



The possibility of Duck Hepatitis Virus type 1 (DHV-1) transmission from experimentally infected ducklings to goslings

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ABSTRACT

The present work aimed to investigate the possibility of DHV-1 transmission from infected ducklings to goslings and to spot the light on gosling susceptibility to such virus infection where they may play a neglected role in the disease outbreaks. The work included experimental infection of a group of one-week old duckling with the virulent Duck Hepatitis Virus – 1 (DHV-1) that was housed in contact with one-week old gosling group. It was found that 2 out of 10 in contact goslings exhibited clinical signs of DHV-1 infection by the 5th day post housing with infected ducklings showing dullness and weakness then recovered within one week. Survived goslings exhibited detectable serum neutralizing antibody titers by the 1st week post infection. These findings indicate that gosling can attract DHV-1 infection from infected ducklings.

Keywords: Duck hepatitis virus type- 1 (DHV-1); gosling; duckling.

(<http://www.bvmj.bu.edu.eg>)

(BVMJ-35(1): 145-151, 2018)

1. INTRODUCTION

Duck hepatitis virus (DHV) is a fatal rapidly spreading viral infection of young ducklings, characterized primarily by hepatitis and high potential mortality if not controlled. The disease is caused by at least three different viruses (Woolcock and Tsai, 2013)

Duck Hepatitis Virus (DHV), formerly designated as duck hepatitis A virus type 1 “DHAV-1”, is a member of the genus Avihepatovirus, family Picornaviridae (Ding and Zhang, 2007). DHAV-2 was isolated in Taiwan by (Tseng and Tsai, 2007) and DHAV-3 was isolated in South Korea (Kim et al., 2007) and in China (Fu et al., 2009). DHAV-2 and DHAV-3 are now reclassified

and named as Duck Astrovirus types 1 and 2 respectively (Fu et al., 2009).

The most of presented studies revealed that geese could be experimentally infected by DHAV-1 as where experimental infections in goslings have been reported by Akulov et al., (1972). In experimentally infection with DHV-1, goslings showed high mortality (Hwang, 1974).

The aim of the present work was directed to study the susceptibility of gosling to infection with DVH-1 if kept with experimentally infected duckling with DHV-1.

2. MATERIALS AND METHODS

2.1. Viruses:

2.1.1. Virulent duck hepatitis virus-1 (DHV-1):

A local isolate of duck hepatitis virus-1 (DHV-1) was supplied by Veterinary Serum and Vaccine Research Institute (VSVRI) Abbasia, Cairo. It had a titer of $10^8 EID_{50}/ml$. This virus was egg adapted and was used for experimental infection of ducklings.

2.1.2. Cell culture adapted DHV-1:

Vero cell culture adapted DHV-1 (El-Koffy, 1997) with a titer of $6 \log_{10} TCID_{50}/ml$ was supplied kindly by VSVRI and used for detection and titration of DH antibody in sera of infected birds using serum neutralization test.

2.2. Experimental birds:

Fifteen balady goslings with 7 days old and 15 balady ducklings of 7 days old were provided and reared under strict hygienic measure in isolated disinfected wire floored isolators and were screened using serum neutralization test (SNT) and found to be free from duck hepatitis antibodies.

2.3. Specific pathogen free embryonated chicken eggs (SPF-ECE):

Fifteen specific pathogen free embryonated chicken eggs (SPF-ECE) 10 days old were supplied by Nile Company for SPF egg production, Qoum Oshim, Fayom, Egypt. They were used in trials for virus recovery from tissue samples of infected dead birds.

2.4. Vero Cell culture:

African green monkey kidney (Vero) cell monolayer were supplied by VSVRI and used for estimation of Duck Hepatitis antibodies in sera of experimentally infected ducks and geese using SNT.

2.5. Anti-DHV-1 hyper immune serum:

It was kindly supplied by VSVRI and used in virus neutralization test to confirm DHV-1 recovery.

2.6. Experimental design:

Ten ducklings were infected with virulent DHV-1 strain had a titer of $10^8 EID_{50}/ml$ using a dose of 0.5 ml/bird injected intramuscularly according to Woolcock (2008) and housed in contact with 10 goslings while 5 ducklings and 5 goslings were kept isolated healthy as test control. These birds were kept under daily observation for detection of clinical signs and mortality for 10 days.

2.7. Sampling:

2.7.1. Samples for virus recovery:

Specimens of, liver, spleen and kidneys were collected from dead birds for virus recovery after application of the post mortem examination.

2.7.2. Serum samples:

Blood samples for serum collection were obtained from all bird groups one-week intervals post application of the experimental work.

2.8. Preparation of samples for virus recovery:

Collected tissue samples for virus recovery were prepared separately by grinding about one gram of tissue using electric homogenizer in phosphate buffer saline (PBS) pH 7.0-7.2. After three cycles of freezing and thawing, the homogenate was clarified by centrifugation at 2000 rpm for 15 minutes. The supernatant fluid was treated with 100 IU/ml penicillin and 100 $\mu g/ml$ streptomycin then kept at $-80^{\circ}C$ for virus isolation.

2.9. Virus recovery:

Virus isolation was carried out by egg inoculation technique according to Senne (1989) through the Chorio-allantoic membrane inoculation. The inoculated eggs were examined daily by candling for a period of 6 days, deaths occurred within the 1st 24 hours post-inoculation were excluded as non-specific, after that dead embryos were examined, embryonic fluids, CAM, and

embryo without head and legs were harvested for virus identification using virus neutralization test.

2.10. *DHV-1 titration in ECE:*

The allantoic fluid obtained from inoculated ECE by the prepared samples from dead birds through 2 passage was titrated according to Reed and Muench (1938).

2.11. *Virus neutralization test (VNT):*

It was carried out according to Woolcock, (1989) using hyper immune serum against DHV type I through inoculation into allantoic cavity of five ECE (0.1 ml/egg).

2.12. *Serum Neutralization Test (SNT):*

SNT was carried out on Vero cell culture using the micro titer technique to follow up the levels of DH antibodies in the sera of experimental birds according to Bass et al., (1982).

3. RESULTS

3.1. *Clinical examination of infected birds:*

Infected ducklings showed classical signs of DHV-1 (9 out of 10 duckling) represented by depression; weakness and sudden death occur in opisthotonus position (photo-1). Deaths of affected (8) duckling were recorded by 2nd day post infection. These findings are tabulated in table (1).

It was noticed that gosling in contact to infected duckling showed high resistance to infection with DVH-1 showing mild clinical signs started by the 4th day post infection.

These signs were detected in 2 out of 10 gosling represented (table-2) by dullness, depression and weakness (photos-2). Recovery occurred to all goslings 1 week post infection.

Ducklings showed symptoms of infection in 9 out of 10 experimentally infected birds of which 2 were died, while goslings showed symptoms of infection in 2 out of 10 experimentally infected birds with no deaths (table 3).

3.2. *Post-mortem (PM) lesions in dead birds:*

PM lesion of died gosling and duckling showed congestion of internal organs and mottled liver as shown in photos (3, 4).

3.3. *DH virus recovery from dead birds:*

The virus could be recovered from liver and kidneys of died birds after serial passage in SPF embryonated chicken eggs and confirmed by virus neutralization test using DHV hyper immune serum as shown in table (4).

3.4. *Detection of DH antibodies in survived goslings and ducklings:*

Survived goslings and ducklings showed detectable SNT antibody titers from the first week and up to 3 weeks post infection. Survived goslings developed lower mean DH neutralizing antibody titers than that developed in survived duckling (table-5).

Table (1): Clinical response of ducklings to experimental infection with DHV-1

Observed clinical signs on DPI*													
1	2	3	4	5	6	7	8	9	10	11	12	13	14
No symptom			Depression & Weakness										
0/10	0/10	2/10	3/10	4/10	5/10	7/10	9/10	8/10	8/10	8/10	8/10	8/10	8/10

* DPI= days post infection.

** Number of ducklings showing signs of DHV-1.

Table (2): Clinical response of goslings in contact to experimentally infected ducklings with DHV-1

		Observed clinical signs on DPI*											
1	2	3	4	5	6	7	8	9	10	11	12	13	14
No symptom		Weakness, ruffled feather and depression											
0/10	0/10	0/10	0/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10

* DPI= days post infection.

** Number of goslings showing signs of DHV-1.

Table (3): Rate of infection in ducklings after experimental inoculation with DHV-1.

Birds	Number of birds	Diseased birds	Dead birds
Ducklings	10	9	8
Contact goslings	10	2	0

Table (4): Morbidity and Mortality rates in experimentally infected ducklings with DVH-1

Birds	Number of birds	Diseased birds	Dead birds	Morbidity rate	Mortality rate
Infected ducklings	10	9	8	90%	80%

Table (5): DHV recovery ECE from dead infected goslings and ducklings

Type of sample	No. of sample	organ	No. of +ve sample	Embryonic deaths / days post inoculation						Total embryonic deaths	
				1	2	3	4	5	6	No.	%
Infected duckling	8	liver	8	0	0	4	2	2	0	8/8	100
		kidney	7	0	0	4	2	1	0	7/8	87.5
		spleen	6	0	0	4	1	1	0	6/8	75

Table (6): Mean DHV-1 neutralizing antibody titers in goslings and ducklings

Applied Test	Bird serum samples	DH antibody titers/ WPI*		
		1	2	3
SNT**	Experimentally diseased goslings	1	4	16
	Gosling in contact to infected duckling	1	4	16

*WPI= week post infection.

**SNT titer = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100TCID₅₀ of DHV-1



Fig.1. Experimental infected duckling with DHV-1 showing depression and inability to move.



Fig.2. Experimental infected gosling with DH virus showing depression and inability to move.

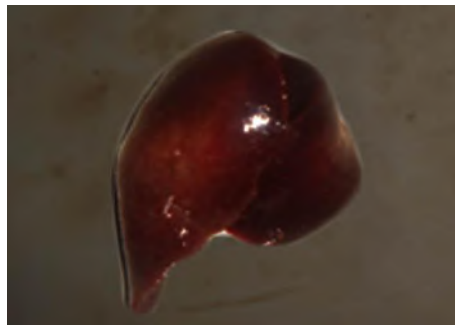


Fig.3. Experimental infected duckling with DH virus showing enlarged heamorrhagic liver



Fig.4. Liver of dead gosling with DH virus showing hemorrhage.

4. DISCUSSION

It was reviewed that gees could be experimentally infected by DHV-1as (Akulov

et al, 1972). Experimentally infection with DHV-1 in young goslings induced high mortality (Hwang, 1974). The present study investigated the susceptibility of gosling to attract DVH-1 when kept with experimentally infected duckling with DHV-1.

The obtained results revealed that gosling kept in contact to infected ducklings with DHV-1 showed high resistance to infection with DHV-1 showing no mortality but showed clinical signs as dullness, weakness and inability to move. Clear clinical signs appeared on infected ducklings showing mortality among 8 out of 10 ducklings showing weakness, dullness, ataxia and loss of balance and sudden death in opisthotonus position. These results came in agreement with those of Maria (2007) who reported that DHV type II affects ducklings less than 6 weeks of age with loose droppings, polyuria, ataxia and convulsions prior to death.

Morbidity and mortality among experimentally infected duckling revealed that mortality rate was 80% in agreement with Erfan et al., (2015) who found that all duck breeds (Pekin, Muscovy, Mallard and Green Winged) were susceptible to the disease with mortality ranged from 15% to 96.7%.and there are nil researches reveal that ducklings may transport infection to goslings showing mortalities.

DHV-1 was recovered from liver, spleen and kidneys of dead goslings through the inoculation into the allantoic cavity of embryonated chicken eggs (ECE) where 3 days post infection the embryos showed stunting; hemorrhage over the whole body and with edema in agreement with El-Ebiary et al. (1992) who mentioned that in case of inoculations of DHV type I in AC of chicken embryos death of embryos occurred between 2nd-4th days after inoculation with stunting, hemorrhage, and edema

On the other hand, diseased experimentally infected ducklings showed serum neutralizing antibody titers of 16 and 64 on the 1st and 3rd week post infection respectively. Parallel to these findings, in contact survived goslings exhibited serum

neutralizing antibody titers 2 and 16 on the 1st and 3rd week post exposure, respectively (table 5) as reported by Mahmoud (1980) and Woolcock (1991) who mentioned that SNT used to estimate the immune response of vaccinated ducklings.

5. CONCLUSION

It could be concluded that infected ducklings with DHV-1 could transmit the infection to less extent to gosling and further studies are in need to fulfillment all points related to the behavior of DHV-1 infection and immunity in goslings.

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