Methods:

1- Virus isolation:

0.2 ml of 10% suspension of the pooled internal organs was inoculated into each of ECE via Y/S and CAM routes. The inoculated ECE were incubated at 37° C and 80% humidity with daily candling till hatching.

2- Virus identification:

Isolated virus was identified by AGPT and Dot-ELISA.

a) AGPT was applied as described by (**Roessler & Rosenberger 1989**). The test was used for detecting virus antigen in 50% homogenates of the pooled internal organs and homogenates of the inoculated ECE.

b) Dot-ELISA was applied according to (**Hawkes *et al.*, 1982**). The test was used for the detection of reovirus antigen in 50% pooled internal organ homogenates and homogenates of the inoculated ECE using antireovirus S1133 hyperimmune serum.

3- Detection of Reovirus antibodies in serum samples:

Antibodies of Reovirus were detected in serum samples by Solid- phase ELISA. The test was applied as described by (**Snyder *et al.*, 1983**).

4- Virus Titration:

Determination of virus infectivity titers was carried out in ECE for pock forming assay, ten-fold dilution of virus suspension are inoculated onto CAM. The actual titration is carried out with enumeration of pock lesions. Egg infective dose (EID_{50}) was determined mathematically by the method of (**Reed & Muench, 1938**).

5- Experimental study:

Fifty one-day-old chicks were divided into two equal groups. The first group was infected via foot pad route with 0.2 ml of 10^6 EID_{50}/ chick and the second group were left uninfected as control group. These chicks were observed for 4 weeks.

RESULTS

History of the disease:

The present study was carried out on a five farms at El- Azab Project for improvement of native breeders, at El-Fayoum Governorate. This farms contains different ages ranging from one day to 10 months old. Dwarfed dead embryos were noticed at the time of hatching leading to decrease of hatchability (about 5%). Besides hatched chicks showed signs of stunted growth, raising of the wings upward taking the appearance of helicopter wing (see Fig. 1), lameness, malfeathering, diarrhea, inappetence, loss of body weight, ruffled feathers and low vitality. Diseased and dead birds were examined for gross lesions, bacteriological and virological examinations. The post mortem examination revealed enteritis, hepatitis, myocarditis, splenitis and the hock joints contained small amounts of straw coloured or blood tinted exudate in few cases.

Flock survey:

Reovirus was isolated from intestine and internal organs of diseased chicks as shown in Table (2).

Virus isolation and identification:

Virus isolation by CAM and Y/S sac routes of inoculation of ECE are shown in Tables (2 & 3). The inoculated suspected material produced small head white pock lesions on the inoculated CAM as shown in Fig. (2). Embryonic mortality was observed 3 – 5 days post inoculation. The inoculated embryos were stunted and markedly hemorrhagic all over the body as shown in Fig. (3). The internal organs were congested and hemorrhagic on comparing with the control embryos. Embryos that survive until 21 days were slightly dwarfed and the liver, spleen and heart were enlarged, containing necrotic foci and abnormalities in the wings (Fig. 4). Control embryos showed no lesions.

Gross lesions:

Various gross lesions are shown in Table (1).

Serological tests (AGPT & Dot ELISA):

For identification of the isolated virus using hyperimmune serum against reovirus S1133 results are shown in Table (4).

Solid phase ELISA:

Reovirus antibodies were detected in breeder sera by Solid phase ELISA as shown in Table (5).

Clinical symptoms and P.M. lesions of experimentally infected chicks:

The infected chicks showed clinical signs and P. M. lesions resembled to those induced by reovirus infection. The clinical symptoms and P. M. lesions are presented in Table (6).

Reisolation of reovirus from internal organs of experimentally infected chicks:

It was successfully carried out throughout the whole period of examination (28 days) as shown in Table (7).

DISCUSSION

Balady Farms at El-Azab project for improvement of native breeder, at El- Fayoum Governorate, contains different ages ranging from one day to 10 months old. Dwarfed dead embryos were noticed at the time of hatching leading to decrease of hatchability (about 5%). Beside hatched chicks showed signs of stunted growth, raising of the two wings upward taking the appearance of helicopter wing (see Fig. 1), lameness, malfeathering, diarrhea, inappetence, swellings of the foot pad and hock joints and loss of body weight, ruffled feathers & low vitality.

Searching for Reovirus antibodies in breeders sera were adopted using KPL-ELISA plates. These breeders did not receive any Reovirus vaccine. Data presented in Table (5) revealed that some breeders had Reovirus antibodies in a positive percentage of 16.22%. The presence of Reovirus antibodies in the breeder sera denote previous infection of the farms with Reovirus.

The potential use of ELISA as a rapid and sensitive profiling tool for avian viral diseases has been adopted by many researches **Slaght *et al.*, (1978)** and **Thayer *et al.*, (1987)**. The decrease in hatchability, the pathological picture of hatched embryos beside the presence of reovirus antibodies in breeder sera triggered us to isolate reovirus. Two routes of inoculation (Y/S and CAM) were used for this purpose, 0.2 ml of 10% suspension of pooled internal organs of infected chicks was inoculated into each ECE. Death of embryos were noticed 4^{th} day post inoculation on using Y/S whereas it occurred at 5^{th} day post inoculation on using CAM as shown in Table (3). Dead embryos showed marked hemorrhages all over the body as shown in Fig. (3), the internal organs were congested and there were pock

lesions on the CAM as shown in Fig. (2). The observed lesions in infected embryos are in agreement with those obtained by (**Deshmukh & Pomeroy, 1969** and **Van der Heide & Kalbac 1975**).

Dot-ELISA proved to the potential tool for rapid and definitive diagnosis of Reovirus as it gives accurate results only after 2 hours. It does not need special equipment (ELISA reader) as in Solid-phase ELISA and the blue dots developed in the NCM can be seen easily with the nacked eye. In contrast, the AGPT need three days for the appearance of clear precipitating lines. The Dot- ELISA proved its sensitivity than AGPT as it detected 100% of positive infected CAM, Yolk sac and 81.8% of the infected organs The sensitivity of Dot- ELISA for the detection of virus antigens was proved by other investigators **Hawkes *et al.*, (1982).**

The hatched chicks showed decrease in body weight and stunted growth. These decrease in body weight and stunted growth seemed to be a feature of reovirus infection (**Kouwenhoven *et al.*, 1988 & Rosenberger *et al.*, 1989**). The mechanism through which avian reovirus reduce the body weight is not yet clear, but it has been hypothesized that when reovirus induced enteritis it interferes with the digestion and/ or intestinal absorption, resulting in malabsorption syndrome (**Kouwenhoven *et al.*, 1978; Rosenberger, 1980** and **Van der Heide *et al.*, 1981**). On the other hand pancreatic inflammation and degeneration has also been incriminated by (**Van der Heide *et al.*, 1981**) who suggested that lack of secretion of digestive enzyme due to pancreatic degeneration would lead to improper absorption of nutrient and presence of undigested feed in the intestinal lumen. Other hypothesis was reported by (**Rossler & Rosenberger 1989**) who suggested that reovirus cause significant physiological and biochemical alterations that can be reflected in growth inhibition of protein and mineral metabolism.

Confirmatory diagnosis was done by experimental infection of twenty five one- day old chicks using the isolated virus in a dose of 0.2 ml of 10^6 EID50 / chick inoculated via foot pad route. Other twenty five chicks of the same age were left uninfected as a control group. The infected chicks showed clinical signs and P. M. lesions resembled to those induced by reovirus infection. The clinical symptoms and P. M. lesions are presented in Table (6). The mortality rate reached about 60% and the clinical symptoms appeared 5 – 7 days till day 14 post inoculation. The association of mortality with reovirus infection was observed in the field cases by some researchers (**Bains *et al.*, 1974; Wyeth *et al.*, 1981** and **Fehervari *et al.*, 1984**) and was experimentally reproduced by others (**Montgomery *et al.*, 1985; Bekhit, 1988** and **Rosenberger *et al.*, 1989**). These mortality may be attributed to

severe hepatic necrosis (**Gouvea & Schnitzer, 1982; Jones & Guneratne, 1984** and **Kibenge *et al.*, 1985**).

Leg lesions were observed in the form of swelling in the foot pad (Fig. 5) and hock joint and diarrhea accompanied by pasty vent. The marked P. M. lesions lie in tenosynovitis. Experimentally infected chicks have not the ability to move and the chicks stand on their hock joint (Fig. 5 & 6) similar leg lesions were also reported by ***Kouwenhoven et al., 1978; Voeten & Van Der Leest, 1979; Bracewell & Wyeth, 1981 and Van der Heide et al., 1981; Page et al., 1982; Bekhit, 1988 & Rosenberger et al, 1989.*** It was hypothesized that leg lesions were induced as a result of losses of some nutritive elements (vit. A, E, D_3 &B_{12}) and some minerals like Ca, Phosphorus which resulted from malabsorption that occurred due to experimental reovirus infection (**Van Der Heide, 1982**).

Reisolation of reovirus from the internal organs of inoculated chicks has been successfully carried out throughout the whole period of examination (28 days) such findings (Table 7) proves the ability of reovirus to persist for long period in certain tissues of the body as documented by **Kibeng *et al.*,(1985).**

Finally, the incidence of reovirus infection in our native breeders especially those used for breeder improvement is considered a dangerous point for realizing this purpose as it impairs such improvement. Controlling of reovirus in these farms is critical and it needs high-qualified planned programs with selection of good protected reovirus vaccines.

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Table (1): Main clinical symptoms and postmortem lesions in diseased birds.

Age/ days	Clinical symptoms	P.M. findings
One day-30 day	stunted growth, helicopter appearance as shown in Fig (1), lameness, diarrhea, loss of body weight and malfeathering	Congestion of the internal organs, hepatitis, splenitis, myocarditis

Table (2): Isolation of virus on ECE by Y/S and CAM routes.

Passages	Dose of infection	Y/S			CAM		
		Total No. of ECE	No. of dead	% +ve	Total No. of ECE	No. of dead	% +ve
1st passage	0.2 ml	10	6	60	10	5	50
2nd passage	0.2 ml	10	8	80	10	7	70
Control	0.2ml saline	10	0	0	10	0	0

From this Table it is very clear that the Y/S route is more sensitive for the pathogenesis of reovirus as it produced mortalities in inoculated embryos 60 & 80% for the 1st and 2nd passage but the CAM produced 50 & 70% respectively.

Table (4): Serological tests for identification of the isolated virus using antireovirus S1133 hyperimmune serum.

Tested samples	No. of tested samples	AGPT			Dot- ELISA		
		+ve	−ve	%+ve	+ve	−ve	%+ve
Y/S	14	8	6	57.1	14	0	100
CAM	12	7	5	58.3	12	0	100
Pooled internal organs	11	4	7	36.3	9	2	81.8
Control Non infected organs	10	0	10	0	0	10	0

Table (3): Pathological lesions of inoculated embryos.

No. of passages	No. of ECE	Pathological lesions	
		Y/S	CAM
First passage	10	Death of 6/10 embryos after 4 days post inoculation. The embryos were markedly haemorrhagic all over the body as shown in Fig (3), the internal organs were congested & haemorrhagic. Embryos that survive until 21 days were slightly dwarfed, the liver, spleen & heart enlarged & contained necrotic foci. There were abnormalities in wings.	Death 5/10 embryos after 5 days P.I. with the appearance of petecheal hemorrhages all over the body. - Appearance of few small pen headed white pock lesion on the CAM as shown in Fig. (2).
Second passage	10	8/10 embryos died after 3 days P.I. with severe petecheal hemorrhages. Embryos that survive until 21 days were slightly dwarfed as shown in Fig. (4) & the liver, spleen & heart were enlarged & contained necrotic foci.	Death 7/10 embryos after 4 days P.I. with the petecheal hemorrhages all over the body. The pock lesions increased in numbers and became very clear.
Control non infected embryos	10	- No hemorrhage - Normal size - No death	- No hemorrhage - Normal size - No death

From Table (3) it is very clear that death of embryos were occurred after 3 & 4 days when using Y/S route, but it occurred after 4 & 5 days when using CAM route and this indicates the Y/S route is the preferable route for virus isolation and studying the pathogenencity of reovirus infection in ECE.

Table (5): Presence of Reovirus antibodies in breeder sera as detected by Solid-phase ELISA.

No. of breeder farms	Tested sera*	Negative	Positive	% positive
1	90	74	16	17.77
2	90	81	8	8.88
3	90	90	0	0.00
4	90	49	41	45.55
5	90	82	8	8.88
Total	450	377	73	16.22

*sera were collected from unvaccinated breeders. Positive sera denote to the previously reovirus infection of the breeders from which the sera were collected.

Table (6): Clinical symptoms and P. M. lesions of experimentally infected chicks.

Group	Mortality %	Clinical signs	P.M. lesions
Infected group (25 chicks)	60	The symptoms appeared 5 days post inoculation including swelling in the foot pad and hock joint and the bird tended to stand on hock joint as shown in Fig. (5) and feather abnormalities	Signs of tenosynovitis as inflammation of tendon and tendon sheath, increase turbidity of synovial fluid and enlarged ,necrosis of liver, spleen and heart.
Non-infected group (25 chicks)	0	Normal	Normal

Table (7): Reisolation of avian Reovirus from experimentally infected chicks via foot pad route.

Group	Bird status	Virus reisolation in ECE Pooled internal organs Days post inoculation					
		4	5	7	14	21	28
Group I (infected)	- Dead	+	+	-	+	-	-
	- Scarified	+	+	+	+	+	+
Group II (non infected)	- Scarified	-	-	-	-	-	-

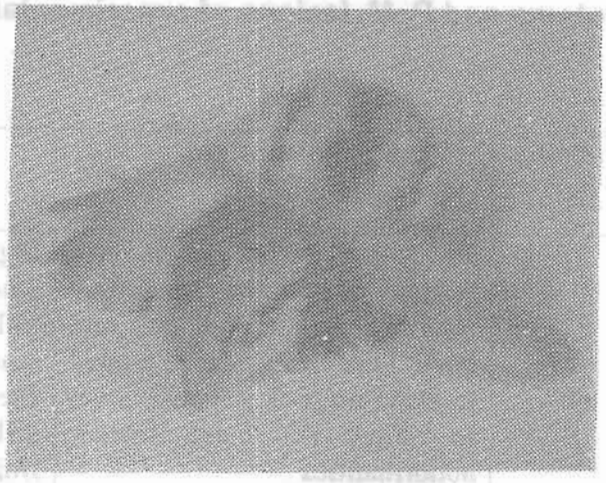

Fig. (1): Chicks showing helicopter appearance

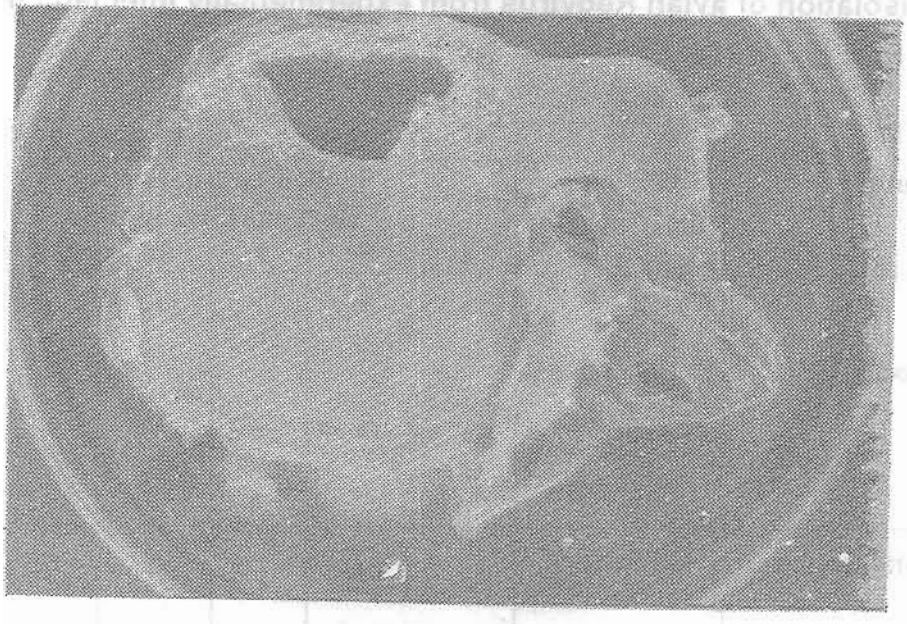

Fig. (2): Pock lesions on the CAM after 5 days P.I.

Fig. (3): Dead embryos after 4 days P.I., the embryos were markedly haemorrhagic.

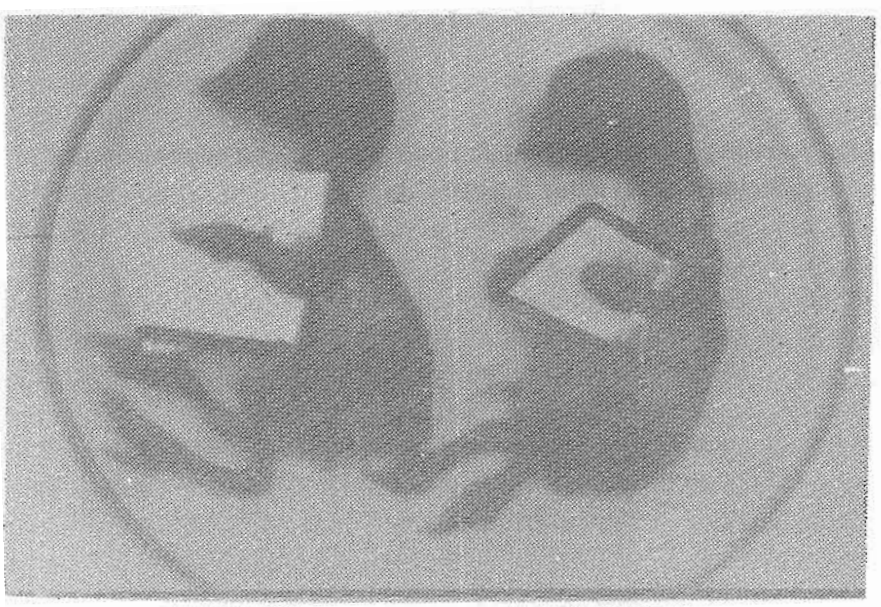

Fig. (4): Dwarfed & markedly haemorrhagic embryos after 21 days old compared with control embryos.

Fig. (5): Swelling in the footpad & hock joint in the experimental infected chicks after 5 days P.I.

الملخص العربى

وباء فيروس الريو فى كتاكيت التسمين البلدية فى محافظة الفيوم

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تم عزل فيروس الريو من كتاكيت التسمين البلدية فى محافظة الفيوم وقد صنف هذا الفيروس المعزول بإستخدام اختبارات الترسيب فى الآجار وبقعة الآليزا وتم الكشف على الأجسام المناعية لهذا الفيروس فى الأمهات بواسطة اختبار الآليزا وقد تم عمل عدوى صناعية لكتاكيت بلدية عمر يوم.