

**Pathogenicity studies of a DHVA-1 isolate
(DVH/EG. Bayoumieh-Sharkia-2015) in three duck breeds
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Abstract

Pathogenicity studies were performed on DHVA-1 isolate designated (DVH/EG. Bayoumieh-Sharkia-2015) through the inoculation of (Pekin, Mallard and Muscovy) Ducklings at (7, 14 and 21) day of age. Body weight, hematological picture, liver functions examination, pathological scoring of liver lesion post challenge., beside the observed mortality and morbidity were the assessment criteria used for evaluation of the pathogenicity of the fore mentioned DHVA-1 isolate.

Mortality was only recorded in the Pekin ducklings. It started 48 hrs. after the S/C inoculation of $10^{3.7}$ EID₅₀/ duckling, mortality reached 50% when ducklings were inoculated at 7 days of age and was 25% when ducklings were inoculated at 14 day of age while no mortality was recorded when they were inoculated at 21 day of age. Dead ducklings were showing opisthotonic posture and had congested hemorrhagic livers on autopsy.

Recorded body weight in inoculated ducklings at 7, 14, and 21 day of age as an indicative for performance., revealed that both ducklings of the Mallard breed and those of the Muscovy breed had a significantly lower body weight compared to their control ducklings although they were not expressing clinical disease or mortality post inoculation with (DVH/EG. Bayoumieh-Sharkia-2015). And it became clear for us that., although the Mallard and the Muscovy breeds were clinically resistant for DHAV-1 when inoculated at 7, 14, and 21 day of age but they had a reduced body weight, alterations in the blood picture, had pathological liver alterations known for DVH beside disturbance in the liver functions.

Abbreviations: SAN=specific antibody negative, ECE= embryonated chicken eggs, DVH=duck virus hepatitis.

Key words: DVH, Pathogenicity, Performance, CBC, Liver functions, liver lesion score, AST, ALT, Albumen.

Introduction

Duck hepatitis (DH) is a highly fatal, rapidly spreading viral infection of young ducklings characterized primarily by hepatitis. The disease can be caused by at least five different viruses. The most pathogenic is a member of the newly proposed Picornavirus genus (*Avihepatovirus*) duck hepatitis A virus 1(DHAV-1) **Woolcock and Hsiang-Jung Tsai (2013).**

Song et al., (2014) Studied the effect of age on

the pathogenesis of DHV-1 in Peking ducks and on the innate immune responses of ducks to infection and they found that Duck hepatitis virus (DHV) affects 1week old but not 3week-old ducks, and it causes a more severe disease in the younger ducks.

Chung-Hsi Chou et al., (2013) found that. Unlike other usual DVH pathogen belonging to the genus of *Avihepatovirus*, strain TW90A would not cause any typical mortality or hem-

orrhagic lesions of hepatitis in ducklings. In their experimental infection trials, neither mortality nor any hepatic lesions were found at any age groups. Nevertheless, body weight gain was found significantly reduced in infected ducklings and it was implied that subclinical infection of DPV may have potential causing economic loss in duck production. And they are currently conducting experiments to determine the pathogenesis causing the lower weight gain during the infection.

Woolcock (2008) mentioned that morbidity for DVH is 100% and mortality is variable in young ducklings infected with DHV -1. In some broods less than 1 wk old, mortality may reach 95%. In ducklings 1–3 wk of age, mortality may be 50% or less. In ducklings 4–5 wk of age, morbidity and mortality are low or negligible.

Woolcock and Hsiang-Jung Tsai (2013) mentioned that **(Tseng and Tsai, unpublished data)** found had recorded that Tsiay duck (*Anas platyrhynchos* var. domestica) and Cherry Valley ducklings were highly sensitive, and Pekin ducklings and hybrid ducklings of Tsiay duck and Pekin duck were relatively sensitive. Muscovy ducklings were resistant, whereas mule ducklings showed intermediate sensitivity to infection.

In the present study it was aimed to study the pathogenicity of the isolated DHVA-1 designated (DVH/EG. Bayoumie-Sharkia-2015) in the commercially available ducklings in Egypt and we were focusing on a different hypothesis (does the clinically resistant duck breeds for DVH such as Mallard and Muscovy develop pathological liver alteration while being clinically normal ?, what is the extent of liver impairment after experimental infection with the (DVH/EG. Bayoumie-Sharkia-2015)?, can the encountered pathological lesion justify the recorded reduced performance of those clinically resistant breeds. ?) and it became clear to us that., although the Mallard and the Muscovy breeds were clinically resistant for DHAV -1 when inoculated at 7, 14, and 21 day of age but they had a reduced body weight, they had alterations in the blood picture, they had patho-

logical liver alterations known for DVH and they have disturbance in the liver functions.

Materials

DVH Field isolate.

A DHVA-1 isolate designated (DVH/EG. Bayoumie-Sharkia-2015) previously isolated from Sharkia province and molecularly characterized by **Bayoumie and Abd EL-Samie (2015)** was used.

Ducklings.

Hundred and twenty ducklings (40 Pekin, 40 Mallard and 40 Muscovy) were used in the present study.

ECE

Nine-day-old SAN -ECE were used for reisolation trials of DHV-1.

Membrane filters:

450 nm Thermo scientific Nalgene syringe filter. Cat-no. 190-2545 (8-0404-40493), 200 nm Thermo scientific syringe filter. Nalgene cat. No. 190-2520 (8-0404-391109). 100 nm Sigma Aldrich syringe filter cat. No. (F-7523) LOT#MKBW3870, PC Code 1002103112, mfr.no:SLVV33RS

Ration

Starter pelleted ration “3000 k Cal MEenergy, 21% protein and 2.88% fat obtained from “commercial source” ration were used ad libitum.

Cal - heparine[®] (AMOUN).

Cal heparine 5000 I.U. B.N.1529798 manf. 7/2015 exp. 7/2018 was used as an anticoagulant.

Methods

Experimental design.

Forty one day old ducklings of each of the Pekin, Mallard and Muscovy duck breeds were used in the present study. Ten ducks were kept as negative control and at each time interval of the experiment at (7, 14 and 21 day) {eight ducklings were inoculated with aforementioned DVH-1 isolate while two ducklings were sacrificed to obtain samples prior to challenge procedure}. At each time interval ducklings were weighted, blood was collected. Portion of blood was collected on Cal heparin and

another portion was processed to obtain sera for determination of liver functions.

Viral reisolation.

Viral re isolation was performed as previously described by **Bayoumie, and Abd EL-Samie (2015)**.

Viral titration.

Titer calculation was preformed according to **Spearman & Karber** in **Cunningham (1973)**.

Hematological examination.

Hematological assay was performed using automated animal hematology analyzer (**SYSMEX-XT-2000 iv**)

Histopathological examination

Specimens from liver and spleen were collected (at the end of each trial) from duckling., after post mortem examination and fixed in 10% buffered neutral formalin solution then dehydrated in gradual ethanol (70-100%), cleared in xylene and embedded in paraffin, five micron sections were prepared and then routinely stained with hematoxylin and eosin

(H&E) according to **Suvarna *et al.*, (2013)**. Then examined.

Clinico -chemical assay.

Serum Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed according to **Henry (1974)** as an indicative for liver functions. Albumen was performed as described by **Bing and Lim (1978) and Webster (1982)**.

Live body weight.

The body weight (BWT), body weight gain (BWG). were used as indicators for performance

Statistical analysis.

Data were statistically analyzed as described by **Snedecor and Cochran (1967)** using SPSS.16 computer program, value was used to determine significance.

Results

Results are as shown in table (1-10) and plate - 1 containing figures (1-9).

Table (1). Shows the titration of DHVA-1 isolate (DVH/EG. Bayoumieh-Sharkia-2015) in ECE.

10 fold serial dilution	-1	-2	-3	-4	-5	-6
ECE Mortality			5/5	5/5	4/5	2/5
Titer = $6+0.5-4/5 = 10^{5.7}$ ID ₅₀ /0.1ml according to Spearman & Karber in Brian and Kangro(1996) .						

Table (2). Shows the observed mortality of experimentally inoculated ducklings.

Age	Breed					
7 days	Pekings		Mallard		Muscovy	
	Control	Infected*	Control	Infected	Control	Infected
	0/8	4/8 (50%)	0/8	0/8	0/8	0/8
14 day	0/8	2/8 (25%)	0/8	0/8	0/8	0/8
21 day	0/8	0/8	0/8	0/8	0/8	0/8

*= mortality in inoculated ducklings was confirmed to (DVH/EG. Bayoumieh-Sharkia-2015) through reisolation on ECE after passing 100 nm membrane filter Bayoumie, and Abd EL-Samie (2015). 2 ducks were scarified of the group of 10 to obtain samples before challenge.

Table (3). Shows the Starting body weight at 7, 14 and 21days. And body weight at the end of experimental trial post challenge with DHVA-1 isolate (DVH/EG. Bayoumieh-Sharkia-2015), and their figure illustration.

Table (3-a). Shows starting body weight at 7 days and at the end of experimental trial.

B	G		Starting 7 D B.wt	End of trial
Pekin	C	av	87.55±4.47	185.5±10.5 *
		ooo	14.81	14.85
	Inf	av		178.5±9.22
		ooo		22.58
Mallard	C	av	93.68±3.25	228.5±7.5 **
		ooo	10.78	10.61
	Inf	av		177.71±8.22
		ooo		21.74
Muscovy	C	av	125,2±14,4	263±2,03***
		ooo	32,41	4.53
	Inf	av		119,6±2,91
		ooo		6.5

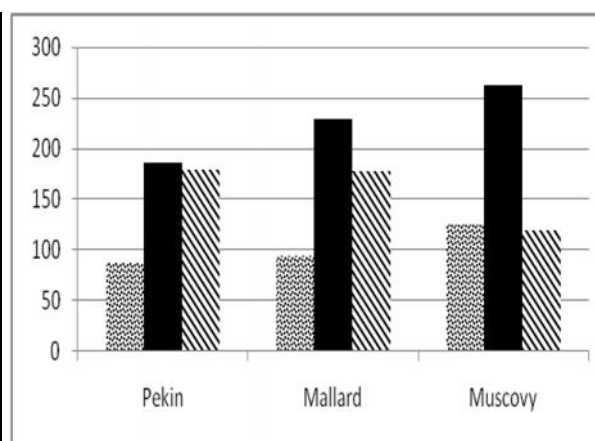


Table (3-b). Shows starting body weight at 14 days and at the end of experimental trial.

B	G		Starting 14 D B.wt	End of trial
Pekin	C	av	245,5±4,91	364±16.35 *
		ooo	13,89	36.55
	Inf	av		359.5±9.91
		ooo		28.04
Mallard	C	av	341,25±18,12	429.6±2.23*
		ooo	51,26	4.98
	Inf	av		360±5.21
		ooo		14.74
Muscovy	C	av	421.2±20.54	540±53.76 **
		ooo	45.93	120.21
	Inf	av		452.4±26.41
		ooo		59.06

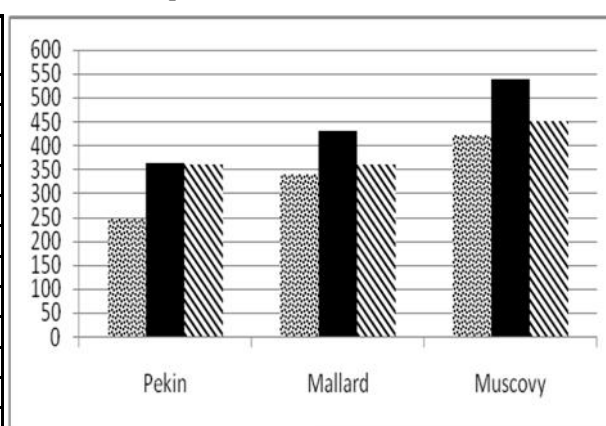
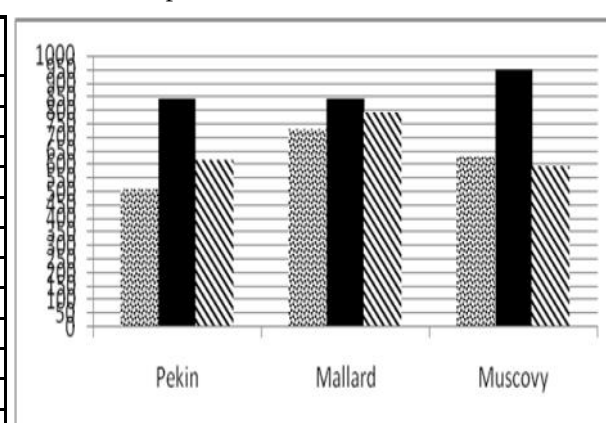


Table (3-c). Shows starting body weight at 21 days and at the end of experimental trial.

B	G		Starting 21 D B.wt	End of trial
Pekin	C	av	509.25±33.63	844.55±20.21*
		ooo	95.13	67.02
	Inf	av		612.38±40.35
		ooo		114.13
Mallard	C	av	725.38±45.58	841.43±36.67 *
		ooo	128.91	97.03
	Inf	av		790.75±46.37
		ooo		131.16
Muscovy	C	av	626,13±56,72	949±127,07 **
		ooo	160,43	220,09
	Inf	av		594,4±44,1
		ooo		98,61

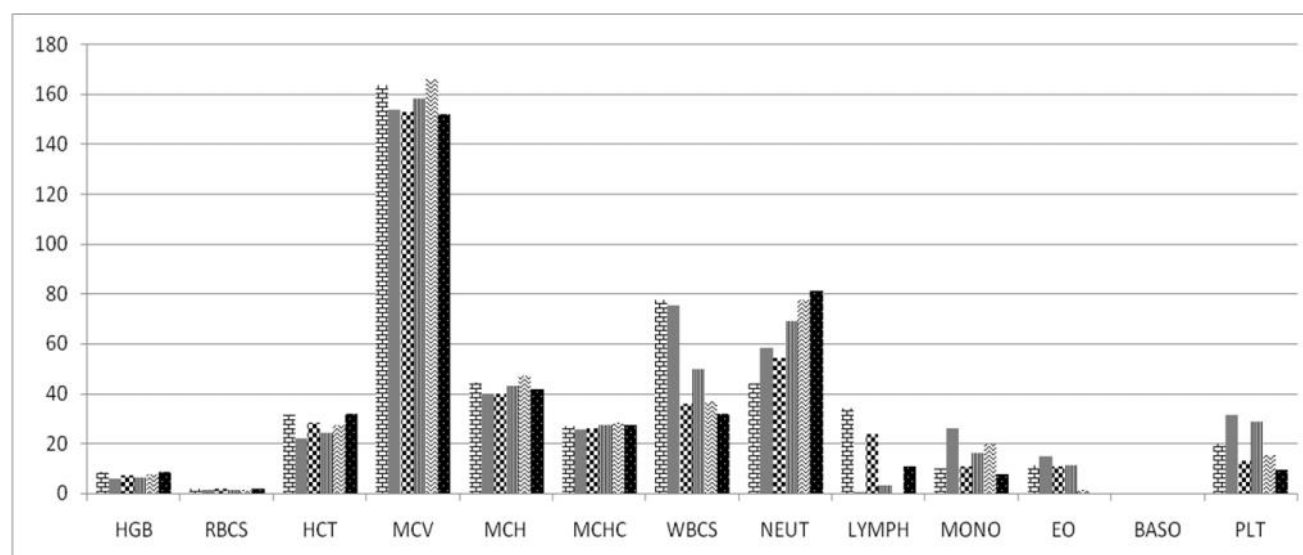


Starting weight body weight of Control body weight of infected

C= control, Inf= infected, * P< 0.05 ** P< 0.01 *** P< 0.001, av=Mean ± SE, ooo. = st deviation , B=breed, G=group.

Table (4). Shows the hemogram post challenge with DHVA-1 isolate (DVH/EG. Bayoumieh-Sharkia-2015) at 7 days of age and their figure illustration.

		Pekin				Mallard				Muscovy			
		C		Inf		C		Inf		C		Inf	
		av	°°°	av	°°°	av	°°°	av	°°°	av	°°°	av	°°°
CBC- post challenge at 7 days	HGB(g/dl)	8.63±0.67 **	1.16	5.7±0.49	0.85	7.33±0.74 *	1.29	6.57±0.23	0.4	7.75±0.15 **	0.21	5.75±0.05	0.07
	RBCS(10 ⁶ / ul)	1.93±0.1 *	0.17	1.45±0.2	0.35	1.84±0.05 *	0.08	1.54±0.16	0.27	1.65±0.01 **	0.02	2.09±0.05	0.07
	PCV(%)	31.7±1.85 **	3.21	22.2±2.57	4.45	28.17±0.4 *	0.7	24.17±1.67	2.9	27.3±0.2 **	0.28	31.75±0.65	0.92
	MCV(fl)	163.83±1.84 *	3.19	153.83±4.08	7.07	153.23±3.49 *	6.05	158.4±7.83	13.56	166±2.7 **	3.82	151.95±0.55	0.78
	MCH(pg)	44.57±1.96 *	3.39	39.8±2.17	3.75	40.03±4.72 *	8.17	43.37±3.2	5.55	47.1±0.5 **	0.71	41.85±0.75	1.06
	MCHC(g/dl)	27.2±0.93 *	1.61	25.83±0.8	1.39	26.03±2.53 *	4.39	27.3±1.01	1.75	28.4±0.8 *	1.13	27.6±0.4	0.57
	WBCS(10 ³ / ul)	77.57±55.07 *	95.39	75.42±16.18	28.02	35.81±16.24 *	28.13	49.88±22.15	38.36	36.98±4.36 *	6.17	32.14±1.32	1.87
	NEUT (%)	44.33±20.26 *	35.1	58.53±13.26	22.96	54.3±20.92 *	36.23	69.27±11.73	20.32	77.65±5.05 *	7.14	81.45±0.55	0.78
	LYMPH (%)	34.27±28.68 *	49.67	0.47±0.47	0.81	23.97±22.57 *	39.09	3.1±2.3	3.99	0.65±0.65 **	0.92	10.9±1.9	2.69
	MONO (%)	9.87±4.7 *	8.14	25.97±7.85	13.6	11.03±5.71 *	9.89	16.3±8.16	14.13	19.9±5.3 *	7.5	7.65±1.35	1.91
	EO (%)	11.53±5.01 *	8.67	15.03±5.33	9.24	10.7±3.94 *	6.82	11.33±5.33	9.24	1.65±1.05 *	1.48	0	0
	BASO (%)	0	0	0	0	0	0	0	0	0	0	0	0
	PLT(10 ³ /UL)	19.67±8.84 *	15.31	31.33±21.53	37.29	13±5.69 *	9.85	29±15.57	26.96	15.5±1.5 *	2.12	9.5±2.5	3.54

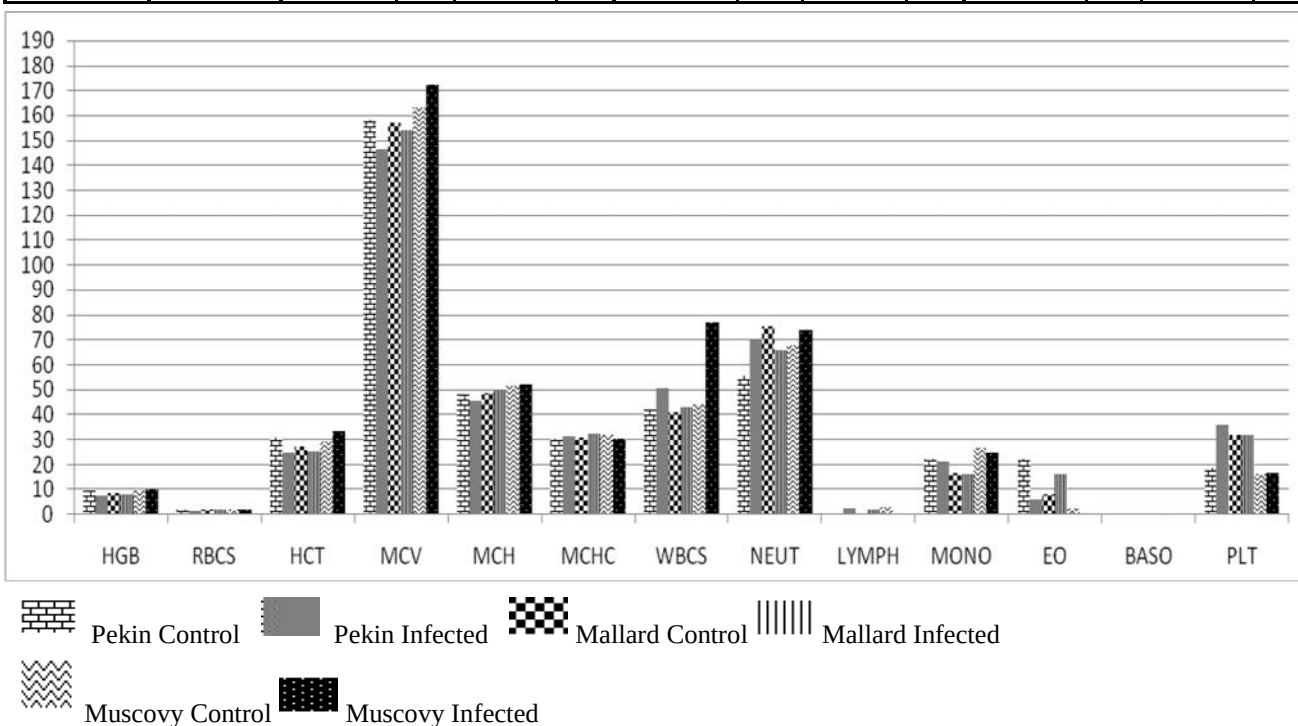


 Pekin Control
  Pekin Infected
  Mallard Control
  Mallard Infected
  Muscovy Control
  Muscovy Infected

C= control, Inf= infected, * P< 0.05 ** P< 0.01 *** P< 0.001, av=Mean ± SE, °°° = st deviation,

Table (5). Shows the hemogram post challenge with DHVA-1 isolate (DVH/EG. Bayoumieh-Sharkia-2015) at 14 days of age and their figure illustration.

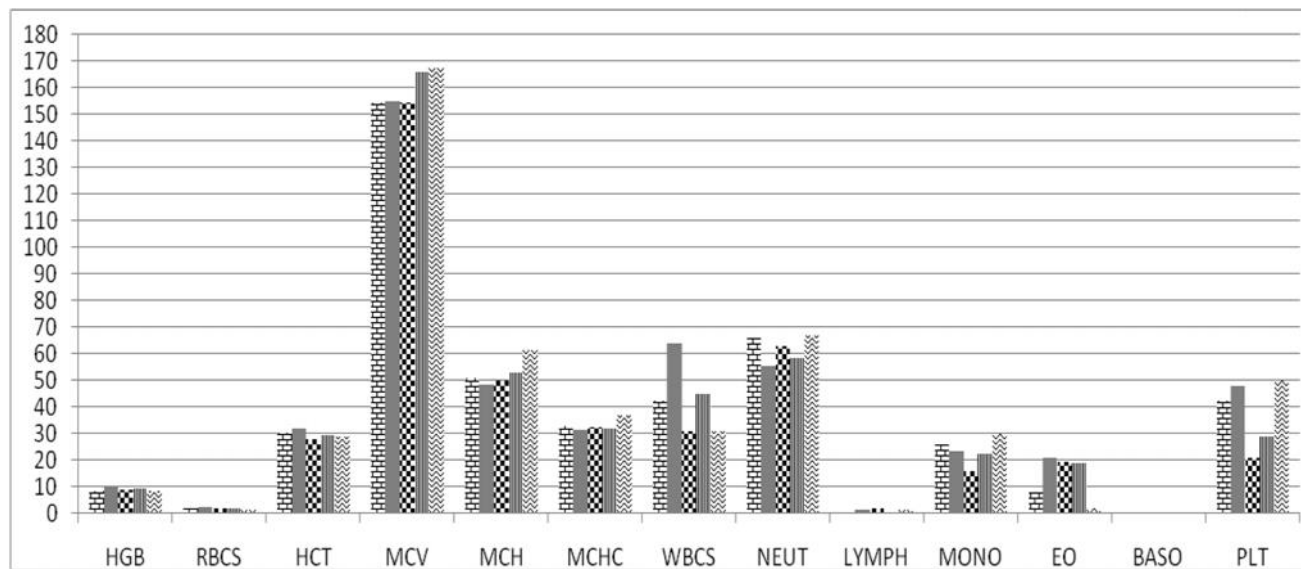
		Pekin				Mallard				Muscovy			
		C		Inf		C		Inf		C		Inf	
		av	°°°	av	°°°	av	°°°	av	°°°	av	°°°	av	°°°
CBC- post challenge at 14 days	HGB(g/dl)	9.35±0.65*	0.92	7.77±0.48	0.84	8.4±0.4 *	0.57	8.1±0.46	0.79	9.3±0.36 *	0.62	10.13±0.89	1.54
	RBCS(10 ⁶ / ul)	1.95±0.13 *	0.19	1.7±0.1	0.18	1.74±0.09 *	0.13	1.68±0.16	0.28	1.8±0.07 *	0.12	1.94±0.2	0.35
	PCV (%)	30.65±1.65 *	2.33	24.93±1.82	3.16	27.25±1.55 *	2.19	25.37±2.56	4.44	29.3±1.25 *	2.16	33.47±3.29	5.69
	MCV(fl)	157.75±2.45 *	3.46	146.33±5.0 ₅	8.74	157.05±0.35 *	0.49	153.97±4.47	7.74	163.13±4.19 *	7.26	172.43±1.89	3.28
	MCH(pg)	48.1±0 *	0	45.63±0.88	1.53	48.45±0.35 *	0.49	49.57±2.17	3.75	51.77±1.18 *	2.05	52.33±0.98	1.69
	MCHC(g/dl)	30.5±0.5 *	0.71	31.23±0.52	0.9	30.85±0.25 *	0.35	32.27±1.62	2.81	31.73±0.14 **	0.25	30.33±0.27	0.47
	WBCS(10 ³ / ul)	41.91±10.68 *	15.11	50.69±6.94	12.02	41.14±0.86 *	1.21	43.03±8.45	14.64	44.19±15.37 **	26.62	76.67±7.02	12.16
	NEUT (%)	55.55±15.65 *	22.13	70.17±2.52	4.36	75.45±7.65 *	10.82	65.93±12.53	21.71	67.73±3 *	5.19	74.1±8.34	14.45
	LYMPH (%)	0	0	2.4±1.23	2.13	0	0	2.1±1.91	3.3	3.03±0.62 **	1.07	0.63±0.35	0.6
	MONO (%)	22.1±2.8 *	3.96	21.5±1.16	2.01	16.6±2.2 *	3.11	15.93±1.82	3.15	26.67±3.94 *	6.82	24.53±7.55	13.08
	EO (%)	22.35±12.85 *	18.17	5.93±1.5	2.6	7.95±5.45 *	7.71	16±10.24	17.74	2.57±0.61 *	1.06	0.7±0.47	0.82
	BASO (%)	0	0	0	0	0	0	0.03±0.03	0.06	0	0	0.03±0.03	0.06
	PLT(10 ³ /UL)	18.5±3.5 *	4.95	36±14.05	24.33	32±2 *	2.83	32±11.02	19.08	16±10.02 *	17.35	16.67±9.21	15.95



C= control, Inf= infected, * P< 0.05 ** P< 0.01 *** P< 0.001, av=Mean ± SE , °°° = st deviation ,

Table (6). Shows the hemogram post challenge with DHVA-1 isolate (DVH/EG. Bayoumieh-Sharkia-2015) at 21days of age and their figure illustration.

CBC- post chal- lenge at 21 days		Pekin				Mallard				Muscovy			
		C		Inf		C		Inf		C		Inf	
		av	°°°	av	°°°	av	°°°	av	°°°	av	°°°	av	°°°
	HGB(g/dl)	8,27±0,35 *	0,6	9,83±0,47	0,81	8,83±0,7 *	1,21	9,3±0,65	1,13	8,23±0,72 *	1,24	10,05±0,65	0,92
	RBCS(10 ⁶ / ul)	1,64±0,12 *	0,2	2,03±0,12	0,2	1,78±0,14 *	0,25	1,77±0,16	0,28	1,34±0,14 **	0,24	2,01±0,06	0,08
	PCV(%)	30,07±0,58 *	1,01	31,57±2,15	3,73	27,63±2,66 *	4,6	29,33±2,6	4,51	29±0,58 *	1	32,45±1,75	2,47
	MCV(fl)	154,43±2,41 *	4,18	155,07±2,16	3,74	154,37±2,49 **	4,32	166,2±3,19	5,53	167,5±1,36 *	2,36	161,7±4,3	6,08
	MCH(pg)	50,6±2,23 *	3,86	48,4±0,58	1	49,57±0,58 *	1	52,93±2,48	4,29	61,53±1,15 **	2	50,05±1,85	2,62
	MCHC(g/dl)	32,73±1,15 *	1,99	31,23±0,69	1,19	32,07±0,53 *	0,91	31,83±0,94	1,62	36,73±0,53 ***	0,91	30,95±0,35	0,49
	WBCS(10 ³ / ul)	42,5±14,72 *	25,5	63,59±1,84	3,18	30,91±4,15 *	7,19	44,84±7,37	12,76	30,91±4,15 *	4,15	31,62±5,06	7,16
	NEUT(%)	66,1±6,41 *	11,1	55,13±9,31	16,12	63,1±6,63 *	11,49	58,27±12,12	21	67,13±6,27 *	10,86	73,65±9,15	12,94
	LYMPH(%)	0,27±0,22 *	0,38	1±0,85	1,48	1,7±0,98 *	1,7	0,37±0,37	0,64	1,5±0,49 *	0,85	3,15±3,15	4,45
	MONO(%)	25,73±3,5 *	6,06	23,07±2,17	3,76	15,8±4,1 *	7,1	22,47±10,82	18,74	29,63±4,36 *	7,55	17,9±5	7,07
	EO(%)	7,8±3,25 *	5,63	20,73±11,73	20,32	19,17±9,37 *	16,23	18,87±16,21	28,07	1,73±1,48 *	2,57	5,3±1	1,41
	BASO(%)	0,07±0,03 *	0,06	0,07±0,03	0,06	0,23±0,23 *	0,4	0,03±0,03	0,06	0±0	0	0±0	0
	PLT(10 ³ /UL)	42,33±22,98 *	39,8	47,67±6,96	12,06	21±10,81 *	18,73	28,67±15,07	26,1	50±6,93 *	12	36,5±6,5	9,19

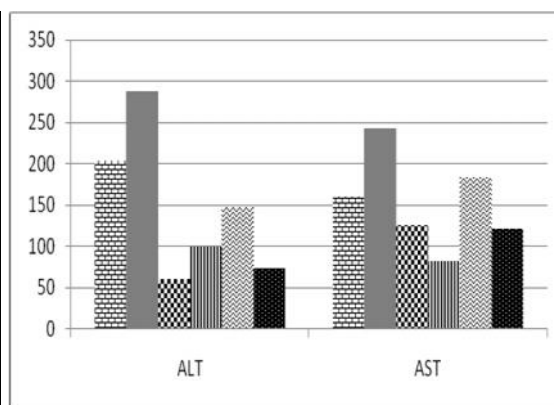


Pekin Control
 Pekin Infected
 Mallard Control
 Mallard Infected
 Muscovy Control
 Muscovy Infected

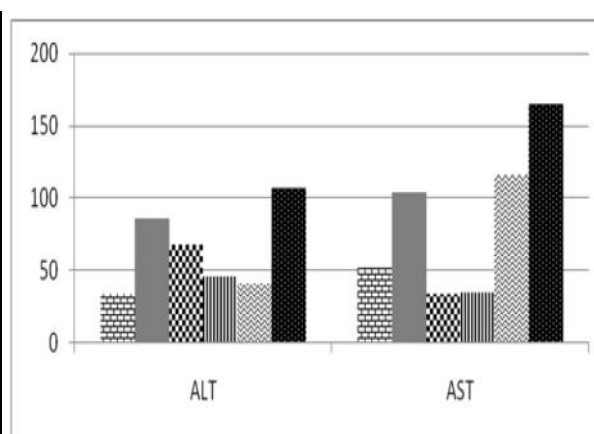
C= control, Inf= infected, * P< 0.05 ** P< 0.01 *** P< 0.001, av=Mean ± SE , °°° = st deviation ,

Table (7). Shows ALT and AST., at the end of experimental trial (at 7, 14 and 21days) post challenge with DHVA-1 isolate (DVH/EG. Bayoumieh-Sharkia-2015)., and their figure illustration.**Table (7-a).** Shows ALT and AST at the end of experimental trial at 7 days and their figure illustration.

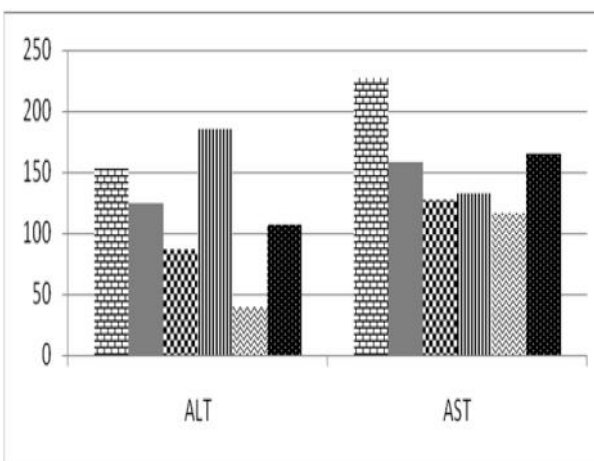
			AST	ALT
Pekin	C	av	159,67±22,81 *	204,33±24,66 *
		ooo	39,5	42,72
	Inf.	av	242,33±97,84	287,67±82,34
		ooo	169,47	142,61
Mallard	C	av	125,33±30,63 *	61±8,02 *
		ooo	53,05	13,89
	Inf.	av	82±17,01	99,33±26,18
		ooo	29,46	45,35
Muscovy	C	av	183,67±46,19 *	147,67±32,54 *
		ooo	80	56,36
	Inf.	av	121±20,31	73±18,15
		ooo	35,17	31,43

**Table (7-b).** Shows ALT and AST at the end of experimental trial at 14 days and their figure illustration.

			AST	ALT
Pekin	C	av	51,33±11,7 *	33±2,64 *
		ooo	20,26	4,58
	Inf.	av	103,33±42,78	85,33±22,83
		ooo	74,1	39,55
Mallard	C	av	33,33±5,81 *	68±10,41 *
		ooo	10,07	18,03
	Inf.	av	34±2	45±14
		ooo	2,83	19,8
Muscovy	C	av	116±25,24 *	40±4,04 *
		ooo	43,71	7
	Inf.	av	165±32,19	106,33±33,52
		ooo	55,75	58,05

**Table (7-c).** Shows ALT and AST at the end of experimental trial at 21 days and their figure illustration.

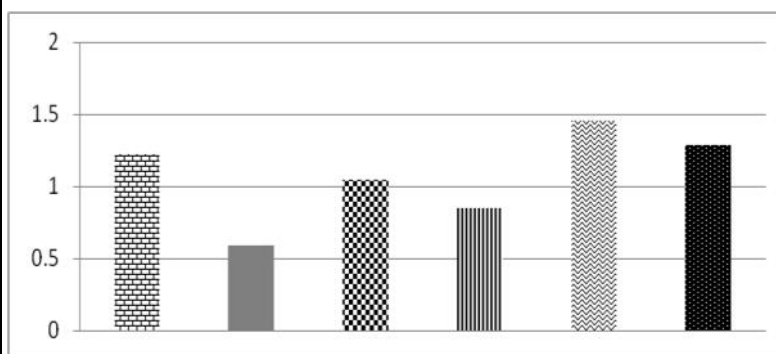
			AST	ALT
Pekin	C	av	227,67±96,29 *	153,33±20,92 *
		ooo	166,78	36,23
	Inf.	av	157,67±50,78	124,67±32,83
		ooo	87,95	56,87
Mallard	C	av	127,67±48,7 *	87±33,5 *
		ooo	84,35	58,03
	Inf.	av	132±18,68	184,67±50,41
		ooo	32,36	87,31
Muscovy	C	av	116±25,24 *	40±4,04 *
		ooo	43,71	7
	Inf.	av	165±32,19	106,33±33,52
		ooo	55,75	58,05



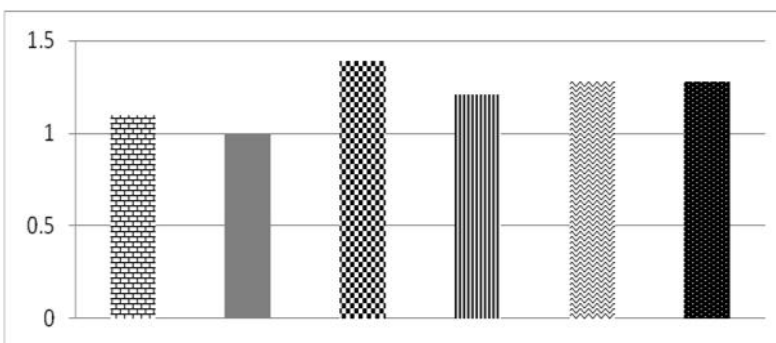
* P< 0.05, ** P< 0.01, *** P< 0.001, ooo st deviation, av=Mean ±SE, C=control, Inf=infected

Table (8). Shows Albumin., at the end of experimental trial (at 7, 14 and 21days) post challenge with DHVA-1 isolate (DVH/EG. Bayoumieh-Sharkia-2015), and figure illustration.**Table (8-a).** Shows Albumin at the end of experimental trial at 7 days and figure their illustration.

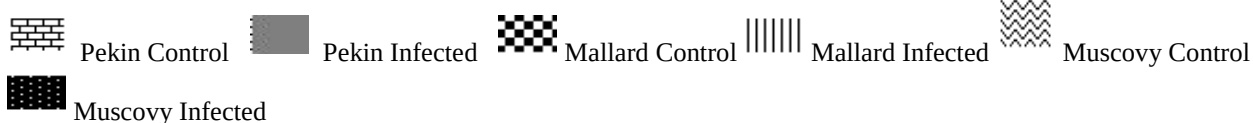
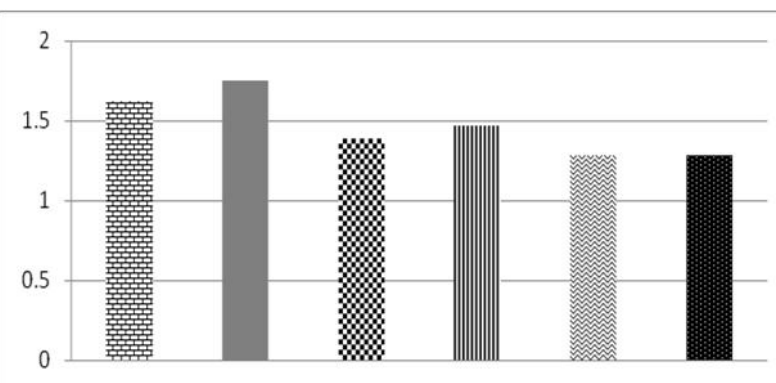
Albumin			
Pekin	C	av	1,22±0,02
		ooo	0,04
	Inf.	av	0,59±0,03
		ooo	0,06
Mallard	C	av	1,05±0,1 *
		ooo	0,18
	Inf.	av	0,85±0,06
		ooo	0,1
Muscovy	C	av	1,46±0,02 *
		ooo	0,04
	Inf.	av	1,29±0,07
		ooo	0,12

**Table (8-b).** Shows Albumin at the end of experimental trial at 14 days and figure their illustration.

Albumin			
Pekin	C	av	1,1±0,16 *
		ooo	0,27
	Inf.	av	0,99±0,04
		ooo	0,07
Mallard	C	av	1,39±0,08 *
		ooo	0,14
	Inf.	av	1,21±0,01
		ooo	0,02
Muscovy	C	av	1,28±0,06 *
		ooo	0,11
	Inf.	av	1,28±0,04
		ooo	0,07

**Table (8-c).** Shows Albumin at the end of experimental trial at 21 days and figure their illustration.

Albumin			
Pekin	C	av	1,62±0,16 *
		ooo	0,28
	Inf.	av	1,75±0,02
		ooo	0,03
Mallard	C	av	1,39±0,12 *
		ooo	0,2
	Inf.	*	1,47±0,11
		ooo	0,19
Muscovy	C	av	1,28±0,06 *
		ooo	0,11
	Inf.	av	1,28±0,04
		ooo	0,07



* P< 0.05, ** P< 0.01, *** P< 0.001, ooo st deviation, av=Mean ±SE, C=control, Inf=infected

Table (9). Shows the pathological alterations in the different experimental groups post challenge with DHVA-1 isolate (DVH/EG. Bayoumieh-Sharkia -2015).

group	Hepatic cell lysis	Hepatic cell necrosis	Portal macrophage	Portal Granulocytic infiltration	Hemorrhage	Congestion	Cell swelling	Reticular cell activation	Microste atosis	Other pathologic Lesion.
9-a										
Pekin -7	C	-	-	-	-	-	-	-	-	-
	Inf	-	-	-	+	+	+mild acute	-	+	Chronic cholangitis
Mal-lard-7	C	-	-	-	-	-	-	-	-	-
	Inf	-	+	-	-	+	+mild acute	-	+	Proliferation of bile ductules.
Mus-covy -7	C	-	-	-	-	-	-	-	-	-
	Inf	-	-	-	+	+	+	-	-	-
9-b										
Pekin -14	C	-	-	-	-	-	-	-	-	-
	Inf	-	-	+	-	++	-	-	++	bile duct proliferation ,portal fibrosis and hemosiderrosis
Mal-lard-14	C	-	-	-	-	-	-	-	-	-
	Inf	-	+	-	+	+	+	-	+	-
Mus-covy-14	C	-	-	-	-	-	-	-	-	-
	Inf	-	+	-	+	-	++	-	-	proliferation of bile ductules
9-c										
Pekin -21	C	-	-	-	-	-	-	-	-	-
	Inf	-	+	-	+	-	+	-	-	-
Mal-lard-21	C	-	-	-	-	-	-	-	-	-
	Inf	-	+	-	-	-	-	-	++	bile duct proliferation
Mus-covy-21	C	-	-	-	-	-	-	-	-	-
	Inf	-	+	-	+	+	+	-	+	bile duct proliferation

C= control, Inf= infected.

Table (10). Shows the performance of the negative controls of the grown duck breeds.

Table (10-a). Non infected birds.

Age	Av. of Body Weight of healthy birds		
	Pekin	Mallard	Muscov
7D	87.55	93.68	125.2
10D	185.5	228.5	263
14D	245.5	341.25	421.2
17D	364	429.6	540
21D	509.2	725.38	626.1
24D	844.5	841.43	949

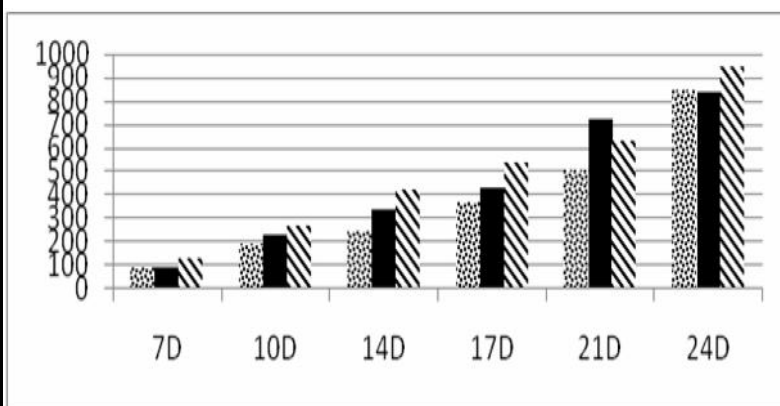
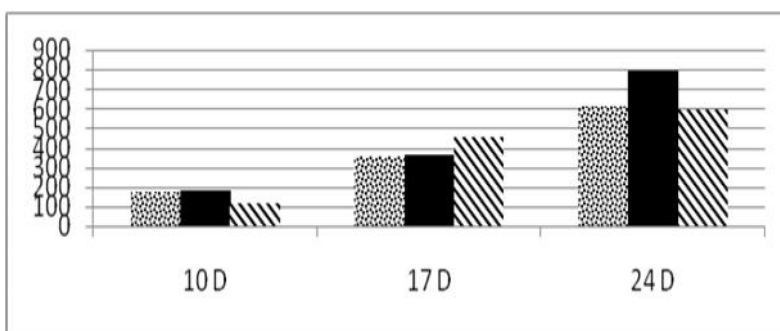


Table (10-b). Infected birds.

	Av. of Body Weight post infection		
	Pekin	Mallard	Muscov
10 D	178,5	177,71	119,6
17 D	359,5	360	452,4
24 D	612,38	790,75	594,4



 Pekin
  Mallard
  Muscovy

Plate -1: Showing the pathological alterations of liver and spleen at the end of challenge trials.

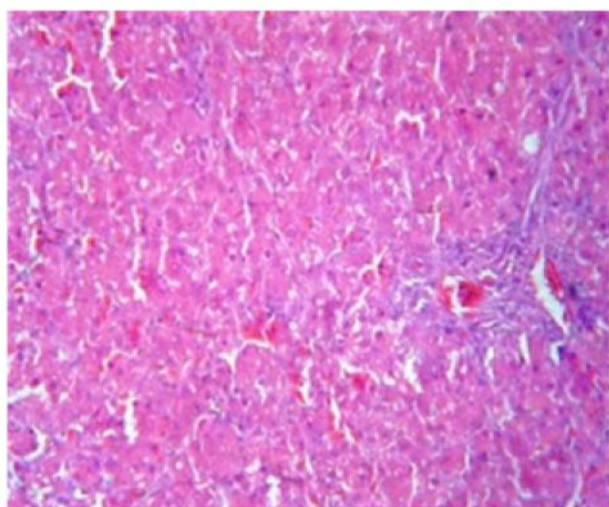


Figure 1. Liver of control Muscovy duckling at 7days showing normal hepatic parenchyma. H&E X1200.

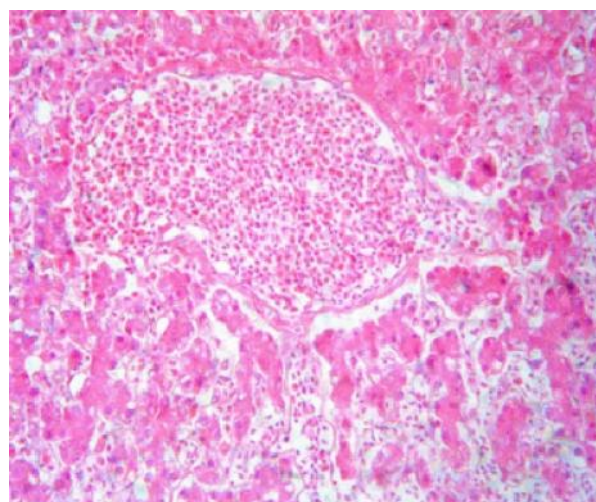


Figure 2. Liver of Pekin duckling at the end of 14 day challenge trial showing severe congestion of blood vessels and hepatic sinusoids. H&E X1200.

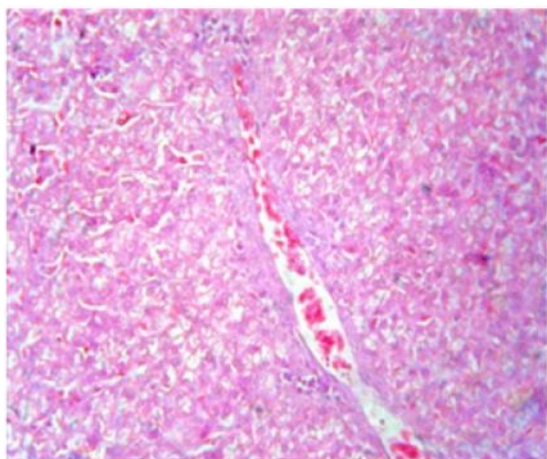


Figure 3. Liver of Mallard duckling at the end of 14 day challenge showing acute cell swelling of the hepatic cells. H&E X 1200.

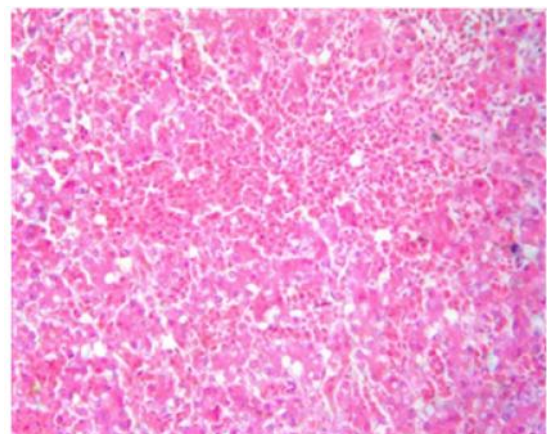


Figure 5. Liver of Mallard duckling at the end of 14 day challenge trial showing hemorrhages and congestion in the hepatic tissue. H&E X1200.

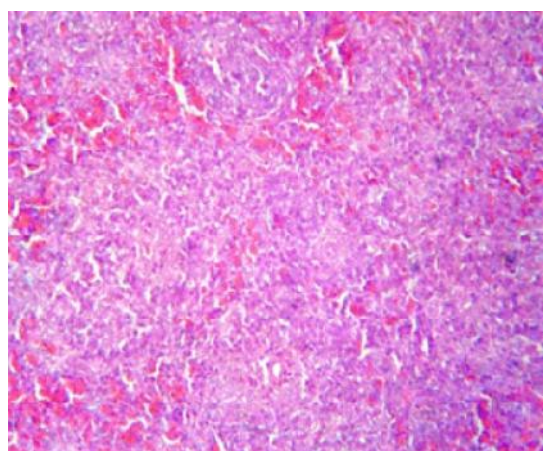


Figure 7. Spleen of Pekin duckling 21days old showing normal splenic parenchyma. H & E X1200.

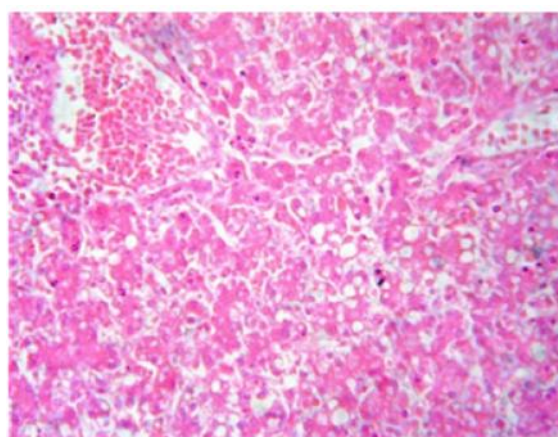


Figure 4. Liver of Mallard ducklings at the end of 21 days trial showing microsteatosis of the hepatic cells and congestion of blood vessels. H&E X1200.

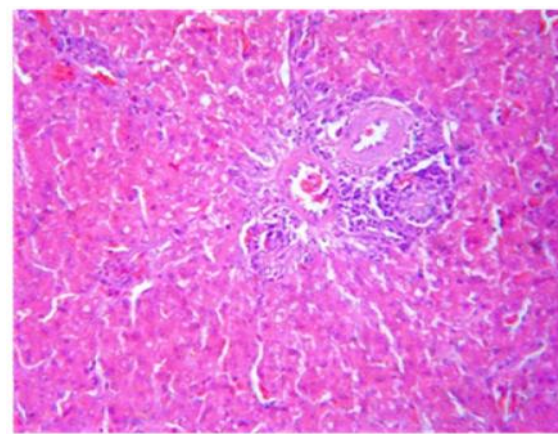


Figure 6. Liver of Muscovy duckling at the end of 14 day challenge trial showing portal mononuclear cell with granulocyte infiltrations and proliferation of bile ductules. H&E X1200.

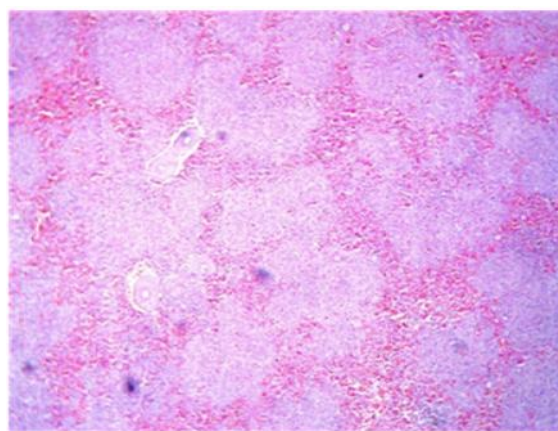


Figure 8. Spleen of Pekin ducklings at the end of 21 days trial showing. Showing intense lymphoid depletion of white pulp. H & E X1200.

Discussion

Previous studies discussing the pathogenicity of duck virus hepatitis (DHV-1) were focusing on one or two prospective namely age and induced mortality following challenge. **Song et al., (2014)** studied the effect of age on the pathogenesis of DHV-1 in Peking ducks and they examined their innate immune responses. They found that Duck hepatitis virus (DHV-1) affects 1-week-old but not 3-week-old ducks and it causes a more severe disease manifestation in the younger ducks. In the present study when challenge was performed at 7 days of age the pekin ducklings showed 50% mortality when inoculated subcutaneously with the DHVA-1 isolate termed (DVH/EG. Bayoumieh-Sharkia-2015). The inoculum was containing $10^{3.7}$ EID₅₀/0.1 ml/ duckling., this breed showed the least affected body weight reduction post challenge compared to the remaining challenged breeds (Mallard and Muscovy). And it worth to mention that Muscovy ducklings' had shown the severest reduction in body weight of all of the inoculated breeds (table 3-a,b,c and their figure illustration) although they remained clinically normal without mortality (table -2).

In the present study when challenge was performed at 14 days of age 12.5% mortality was observed in the Pekin breed only. **Jin et al., (2008)** had similar results. The pekin breed again showed the least body weight reduction post challenge while the Muscovy breed showed the severest body weight reduction post challenge while remaining clinically normal (table 3-a,b,c and their figure illustration). **Song et al., (2014)** interpreted these results based on their study of the up regulation and gene expression of the cytokine storm component which include {(Toll-like receptor (TLR-7, TLR3), retinoic acid-inducible gene – I (RIG-I), melanoma differentiation associated gene 5 (MDA-5), interleukin (IL)-6, interferon (IFN)- α , interferon-induced transmembrane protein 1 (IFITM1), interferon-stimulated gene 12 (ISG-12), and 2'-5' oligo adenylate synthetase-like gene (OASL)} in the livers of 3 W ducks, and

they found that IL-6, OASL and ISG12 mRNA levels were more than 100-fold higher in infected 3 week old ducklings than in non - infected ducks of the same age. These genes were induced much less in infected 1day ducklings. They were able to present evidence that a lower level of viral replication in the hepatocytes of 3 week ducks, whose basal level of cytokines is higher than that in 1D ducklings, may be related to the strong innate immunity induced. Thus they concluded that duck age plays an important role in the pathogenicity and innate immune responses to DHV **Katze et al., (2002)**, **Akira et al., (2006)**, **Kollmann et al., (2012)**. To date, a number of immune-related genes in ducks have been identified **Elfeil et al., (2012)**, **MacDonald and Speert (2008)**, **Wu et al., (2007)**, **Wu et al., (2008)**. The activation of the host innate immunesystem by viruses is partly mediated by pattern-recognition receptors, including retinoic acid-inducible gene-I (RIG-I)-like receptors, and toll-like receptors (TLRs) **Diebold (2009)**, **Kawai, Akira (2009)** such as RIG-I and melanoma differentiation-associated gene 5(MDA-5). RIG-I has a critical role in the recognition of several RNA viruses, such as highly pathogenic avian influenza virus (HPAI), and is essential for the production of interferons in response to RNA viruses such as paramyxoviruses and influenza virus. MDA-5 is critical for the recognition of picornavirus {**Kato et al., (2006)**, **Laskin and Pendino (1995)**, **Opal (2000)**}. Several pro inflammatory cytokines, such as tumor necrosis factor (TNF)- α and interleukin (IL)-2, are produced in response to DHV-1 infections. **Hu (2006)** suggested that cytokine production may play an important role in DHV-1 pathogenesis. The basal level of cytokines in the liver showed that almost all immune-related genes were expressed at a higher level in older ducks than in younger ducks, opposite to the viral load pattern. This suggests that the lower level of virus replication in the hepatocytes of 3 W ducks was due to the higher basal level of cytokines, which induces strong innate immunity. They found a

considerable difference between 1D and 3 W ducks. DHV infection elicited innate immune response in both infection groups at 1D and 3 W, but this response was stronger in the older ducks. RIG-I and MDA-5 are cell-associated pattern-recognition receptors in the innate immune system that are involved in the recognition of microbes **Kawai, Akira (2009)** beside **Tal and Iwasaki (2009)**.

But on the contrary to the previous findings of **Song *et al.*, (2014)**. We can conclude that it is not the age alone during challenge with DHV-1. that manipulates the pathogenicity picture of DHV-1., but also duck breed too, and we thought that it is more wiser to consider age, breed, liver functions, liver pathology, complete blood picture and performance study., because clinically resistant breeds (Mallard and Muscovy) at 7 and 14 day of age showed significant reduction of body weight while being resistant for the disease and it became clear we should consider studying the cytokine storm components in relation to breed resistance and age. ,and it became clear too that we should consider that it was possible to induce a higher mortality if we consider challenging at 1 day since **Woolcock (2008)**, **Clank (1993)** and **Campbell (1984)** Stated that morbidity in DVH is 100% and mortality is variable in young ducklings infected with DHV - 1. In some broods less than 1 wk old, mortality may reach 95%. In ducklings 1–3 wk of age, mortality may be 50% or less. In ducklings 4–5 wk of age, morbidity and mortality are low or negligible. In the present study when challenge was performed at 21 days of age no mortality was observed but again the Muscovy breed showed the severest body weight reduction (table 3-a, b, c and their figure illustration). Results were similar to the previous findings of **Chung-Hsi Chou *et al.*, (2013)**, **Song *et al.*, (2014)**, **Woolcock (2008)**, **Clank (1993)** and **Campbell (1984)**.

Woolcock (2008) Stated that Onset and spread of DVH type 1 are very rapid, with practically all mortality occurring within 3–4 days. Affected ducklings at first fail to keep up with the brood. Within a short time, they stop moving

and squat down with eyes partially closed. Birds fall on their sides, kick spasmodically with both legs, and die with heads drawn back. Death occurs within an hour or so after signs are noted. During the height of severe outbreaks, the rapidity with which ducklings die is astonishing. For this reason we were monitoring experiment and terminating it in a four days period post-challenge.

Woolcock and Hsiang-Jung Tsai (2013) Stated that disease reports based on postmortem findings frequently lack the clinical pathologic data that would be of value to the clinician. With many diseases, a clinician will be able to demonstrate disruption of functional integrity (pathobiology) of an organ by means of associated clinical pathologic changes.

Campbell (1994), **Campbell (1988)**, **Dein (1986)**, **Hawkey and Dennett (1989)** stated that Evaluation of the avian hemogram involves counting the various blood cells per microliter of blood as well as cytological evaluation of the cells and mentioned that avian blood does not store well and that hematologic results obtained soon after collection are preferred over those performed several hours later. For this reason we had used the automated cell counter (animal hematology analyzer- **SYS-MEX-XT-2000 iv**) as it provides a quick reliable method for obtaining the hematological picture.

The mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) are useful in the characterization of the erythrocytes, and especially in the evaluation of anemia. The normal PCV of birds ranges between 35 and 55 percent **Campbell (1994)**. In the present study the PCV % in all of the inoculated duck breeds at the end of the 7 days trial., was significantly lower than their reciprocal control which is indicative of anemia., and this was also observed at the end of the 14 days trial for the Pekin and mallard breeds only., while the Muscovy breed had showed a significant increase in the PCV% in the infected group compared to their control at the end of 14 and 21 trials. A PCV less than 35 percent is indicative of anemia, and a PCV

greater than 55 percent is suggestive of dehydration or polycythemia. In the present study control non infected birds were incorporated for each reciprocal duck breed used in the present study to avoid the wide variation range in the PCV value mentioned by **Campbell (1994)**. **Campbell (1994)** mentioned that an anemic bird showing a ten percent or greater degree of polychromasia is exhibiting a significant regenerative response and the presence of immature erythrocytes in the peripheral blood along with an increase in polychromasia is indicative of a marked regenerative response., but the short time period (four days) of each age trial and the significant increase in the AST, ALT may lead us to justify dehydration., due to liver affection rather than regeneration.

Campbell (1994) stated that there is wide variation in the normal leukograms among birds of the same species. This was actually found in the different duckling breeds in the present study (table 4, 5 and 6 with their figure illustration). The same author suggested that preparing normal reference values on healthy individual birds is the best method for evaluating blood parameters of a bird during illness., but this was not prepared for., and instead of it., the results of Leukogram of the infected breeds used in the present study were compared to their reciprocal control at every time point of challenge.

The general causes of a leukocytosis include infection, trauma, and hemorrhage. The differential leukocytic count aids in the assessment of the leukocytosis. Because when leukocytosis is caused by inflammation a heterophilia will be present because avian heterophils performs the inflammatory function of neutrophils in mammal **Maxwell and Trejo (1970)**, **Hodges (1974)**, **Penninall and spitznagel (1975)**, **Daimon and caxton-Martins (1977)** and **MacRae and Powell (1979)**. The magnitude of the heterophilia usually indicates the magnitude or severity of the initiating inflammatory process. The conjunction of leukocytosis and heterophilia in the present study was not clear if it was attributable for viral infection with DVH or it was the result of hemor-

rhage which was recorded during pathological examination of specimens (table – 9).

In the course of DVH, pathological changes are localized mainly in the liver **Woolcock and Hsiang-Jung Tsai (2013)** mentioned that microscopic changes in uncomplicated, experimentally induced DHAV-1 infections have been studied early by **Fabricant et al., (1957)** where the primary changes in the acute disease consisted of necrosis of hepatic cells, diffuse fatty liver degeneration, scattered bleeding, liver hemangiectasis surrounded by lymphocytic infiltration, necrosis, lymphoid aggregates, mild bile duct epithelial cell proliferation, and vacuolated degeneration of liver cells. The survivors had a more chronic lesions showed a widespread bile duct hyperplasia with varying degrees of inflammatory cell response and hemorrhages occurs. **Calnek (1993)**, **Liu et al., (2011)** and **Zhang et al., (2012)** reported regeneration in liver parenchyma in survivors. Experimentally **Song et al., (2014)** found that all SH-infected ducklings exhibited a systematic cell swelling and vesicular degeneration (hydropic degeneration) in the liver at 16 h p.i. more severe blisters, degeneration, local steatosis and a small amount of local heterophilic infiltration in the small blood vessels were observed at 24 h p.i. moreover the hepatocyte morphology was characterized by fuzzy cell boundaries, necrosis, collapse, perivascular lymphocytic infiltration and wide spread of large numbers of granulocytes and many necrotic focal points in liver specimens at 36 h p.i. However, fewer pathological changes were observed in liver specimens from strain FC64-infected ducklings compared with SH infected ducklings. The livers of FC64-infected ducklings appeared only slightly inflamed, with vesicular degeneration and a small number of inflammatory lesions. These results confirmed that the livers of SH infected ducklings had more lesions than those infected with FC64. In the present study we were not focusing on the registration of the existence of pathological alterations due to in liver or spleen because it is already known from early researchers such as **Fabricant et al., (1957)** and those following

such as **Calnek (1993)**, **Liu *et al.*, (2011)** and **Zhang *et al.*, (2012)** and **Song *et al.*, (2014)**. In the present study we were focusing on a different hypothesis (does the clinically resistant duck breeds for DVH such as Mallard and Muscovy develop pathological liver alteration while being clinically normal?, what is the extent of liver impairment after experimental infection with the (DVH/EG. Bayoumieh-Sharkia-2015)?, can the encountered pathological lesion justify the recorded reduced performance of those clinically resistant breeds as seen in (table– 3)?). And it became clear for us that., histopathological changes in Pekin duckling in the present study included congestion, hemorrhage microsteatosis, portal granulocytes or macrophages infiltration beside proliferation of bile ductules., the microscopic lesions in the Muscovy duckling included acute cell swelling, congestion, hemorrhages and microsteatosis these changes were detected when ducklings were inoculated at 7,14 days only while no detectable changes could be seen in the Muscovy duckling inoculated at 21 days. The microscopic lesions in mallard duckling were acute hepatic cell swelling, microsteatosis, hemorrhages accompanied with portal macrophages with proliferation of bile duct. although the Mallard and the Muscovy breeds were clinically resistant for DHAV-1 when inoculated at 7, 14, and 21 day of age but they had a reduced body weight as seen in (table – 3a, b and c with their figures), they had alterations in the blood picture as seen in (table 4, 5 and 6 with their figures), they had pathological alterations known for DVH as seen in (table– 9 a, b and c beside plate– 1 containing fig. 1-8). It also worth to mention that spleen of the examined infected groups was consistently showing lymphoid depletion of the white pulp, reticuloendothelial cell infiltration, congestion, granulocytic infiltration plate– 1 fig 8. Beside hemosidrosis. **Hochleithner (1994)** stated that managing abnormalities in birds requires an understanding of how disease processes change the biochemical functions of the body because the clinical signs of illness in birds are frequently subtle,

clinical chemistries are necessary to evaluate cellular changes. **Ahmed *et al.*, (1975)** reported that in clinical cases of DHAV-1 infection, there were lower serum levels of total protein and albumen and elevated levels of alkaline phosphatase, ALT, bilirubin, and creatinine. The serum levels of ALT and AST were increased in relation to severity of infection. **Mennella *et al.*, (1977)**. Mentioned that even in clinically in apparent DHAV-1 infection in mallards, liver enzyme patterns were altered, with a consequent alteration in metabolism of dichlorodiphenyltrichloroethane (DDT) **Buynitzky *et al.*, (1977)**, **Buynitzky *et al.*, (1978)** and **Fu *et al.*, (2009)**. This may partially explain the interrelationship between chlorinated hydrocarbons and DHAV-1 **Friend and Trainer (1974 a, b)**. In general, AST activities in birds greater than 230 UH are considered abnormal, Abnormal activities have been linked to vitamin E, selenium or methionine deficiencies liver damage particularly in viral disease **Ericsson, Noir (1973)**, **Ploucha *et al.*, (1981)**. **Song *et al.*, (2014)** had made biochemical detection of serum aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), c-glutamyltransferase (GGT) and total bilirubin (T-Bil) Levels of the serum biochemical markers AST, ALP, ALT and T-Bilirubin are associated with liver function (**Hu & Cheng, 1996**). As liver lesions were observed in SH-infected ducklings, each of these biochemical markers of liver function were measured in ducklings infected with SH or FC64. Mock infected liver specimens were included as negative controls. there were higher serum ALT, AST, ALP, GGT and T-Bil levels in liver specimens from SH-infected ducklings than those from FC64- or mock infected ducklings, indicating that liver function was severely impaired by SH infection. There were no significant differences in AST, ALT, ALP, GGT and T-Bil levels between FC64- and mock-infected ducklings, demonstrating that the liver function of FC64-infected ducklings was not impaired.

Conclusion

It became clear for us that., although the Mal-

lard and the Muscovy breeds were clinically resistant for DHAV-1 when inoculated at 7, 14, and 21 day of age but they had a reduced body weight, they had alterations in the blood picture, they had pathological liver alterations known for DVH and they have disturbance in the examined liver functions from these data we find it necessary to protect these resistant breeds from DVH-1 attack through vaccination and because the disease can occur early at 1 day of age. So we can conclude that nothing is left to protect these breeds but breeder vaccination using an inactivated vaccine with homology to the circulating virus in the localities to guard the anticipated performance of their progeny., beside proper application of hygienic standers to avoid early exposure. The performance of the reared duck breeds encourages financing and expansion in such rearing for its outcome of clean meat fit for human consumption.

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دراسات علي ضراوة معزولة فيروس التهاب الكبد الفيروسي في البط

(DVH/EG. Bayoumieh-Sharkia-2015)

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المخلص العربي

في هذه الدراسة قمنا بدراسة مدي ضراوة معزولة فيروس التهاب الكبد الفيروسي (DVH/ EG. Bayoumieh-Sharkia-2015) السابق عزلها في دراسة سابقة. و قد تم دراسة الضراوة من خلال حقن المعزولة تحت الجلد في سلالات البط البيكيني و المولر و المسكوفي في عمر (٧ و ١٤ و ٢١ يوم) من التجربة. و قد قمنا بدراسة متوسط وزن الجسم قبل البدء و في نهاية فترة التجربة بعد الحقن و قمنا بعمل الصورة الدموية وفحصنا وظائف الكبد ، وسجلنا التغيرات الباثولوجية الحادثة في الكبد وفي بعض قطاعات الطحال بعد التحدي لكل سلالة علي حدة وذلك الي جانب تسجيل الوفيات والاعراض المرضية وقد سجلنا حدوث وفيات في فراخ البط البيكيني فقط بعد ٤٨ ساعة من حقن المعزولة تحت الجلد وكانت الوفيات ٥٠% في المجموعة المحقونة في عمر ٧ أيام من العمر، وكانت الوفيات ٢٥% في المجموعة المحقونة في عمر ١٤ يوم و لم تحدث وفيات في المجموعة المحقونة في عمر ٢١ يوما.

من خلال دراسة نتائج متوسطات الازان لاحظنا ان البط البيكيني بالرغم من حدوث نفوق به دون غيره الا ان انه كان اقل تأثراً من حيث انخفاض الوزن و قد اظهر نوع البط المسكوفي و المولر انخفاض شديد المعنوية في متوسط الوزن بالمقارنة بضابط التجربة و بادئها.

من خلال هذه الدراسة نجد أنه من الضروري حماية السلالات المقاومة ظاهرياً لأعراض الإصابة بـ DVH-١ من خلال التحصين. وأنه للحدوث المبكر لهذا المرض في عمر يوم فإن فكر المقاومة بالتحصين يجب توجيهه الي تحصين امهات هذه السلالات ضد المعزولات الحقلية وكذلك تطبيق ممارسات الأمان الحيوي في أماكن التربية للحد من الإصابة المبكرة. كذلك وجدنا انه من الضروري تشجيع التوسع في هذه تربية قطعان البط المختلفة لسرعة نموه و معدلات ادائه الإنتاجي المرتفعة.