رقم البحث (10)

VIROLOGICAL STUDIES ON DUCK HEPATITIS VIRUS TYPE-1 IN DUCKLINGS AT DAKAHLIA AND DAMIETTA PROVINCES

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ABSTRACT

In this study Duck Hepatitis Virus type I (DHV type I) was isolated from different organs; liver, kidney, bile duct, spleen, and pancreas and feces of ducklings.The virus was isolated via allantoic cavity (AC) route of 8-10 days old ECE. Two blind passages were carried out for each sample. Specific hyperimmune serum against standard DHV type I was prepared.The isolated DHV type I was identified using the specific prepared hyperimmune serum by using agar gel precipitation test (AGPT) and virus neutralization test (VNT), and. Experimental infection of isolated DHV type I into 100 susceptible ducklings was done and identified by indirect fluorescent antibody test (IFAT)IFAT.

Key Words: Duck Hepatitis Virus, isolation, identification.

INTRODUCTION

Duck virus hepatitis (DVH) is an acute highly fatal and rapidly spreading viral infection of young ducklings. It is one of those diseases affecting ducklings lead to a great threat to duck industry. Ducks are the natural host of this disease.

DVH characterized primarily by rapid onset and high mortality as high as 90% with short incubation period from 24 to 48 hours. The affected ducklings become listless, develop spasmodic contractions of legs, and die within 1-2 hours in typical opisthotonos (nervous manifestations) with diarrhea, gross lesions are mainly in liver that is enlarged, pale, and shows pathognomonic petechial and ecchymotic hemorrhages (**Fabricant et al. 1957, Toth. 1969b, Farmer et al. 1987, Liao et al 1989**).

DVH is caused by at least two serologically distinct Picornaviruses; DHV type I (**Levine and Hofstad. 1945, Levine and Fabricant. 1950.**) and DHV type III (**Toth. 1969b.**), as well as an Astrovirus; DHV type II (**Gough and Stuart. 1993).** Hepadna viruses; DHBV & WHV (**Fernholz et al. 1993a.**) have been found in domestic ducks in China and USA. The clinical progression varies with the type of the virus.

Adenovirus and Parvovirus are associated with hepatitis in Muscovy ducklings and goslings; Derzsy's disease occur in goslings and Muscovy ducklings less than one month old (**Derzsy. 1967, Takehara et al. 1994.)**.

In Egypt, DHV- type I is a widely spread disease causing high economic losses in young ducklings (**Refaie 1967, Shalaby 1972, and Sokkar et al 1975**). It contains single stranded RNA genome, non-enveloped roughly spherical in shape, it related to genus enterovirus, family Picornaviridae (**Tauraso et al 1969**).

MATERIAL AND METHODS

3.1- Samples collection:

Sixteen samples were collected aseptically for virus isolation each of them consisted of Liver, bile duct, pancreas, spleen, kidney, and feces from freshly dead and diseased Balady and Sudani ducklings, 1 day-4 weeks old from special farms Dakahlia and Damietta Provinces.

3.2- Preparation of specimens for virus isolation:

The samples were collected aseptically in sterile Petri-dishes. These organs were cut into small pieces and grinded in a sterile mortar containing sterile sand, to form 10% suspension in phosphate buffer saline (PBS) (**Thomas. 1969**).

3.3- Isolation of DHV type I:

Embryonated chicken eggs of 8-10 days old inoculated via allantoic cavity according to **Senne, 1989.** Two blind passages were carried out then allantoic fluids were collected and concentrated.

3.4- Propagation of Standard attenuated vaccinal DHV type I:

Reconstitution of attenuated DVH type I vaccine was done and used for preparation of hyperimmune serum against DHV type I.

3.4- Concentration of collected allantoic fluid:

Allantoic fluid suspensions were centrifuged at (30 000 r.p.m.) for 20 min at 4°C. The pellet containing the virus was re-suspended in 10% of the original volume of PBS and stored at -20°C till use.

3.5- Titration of isolated DHV type I:

The isolated DHV type-I (2nd passage) was titrated according to **Anon, 1971, Villegas and Purchase, 1989**. EID_{50} was calculated according to method of **Reed and Muench, 1938.**

3.6- Titration of Standard attenuated vaccinal DHV type I:

Standard attenuated vaccinal DHV type I obtained from VSVRI was titrated according to **Anon, 1971**. Then EID_{50} was calculated according to method of **Reed and Muench, 1938.**

3.7- Preparation of hyperimmune sera against DHV type I:

Five baladi rabbits (5 months old) were inoculated with the titrated attenuated vaccinal DHV type I,rabbits were slaughtered and blood was collected for separation of HIS, and then purified according to **Manal El-Hossiny, 2002.** Then the HIS stored at -20°C till use.

3.8- Identification of isolated DHV type I by Serological tests:

3.8.1- Agar gel precipitation test (AGPT):

It was carried out according to **Hussein et al. 1993.**

3.8.2- Virus neutralization test (VNT):

It was carried out by Alpha method (constant serum varying virus) according to **Woolcock 1989**.

3.9- Experimental Infection:

Twenty- Pekin ducklings of 5 days old were used and classified into three groups: (A) group contained 5 ducklings and (B) group contained 10 ducklings and (C) group contained 5 duckings as a control group, and then they were kept in separate units under the same condition and the same ration. Group A was infected with 2nd passage isolated DHV type I ($10^{7.5}$ EID_{50}/ml) 0.5 ml IM per duckling. Ducklings were observed daily for a period of 10

days for clinical signs of DHV type I, mortality, and PM lesions. The organs were collected from the dead ducklings, pooled and inoculated in ECE for re-isolation of the isolated virus. Group B was infected with isolated DHV type I ($10^{7..5}$ EID_{50}/ml) where 0.5 ml inoculated I/M per duckling. This group was used for IFAT detection of the virus for 7 days by slaughtering one duckling/ day and applying the IFAT on its organs (cryostat method).

3.10- Indirect Fluorescent Antibody Technique (IFAT) for detection of DHV type I antigen:

Cryostat sections were prepared and stained with goat anti-rabbit IgG FITC conjugate according to **(Mishra and Mallick, 1997**) and read immediately under fluorescent microscope. The slides were kept in refrigerator between viewing to avoid fading of fluorescence.

RESULT

4.1- Isolation of DHV type I field isolate from collected samples in ECEs:

Each collected sample from the field was inoculated into 5 ECEs/ each organ sample for virus isolation. The numbers and time of deaths of the embryos post inoculation were recorded in table (1). The inoculated embryos died mostly at the 2nd and 3rd days PI for liver samples.

Table (1): The results of DHV type I field isolate isolation in ECEs.

Inoculated sample	No. & type of embryonating eggs	Embryonic deaths for 6 days post inoculation						Total embryonic deaths
		1st	2nd	3rd	4th	5th	6th	
L (liver)	5 ECE	-	2	2	1	-	-	5/5
B (bile duct)	5 ECE	-	-	-	1	2	2	5/5
P (pancreas)	5 ECE	-	-	2	-	2	1	5/5
K (kidney)	5 ECE	-	1	2	-	2	-	5/5
S (spleen)	5 ECE	-	-	1	2	1	1	5/5
Fs (feces)	5 ECE	-	2	1	2	-	-	5/5

The embryos which died between 2-3 days PI (liver, feces & kidney samples) showed stunted growth, edema in the body especially around the head. There were also skin hemorrhage and general congestion all over the body (Figure 1 a).

The embryos which died between 5-6 days PI (bile, pancreas & spleen) showed enlarged, congested liver with reddish-brown mottling and hemorrhage, severe necrosis most of the liver, enlarged gall bladder with excess bile secretion, greenish secretions in intestine, swollen mottled spleen and pale swollen kidneys with injected blood vessels. Also egg yolk and AF were green in color (Figure 1b).

4.2- Titration of both DHV type I field isolate and standard vaccinal DHV type I in ECEs:

Titration of DHV type I field isolate from liver sample and standard vaccinal DHV type I in ECE according to method of **Reed and Muench, 1938**, are shown in table (2).

Table (2): The results of titration of DHV type I field isolate and the standard vaccinal strain.

Inoculated sample	No. of ECE	Titer $\log_{10}$ EID_{50}/ml
The liver homogenate containing the virus	5 ECE	5.75
Alantoic fluid containing the virus (1st passage)	5 ECE	6.5
Alantoic fluid containing the virus (2nd passage)	5 ECE	7.5
Standard DHV type I	5 ECE	6.75

4.3- DHV type I field isolate identification:

Identification of DHV type I concentrated field isolates was done by using prepared HIS against standard vaccinal DHV type I as follows:

4.3.1- Agar gel precipitation test (AGPT):

The positive precipitin lines appeared between the hyperimmune serum and the suspected field isolate antigen of liver samples (2nd passage) after 48-72 hrs. Also precipitin line was observed with standard vaccinal DHV type I.

4.3.2- Virus neutralization test (VNT):

Both susceptible ducklings and ECE which were inoculated with the mixture of field isolate and hyperimmune serum against standard vaccinal DHV. In the same time these hosts were inoculated by isolated DHV alone and neutralization index (NI) was calculated. The result showed that NI was 2.

Comparison between the serological tests used for identification of the field isolate of liver samples from infected ducklings was performed and the results were recorded in table (3) in which 13, 2 and 13 samples gave positive results in virus isolation, AGPT and NT, respectively.

Table (3): Comparison between the serological tests used for identification of the field isolate of liver samples from infected ducklings:

No. of samples	Virus isolation	AGPT	NT
1	+ve	+ve	+ve
2	+ve	+ ve	+ve
3	+ve	**-ve**	+ve
4	+ve	**-ve**	+ve
5	+ve	**-ve**	+ve
6	+ve	**-ve**	+ve
7	+ve	**-ve**	+ve
8	**-ve**	**-ve**	**-ve**
9	+ve	**-ve**	+ve
10	**-ve**	**-ve**	**-ve**
11	+ve	**-ve**	+ve
12	+ve	**-ve**	+ve
13	+ve	**-ve**	+ve
14	+ve	**-ve**	+ve
15	+ve	**-ve**	+ve

4.4- Experimental infection of isolated DHV type I:

The experimental inoculation of 2nd passage of isolated DHV type I ($10^{7.5}$ EID_{50}/ml) in 5-days old ducklings showed the typical signs of DVH type I 24-48 hrs. PI as weakness, dullness, failed to keep up with the group, and then they stopped walking and squatted with the eyes partially closed. Finally they fell on their sides with spasmodic paddling leg movement and the head drown over the back "opisthotonus position" (Figure 2 a,b,c,d).

The mortality occurred at 3-4 days after appearance of the clinical signs. Also the post mortem lesions seen in dead inoculated ducklings were found in liver, bile duct, spleen, kidneys and pancreas were typical lesions of DHV type I.

4.5- Re-isolation of the DHV type I from experimentally infected ducklings in ECE:

The samples from experimentally infected ducklings were collected, prepared as the original field samples and showed results similar to that of isolated DHV type I from naturally infected ducklings in ECEs.

4.6- Indirect fluorescent antibody technique for detection of DHV type I from experimentally infected ducklings:

Detection of DHV type I from experimentally infected ducklings showed greenish-yellowish color under fluorescent microscope in all infected cells of different organs by the cryostat method (Figure 3 a,b) and the results were recorded and showed that the virus is most prominent in duckling organs 3-5 days PI as shown in table (4).

Table (4): Shows IFAT result of experimentally infected ducklings for 7 days PI.

Day PI	Liver	Kidney	Pancreas	Spleen
1st	++	+	+	+
2nd	+++	++	++	+
3rd	++++	+++	++	++
4th	+++++	+++	++	++
5th	+++++	+++	++	++
6th	+++++	+++	++	++
7th	++++	+++	++	++

The results recorded in table (4) revealed that intensity of the reaction was clear in both liver and kidney but less in both spleen and pancreas.

Fig 1

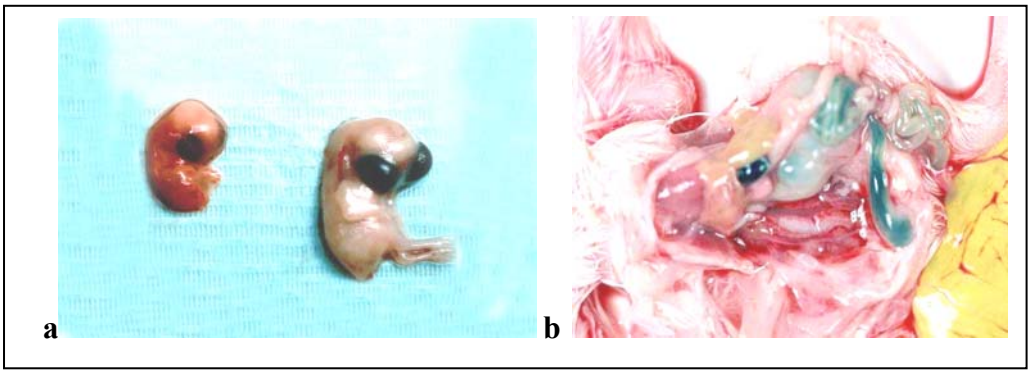

Fig 2

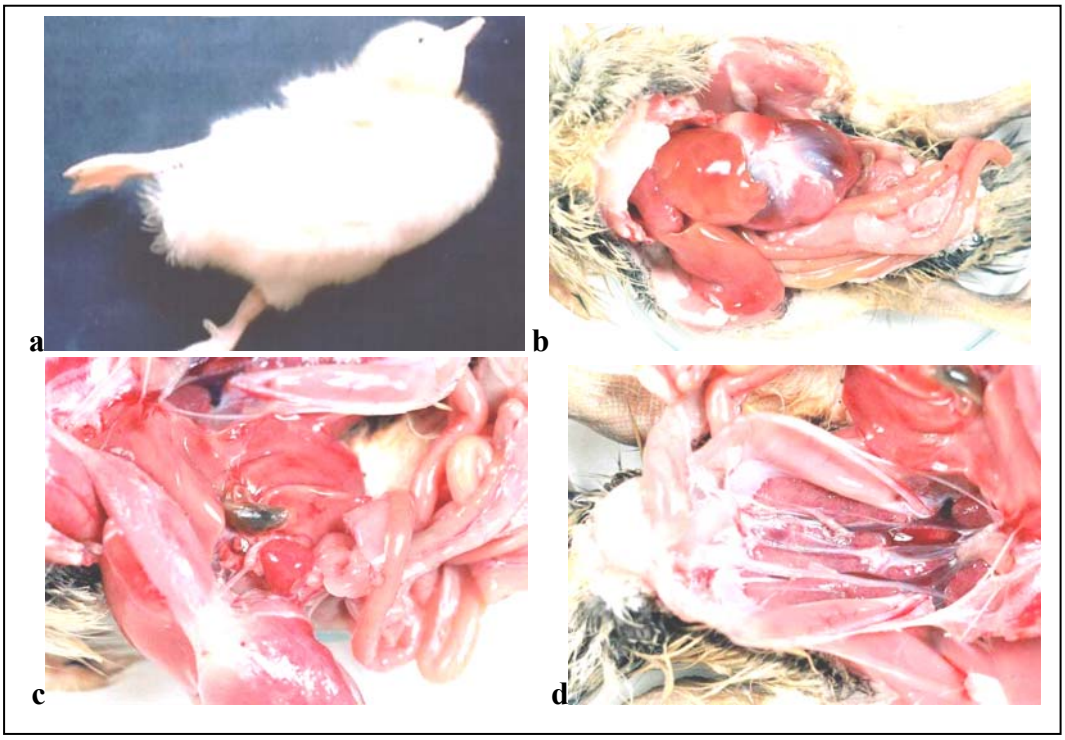

Fig 3

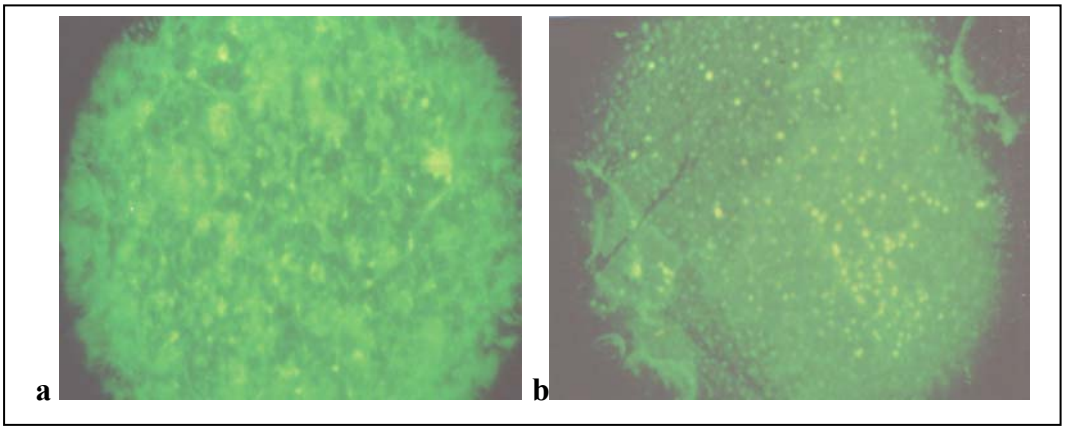

Figure (1):

a- (Left) embryo which died between 2-3 days PI showing stunted growth, edema in the body especially around the head. There were also skin hemorrhage and general congestion all over the body. (Right) normal embryo.

b- Embryo which died between 5-8 days PI showing pale, swollen kidneys with congested blood vessels.

Figure (2):

a- DVH-infected duckling showing the opisthotonus position.

b- DVH-infected duckling showing the enlargement of bile duct and enlarged mottled spleen.

c- DVH-infected duckling showing the enlarged, congested hemorrhagic liver and necrosis with some hemorrhages in enlarged pancreas.

d- Sample No. 16 showing Ascitis in infected Muscovy duckling.

Figure (3):

a- Infected liver showing the greenish-yellowish color under fluorescent microscope.

b- Infected spleen showing the greenish-yellowish color under fluorescent microscope.

DISCUSSION

In this study; a trial for DHV type I isolation from different randomly collected samples from the freshly dead ducklings and diseased ducklings showing clinical signs similar to DHV type I in ducklings below one month old. These samples were collected during (2005-2006) from private duck farms and small groups in villages of Dakahlia and Damietta. Sixteen samples were collected, each sample contains liver, bile duct, spleen, pancreas, kidney, and fecal samples; so 96 samples were prepared and used for virus isolation using ECE aged 8-10 days-old via Ac route. Two blind egg passages were carried out for each sample.

All samples were positive DHV type I except No.8 &10.The positive DHV samples showed stunted growth, edema in the bodyespecially around the head. There were also skin hemorrhage and general congestion all over the body in embryos which died between 2-3 days PI, while embryos which died between 5-6 days PI showed enlarged, congested liver

with reddish-brown mottling and hemorrhage, severe necrosis most of the liver, enlarged gall bladder with excess bile secretion, greenish secretions in intestine, swollen mottled spleen and pale swollen kidneys with injected blood vessels. Also egg yolk and AF were green in color these results were similar to those obtained by **Fitzgerald et al 1968, Woolcock 1996, and Woolcock 1998**. Embryos which died between 2-3 days PI were inoculated with liver, kidney, and fecal samples (high virus titer), while embryos which died between 5-6 days PI were inoculated by pancreas, bile duct, and spleen (low virus titer) this is may be due to the propagation of the virus takes place mainly in the hepatocytes then kidney and excreted mainly in feces, so the virus titer is high. This result agreed with those obtained by **Abd El-Naby 1998 and El-Koffy 1997,** who recorded ECE isolation and titration of DHV in liver suspension that were 10^{7} EID_{50}/ml, and $10^{8.31}$ EID_{50}/ml respectively. Liver samples that gave clear lesions and deaths in ECE within 2-3 days were titrated according to (**Reed and Muench 1938**). The isolated virus titer was $10^{5.75}$ EID_{50} /ml at virus suspension of liver sample, $10^{6.5}$ EID_{50} /ml at 1st passage, and $10^{7.5}$ EID_{50} /ml for 2nd passage. The 2nd passage was used in identification of the virus and in experimental infection.

Identification of the isolated virus took place by AGPT & VNT. The obtained results revealed that by using AGPT for detection of DHV type I directly from field's samples before inoculation in ECE showed that liver samples gave positive results and the other samples were negative, while VNT gave positive results with all samples. This may be due to VNT is able to detect little antigen per ml. So the negative results obtained by the use of AGPT may be due to low concentration of the virus in the field's samples. These results obtained were agreed with **Wachendorfer 1965, Shalaby 1972, Mahmoud 1980, Toth and Norcross 1981b**.

Experimental infection of isolated DHV type I ($10^{7.5}$ EID_{50}/ml) in ducklings showed the typical signs of DVH type I 24-48 hrs. PI as weakness, dullness, failed to keep up with the group, and then they stopped walking and squatted with the eyes partially closed. Finally they fell on their sides with spasmodic paddling leg movement and the head drown over the back "opisthotonus position". The mortality occurred 3-4 days of the first appearance of the symptoms. Also the post mortem lesions seen in dead inoculated ducklings were found in the liver which was enlarged, containing punctuate or ecchymotic hemorrhages and in some cases the liver appeared congested. The bile duct was enlarged "bile duct hyperplasia" with increase of the bile flow. The spleen in some cases was enlarged and mottled while in others

showed lesions similar to that of the liver. The kidneys were swollen, hemorrhagic, and the renal blood vessels were congested. The pancreas showed pancreatic necrosis and some what hemorrhagic. Similar results were obtained by **Woolcock 1989, Woolcock 1996, Woolcock 1998, and El-Koffy 1997**.

Re-isolation of the virus from different organs of the experimentally infected ducklings via AC of 8-10 days ECE and identified by IFAT. The isolated DHV type I was detected in liver, spleen, pancreas, and kidney of experimentally infected ducklings of isolated DHV type I using cryostat method of IFAT. In which the intensity of the reaction was clear in both liver and kidney but less in both spleen and pancreas. These results were in agreement with those obtained by **Vertinskii et al 1968** who detected the virus from the internal organs from both naturally and experimentally infected ducklings.

IFAT is a rapid diagnostic tool for DHV type I.

The results revealed that DHV type I causes nervous manifestations with diarrhea in young ducklings less than one month of age; the virus was isolated from liver, bile duct, pancreas, spleen, and kidney, and also feces of ducklings suffering from these signs. IFAT is the most rapid definitive diagnostic tool for DHV type I.

REFERENCES

Abd El-Naby Younis Metwally. (1998): Some Studies on Viral Diseases of Ducks with Special Reference to Duck Virus Hepatitis and Duck Virus Enteritis. A Thesis Ph. D., Poultry and Rabbit Dis., Zagazig Univ.

Anon (1971): Method for examining and for identifying and quantifying avian pathogens. National Academy of Sciences, achington, D. C.

Derzsy, D. (1967): A viral disease of goslings. I. Epidemiological, clinical, pathological and etiological studies. Acta. Vet. Acad. Sci. Hung. 17:443-448.

El-Koffy, M.A. (1997): Studies on the preparation and evaluation of a vaccine prepared from a local strain of duck virus hepatitis. Thesis Ph.D., Poultry dis., Cairo Univ.

Fabricant, J., Rickard, C.G., and Levine, P.P. (1957): The pathology of duck virus hepatitis Avian Dis. 1:256-275, Avian Viruses.

Farmer, H., Chalmers, W.S.K., and Woolcock, P.R. (1987): The duck fatty kidney syndrome-An aspect of duck hepatitis. Avian path. 16:227-236, Viral Infections of Waterfowl, Chapter 13, edited by Peter R. Woolcock.

Fernholz, D., Wetz, H., and Will, H. (1993a): Hepatitis B Viruses in birds. In J. B. McFerran and M. S. McNulty, eds. Virus infections of birds. Elsevier Science Publishers, B. V., Amsterdam; 111-119.

Fitzgerald, J.E., Hanson, L.E., and Simon, J. (1968): Histopathological changes induced with Duck Hepatitis Virus in the developing chicke embryo. Department of Vet. Pathol. and Hygiene College of Vet. Med. Univ. of Illinois, Urbana, Illinois 48106.

Gough, R.E. and J.C. Stuart. (1993): Astroviruses in ducks (DVH type II). In J. B. McFerran and M. S. McNulty (eds.). Virus infections of birds. Elsevier Science Publishers, B.V., Amsterdam. 505-508.

Hussein, N.A., El-Ebiary, E.A., Nawwar, A.M., and Wassel, M.S. (1993): Serological studies on the immune response against duck hepatitis virus vaccine. Assiut Vet. Med. J. 28(56):140-156.

Levine, P.P. and J. Fabricant. (1950): A hitherto-un-described virus disease of ducks in North America. Cornell Vet.40:71-86.

Levine, P.P. and Hofstad, M.S. (1945): Duck disease investigation. Annual Report New York State Vet. College, Ithaca, New York. P.; 55-56.

Liao, Y.K., Y.S. Lu, and D.F. Lin. (1989): The outbreak and control of duck viral disease in Taiwan. Chiu Department of Epidemiology, Taiwan Provincial Research Institute for Animal Health, Tansui, R.O.C. on Taiwan. 1991-09-01.

Mahmoud, A.A. (1980): The serological response of ducklings vaccinated with a local isolate of duck virus hepatitis attenuated in embryonated chicken eggs. Thesis M. Sc., poultry dis. Cairo Univ.

Mishra, S.S and Mallick, B.B. (1997): Detection of fowl pox virus using immunoperoxidase test and fluorescent antibody technique. Indian Vet..J.74:199-202.

Reed, L.J. and Muench H.H. (1938): A simple method of estimating 50% end point. Am. J. Hyg. 27:493-497.

Refaie, El-R.M. (1967): Studies on some diseases of duckling with special reference to salmonellosis and duck virus hepatitis. Thesis. M. Sc., Assiut Univ.

Senne, D. A. (1989): Virus propagation in embryonating eggs. A laboratory manual for the virus iolation and identification of avian pathogens. The Am. Ass. Of Avian pathologist, 3rd ed. Pp. 176-181.

Shalaby, M.A. (1972): Some studies on viruses affecting ducks with characterization of the isolates. Thesis M. Sc., Poultry dis., Cairo Univ.

Sokkar, I.M.H., Bahagt, A.M., and Refaie, El-R.M., et al. (1975): Some virological and histopathological studies on duck virus hepatitis infection of ducklings at Assiut Province. Egypt. Vet.Med. J., Cairo Univ. 23:75-86.

Takehara, K., Hyakutake, K., Imamura, T., et al. (1994): Isolation**,** identification, and plaque titration of parvovirus from Muscovy ducks in Japan. Avian Dis. 38:810-815.

Taurraso, N. M., Coughill, G. E., and Klutch, M. J. (1969): Properties of the attenuated vaccine strain of duck hepatitis virus. Avian Dis. 13: 321-329.

Thomas, E. Toth. (1969): Studies of agent causing mortality among ducklings immune to duck virus hepatitis. New York State Vet. College, Cornell Univ., Department of Avian Dis., Duck Research Laboratory Eastport, Long Island, New York 11941.

Toth, T.E. (1969b): Studies of an agent causing mortality among ducklings immune to duck virus hepatitis. Avian Dis. 13:834-846.

Toth, T.E. and N.I. Norcross. (1981b): Humoral immune response of the duck to duck hepatitis virus: virus-neutralizing viruses. Virusprecipitating antibodies. Avian Dis. 25:17-28.

Villegas, P. and Purchase, H.G. (1989): Virus titration. A laboratory manual for the isolation and identification of avian pathogenesis. The Am-Assoc. of avian pathologist. 3rd ed. pp. 186-190.

Vertinskii, K.I., Bessarabov, B.F., Kurilenko, A.N., et al. (1968): Pathogenesis and diagnosis of duck viral hepatitis. Veterinariya 7:27-30. Abstr. Vet. Bull. 39:2074.

Vyas, G.N. and Shulman, N.R. (1970): Hem-agglutination assay for antigen and antibody associated with viral hepatitis. Science. 170, 332.

Wachendorfer, G. (1965): Das Agar-prazipitationsverfahren bei derEntenhepatitis, der

Newcastle-Krankheit und besonders der klassischen Schweinepest-Seine Leistungsfahigkeit und Grenzen in der Virusdiagnostik. Zentralbl Veterinamed. 12:55-56, Dis. of Poultry, 10thedition, edited by B. W. Calnek.

Woolcock, P.R. (1989): Duck virus hepatitis. In Purchase H.G., et al (eds.): A Laboratory Manual for the Isolation and Identification of Avian Pathogens 3rd ed. Dubuque, Kendall / Hunt Publishing Co., pp.;152-155.

Woolcock, P.R. (1996): Duck Virus Hepatitis. In Manual of Standards for diagnostic Tests and Vaccines, 3rd ed. Office International des Epizooties, Paris, France. 561-567.

Woolcock, P.R. (1998): Duck Hepatitis. In D.E. Swayne, et al. A LaboratoryManual for the isolation and identification of Avian Pathogens, 4thedition. American Association of Avian Pathologists. P.A. 200-204.

الملخص العربى

دراسات فيرولوجية على فيروس الالتهاب الكبدى للبط نوع -١فى صغار البط فى محافظتى الدقهلية ودمياط

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***قسم الفيروسات – كلية الطب البيطرى- جامعة المنصورة**

تم تجميع عينات من الكبد و الطحال و المرارة و البنكرياس و الكلى و زرق البط المصاب أو النافق حديثا لاعراض تشبه أعراض التهاب الكبد الوبائى. تم عمل فحص بكتيرى على كل العينات المجمعة وتم تحضير كمية من مصل مضاد لفيروس التهاب الكبد الوبائى فى الأرانب. تم عزل الفيروس فى بيض الدجاج المخصب عن طريق حقن الفيروس في التجويف الالنتوسى ثم تمت معايرة الفيروس المعزول. قد تم التعرف على الفيروس المعزول باستخدام اختبارات الترسيب فى الاجار و اختبار الفيروس المتعادل. وأخيرا تم حقن الفيروس تجريبيا فى البط الصغير عمر٥ أيام لعزل الفيروس و كذلك للكشف عن وجود الفيروس في أنسجة البط المحقون باستخدام اختبار الفلورسنت المناعى غير المباشر للعينات المأخوذة من البط .

الفيروس المعزول من البط الصغير المصاب بأعراض عصبية و اسهال مع نسبة وفيات كبيرة هو فيروس التهاب الكبد الوبائى للبط نوع-١ و ذلك بعد تعريفة بالاختبارات السابقة و تبين أن اختبار الفيروس المتعادل أكثر حساسية للتعرف على فيروس التهاب الكبد الوبائى للبط من اختبار الترسيب فى الآجار. و بتتبع السير المرضى للفيروس فى التجويف الألنتوسى لبيض الدجاج المخصب وجد أنه يمكن الكشف عن وجود الفيروس بعد يومين من الحقن. كما تم عزل الفيروس فى البط الصغير عمر٥ أيام وأعطى الفيروس المعزول من البط المحقون تجريبيا نفس أعراض التهاب الكبد الوبائي و تأكد ذلك بالكشف السريع عن وجود الفيروس باستخدام الاختبار الفلورسنتى غير المباشر.