PREVALENCE OF ADENO VIRUS IN SOME POULTRY FARMS IN EL- FAYOUM AND BENI-SUEF GOVERNORATES

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ABSTRACT

Egg drop syndrome (EDS) virus has been isolated from laying birds suffered from decreased egg production accompanied with soft shell or shell-less eggs in EL-Fayoum and Beni-suef Governorates. The isolated virus has identified by haemagglutinatian inhibition (HI) and agar gel precipitation (AGP) tests. In addition it was sensitive to 10% chloroform and heat stable for 40 minutes at $56c^0$.

INTRODUCTION

Since 1976, an economically important syndrome associated with decreased egg production has been described in Netherlands and designated the egg drop syndrome 1976 (EDS-76) by **Van-Eck *et al.*, (1976)**. The condition is characterized by failure to reach the predicated egg production targets or by sudden and severe drop in egg production (10 – 30%) associated with a percentage of soft shell and shell-less eggs with poor internal quality **Mc Ferran, (1980)**. In Egypt of EDS-76 virus was isolated from commercial layers with serious egg problems (**Amina *et al.*, 1989, Ahmed, 1995 and Saber *et al.*, 1996**). The present work aimed to update the prevalence of EDS-76 in some poultry farms in Beni-Suef and EL- Fayoum Governorates.

MATERIAL AND METHODS

I- Materials:

1- Viruses: 1.1. EDS-76 strain 127 was kindly obtained from Animal Health Research Institute, Dokki, Cairo.

1.2. Fowl adeno virus (Celo-virus) was kindly obtained from poultry and rabbit disease Dept. Fac. Vet. Med. Cairo University.

2- Antiserum: Reference EDS. 76 anti serum was obtained from Animal Health Research Institute, Dokki, Cairo.

3- Specimens:

3.1. Organs - portion of liver, spleen, trachea, lung and oviduct were collected from sacrified birds from the effected flocks.

3.2. Droplets were collected from farms of diseased birds

4- Embryonated eggs: commercial embryonated chicken and duck eggs (ECE& EDE) were purchased from private farms.

II – Methods:

1- Virus isolation from collected organ and droplet samples was done according to (**Saber *et al.*, 1996**).

2- Virus identification and characterization.

2.1. Haemagglutination test (H A) was applied according (**Anon, 1971**).

2.2. Haemagglutination inhibition test (HI) was applied according to (**Yamaguchi *et al.*, 1980**).

2.3. Heat stability test was determined according to (**Mc Ferran, 1980**).

2.4. Chloroform sensitivity test was detected according to (**Swain *et al.*, 1992**).

RESULTS

Table (1): Detection of haemagglutinen on egg contents of inoculated EDE after 5 passages.

Inoculum	HA test on			
	CAM extract	AF	Yolk	embryo extract
1- Standard EDS – 76	+	+	-	-
2- Organ extract	-	-	-	-
3- Prepared fecal material	+	+	-	-
4- Prepared uterine discharge	+	+	-	+
5- Standard celo – type adeno – virus	-	-	-	-

From this table it is very clear that the isolated virus agglutinate 1% chicken RBCs like standard EDS – 76 but cello – type adeno virus not agglutinate chicken RBCs. The virus was isolated from fecal droplets, uterine discharge but not from organ extract. The yolk sac route is not sensitive for isolation of the virus.

Table (2): virus isolation on ECE by different routs of inoculation.

Inoculum	Rout of inoculation		
	CAM	Allantoic cavity	Yolk sac
1- Standard virus reference EDS –76	-Thickening of CAM at site of inoculation	- No HA activity or pathological lesion or death of the embryo	No HA activity or pathological lesion or death of the embryo
2- Prepared fecal material.	-Thickening of CAM at site of inoculation	- No HA activity or pathological lesion or death of the embryo - No pathological lesion	- No HA activity or pathological lesion or death of the embryo - No pathological lesion
3-Uterine discharge.	-Thickening of CAM at site of inoculation - Few embryo death, few necrotic area and thickening of CAM. Clear peticheal haemarhage on the skull and toes.	- Dead embryo, curling and stunting – clear peticheal haemorrhage on the skull and toes but no HA activity for chicken RBCs.	Dead embryo. curling and stunting – clear peticheal haemorrhage on the skull and toes but no HA activity for chicken RBCs.
4- A deno virus strain 1 (celo).			

Table (3): Identification of isolated virus by HI and AGPT.

Locality	Type of isolate *		H I #		AGPT #	
		H A	EDS	NDV	EDS	NDV
El - Wasta	Fecal swab	+	+	-	+	-
	Uterine discharge	+	+	-	+	-
Beba	Fecal swab	+	+	-	+	-
	Uterine discharge	+	+	-	+	-
	Reference EDS 76	+	+	-	+	-

* Isolate after 5 passages on EDE.

\# Specific hyper immune sera were used for both viruses.

From this table it is very clear that the isolated virus from El – Wasta and Beba centers that located at Beni – Suef Governorate is EDS 76 as its agglutination activity is inhibited by using EDS – 76 antiserum and gave clear precipitation line with this serum but not with NDV antiserum.

Table (4): Determination of HA titers of isolated virus * at different time post inoculation on EDE.

Days post inoculation	H A activity		
	A F	embryo extract	CAM extract
3	4 -- 8	8	8 – 16
5	2048	8	2048
6	2048	8	2048
8	1024	8	1024
9	1024	16	1024

* Virus isolated from fecal droplets.

From this table it is very clear that the best time for virus harvestation is 5th or 6th day post inoculation on EDE as they gave the highest H A titers.

Table (5): Ability of isolated virus to agglutinate avian and Mammalian erythrocytes.

Type of RBCs	HA activity titer	
	Isolated virus after 5 passage	Standard EDS – 76 virus after 3 passage
Chickens	2048	2048
Ducks	2048	2048
Turkeys	2048	2048
Rat	-	-
Sheep	-	-
Goat	-	-
Guina pig	-	-
Cattle	-	-
Buffalo	-	-

From this table it is very clear that the isolated virus was run in a parallel way for its ability to agglutinated chicken, ducks and turkeys RBCs but not for rat, sheep, goat, guinea pig, cattle and buffalo RBCs.

Table (6): characterization of isolated virus by sensitivity to 10 % chloroform.

Inoculum	Haemagglutination for 1% chicken RB cs	
	Before addition of chloroform	After addition of chloroform
Standard EDS – 76	2048	2048
Uterine discharge	2048	2048
Fecal material	2048	2048

From this table it is very clear that the isolated virus not affected by 10% chloroform as well as standard EDS – 76.

Table (7): characterization of isolated virus by stability to heat.

Heat 56C°/ time	Haemagglutination of 1% chicken RBCs			
	Fecal isolate		Standard EDS – 76 virus	
	before treatment	after treatment	before treatment	after treatment
10 min	2048	2048	2048	2048
20 min	2048	2048	2048	2048
30 min	2048	2048	2048	2048
40 min	2048	2048	2048	2048
60 min	2048	1024	2048	1024
90 min	2048	512	2048	512
12 hrs	2048	512	2048	512
23 hrs	2048	256	2048	256
48 hrs	1024	128	1024	128
72 hrs	1024	64	1024	64
96 hrs	1024	32	1024	32

From this table it is very clear that the isolated virus resisted heat for 40 min at 56 C° and it's HA stability was affected at 56 C° after 60 and 90 min and decreased to a very low titer after 96 hours.

DISCUSSION

Egg drop syndrome is caused by a haemagglutinating adeno virus. This virus is the major etiological agent for reducing egg production during the peak production period in demostic birds (**Kumar *et al.*, 1992**). In Egypt EDS 127 virus was isolated from duck farms associated with detectable HI antibodies (**Hamouda, 1988**). Also EDS 76 virus was isolated from layer flocks showed decrease in egg production located in El – Qaluobia , Monifia and Giza Governorates (**Amina *et al.*, 1989 and Saber *et al.*, 1996**). The present work aimed to survey the EDS 76 virus in Beni – Suef & El – Fayoum Governorates by the isolation and identification of the virus from naturally affected flocks showing decrease in egg production and egg abnormalities. For virus isolation organs extract, fecal swabs, uterine discharge were inoculated into embryonated duck eggs (EDE) by allantoic cavity route beside standard EDS 76 and Celo type a deno virus (table 1). From this table it is very clear that the extracted CAM, AF and embryos of EDE that inoculated by standard EDS – 76 virus, fecal material, uterine discharge agglutinated 1 % chicken RBcs but the EDE that inoculated by organs extract and standard cello type a deno virus failed to agglutinated 1% chickens RBCs. On the other hand the yolk of infected EDE with standard EDS 76 virus, organ extract, fecal material and standard cello type adeno virus not agglutinated chicken RBCs. These results agree with those obtained by (**Xue *et al.*, 1995 and Saber *et al.*, 1996**) as they studied the distribution of EDS 76 virus and its pathogenicity in EDE, and showed that, the CAM

allantoic fluid, contained the highest virus titer while little virus could be demonstrated in embryos but not yolks sac. When the inoculum of organ extract, uterine discharge, fecal material inoculated in embryonated chicken eggs by CAM, allantoic cavity, yolk sac routs, the growth of virus was refractory. In CAM route only thickening at the site of inoculation was observed. The HA activity, characteristic pathological lesion or death of embryos were not observed by other two routes. The standard EDS 76 reference virus produced the same results. Adeno virus strain 1 (celo) induced death in few embryos, few necrotic area and thickening of CAM besides clear petecheal haemorrhage on the skull and toes. No agglutination of chicken RBCs was found in the allantoic fluid or yolk, Table (2). The failure of EDS – 76 virus to grow in ECE was also observed and recorded by (**Gough *et al.*, 1982; Higashihero *et al.*, 1983 and shakya & Dhawedkar, 1991**). For identifying the isolated virus, reference EDS 76 antiserum was used in HI and AGP tests. The viruses that isolated from El Wasta and Beba center at Beni-Suef Governorate were agglutinated chicken RBCs and these reactions inhibted by the reference anti EDS 76 serum beside the clear precipitating line that observed in AGPT Table (3). The activity of HA titer of isolated virus from El-Wasta was detected at different time intervals post – inoculation in EDE, as in Table (4). The highest HA titer of this isolate was found at 5^{th} and 6^{th} days post –inoculation in the allantoic fluid and CAM extract as they gave 11 log HA titer. These results indicate clearly that the best activity can be obtained at 5^{th} or 6^{th} days post–inoculation in EDE. The ability of El – wasta isolated EDS virus to agglutinate avain and mammalian RBCs was studied after 5 passages in EDE Table (5). The viruses have the ability to agglutinate chickens, ducks and turkeys RBCs with 11 log HA titers but not agglutinated rat, sheep, goat, guinea pig, cattle and buffalo RBCs. The reference standard EDS 76 produced the same results. The ability of isolated virus to agglutinate erythrocytes of chickens, ducks and turkeys but not agglutinate rat, sheep, goat, guinea pig, cattle and buffalo erythrocytes is agree with those obtained by (**Lu *et al.*, 1985**).

The isolated virus was characterized by studying its sensitivity to 10% chloroform and it's stability to heat, Tables (6 & 7). On adding 10% chloroform to isolated and standard EDS 76 virus (Table 6), no change was found on the HA titers indicated that the isolated virus is nacked virus as they not affected by lipid solvent chloroform. On the other hand the isolated virus was not affected by heating at 56°C for 1 hr, the HA log titer decreased from 11 log, after 40 minutes to 10 log after 60 minutes and 9 log after 90 minutes exposure, this stability of isolated and standard EDS 76 virus confirm that the isolated virus is EDS virus.

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الملخص العربي

مسح عن تواجد فيروس الأدينو في بعض مزارع الدواجن في محافظتي بنى سويف والفيوم

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تم عزل الفيروس المسبب لمتلازمة نقص إنتاجية البيض من الدجاج البياض في بعض المزارع بمحافظتي بنى سويف والفيوم. وقد تم تمييز الفيروس المعزول باستخدام اختبار التلازن الدموي واختبار الترسيب في الأجار واختبار منع التلازن الدموي مع مصل مرجعي وتم أيضا دراسة خصائص الفيروس من خلال حساسيته لـ ١٠ % كلورفورم ومدى ثباته لتأثير الحرارة عند ٥٦م.