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The role of migratory birds in the transmission of some viral diseases

By

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SUMMARY

A total of 169 migratory birds of 4 species (80 quail , 50 coot , 34 pochard and 5 teal) were used in this study, results for detection of precipitating antibodies (AGP) to some viral diseases were positive for Adenovirus in 4 quail , 3 pochard and one coot serum samples. Also AGP antibodies were decreased in 2 samples from coot and one from pochard against (IBDV). No precipitating antibodies detected against infections bronchitis (IB) and newcastle disease (ND) viruses. Separated samples from respiratory tract, gastrointestinal tract and internal organs were collected from each bird, then 5 specimens pooled together, used in viral isolation trials in embryonated chicken eggs through CAM and AS inoculation routes. Identification of the isolates indicated that : twelve NDV strains were isolated from 5 pooled samples from quail , one from coot and 6 from pochard ; as well as Adenovirus from 5 pooled samples quail , 4 coot, 3 pochard and one teal ; and also IBDV from 8 quail , 2 coot and 2 pochard. These isolates were identified by HI and agar gel precipitation tests.

INTRODUCTION

Numerous species of migratory birds from Europe and Asia visit Egypt in Autumn and Spring and stay their wintering season especially

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waterfowls. Many of these migratory birds had great opportunities for direct and indirect contact with free-ranging domestic poultry raised everwhere throughout the country. The most common species of migratory birds in Egypt are quail (*Coturnix coturinix*) ; coot (*Fulica atra*) ; waterfowls , including pochard (*Aythya ferina*) , teal (*Anas crecca*), shoveller (*Anas clypeata*) , pintail (*Anas acuta*) and mallard (*Anas platyhnchos*) and others.

Among poultry viral diseases which can be transmitted by these birds are Influenza viruses , they carry the disease and distribute them by shedding it into the water. Possibilities of recombination between different Influenza virus strains in nature become likely(**Ottis and Bachmann,1983).**

The isolation of Influenza-A viruses and Paramyox viruses (PMVs) from free-flying birds has become an issue of great concern within the poultry industry. Evidence suggests that some of Influenza epidemics and Newcastle disease virus (NVD) infection occur among domestic poultry as a result of primary introduction of the viruses by migrating waterfowl or feral pigeons **(Karunakaran et al.,1983** and **Halvorson et al.,1987). King et al.,(1981)** reported the first evidence of Avian Adenovirus in wild bobwhite quail. Also detection of antibodies against egg drop syndrome (Adeno 127) in the atlantic flyways, USA among ducks, coots and grobes was recorded by **Gulka et al., (1984) .**

In Egypt, trials for virus isolation from migratory birds started in 1978 by El-Dahaby, but were not successful . **Hosny et al.,(1980) and Hadia (1987)** isolated Avian Influenza viruses , while **Mostafa (1979) , Hadia (1977) and Hanan (1991)** isolated NDV.

This work was carried out in a trial to isolate some viruses that might be harbored by migrating birds in Sinai and North region (Port Said & Damietta) with special reference to NDV, Influenza virus , Adenovirus, Reovirus and Infectious bursal disease virus (IBDV). Serological screening for detection of antibodies to some viral diseases in the selected migratory birds has been done.

MATERIAL AND METHODS

• Materials:

Two areas were chosen ; Sinai (along the entire mediternian coast), Port Said and Domietta (Manzala lake including Marshland). Samples were collected during the period from September to December in both 1993 and 1994).

A total of 169 migratory birds were collected ; 80 quails *(Coturnix coturnix)* , 50 coot *(Fulica atra)* , 34 pochard *(Aythya ferina)* and 5 teal *(Anas crecca)*.

A total of 149 serum samples were collected from 80 slaughtered birds in addition to serum samples 35 coot , 4 pochard and 30 quail.

Tracheal and cloacal swabs from each bird were collected and placed into sterile screw-capped vials containing 3 ml of Hank`s salt solution with 10,000 i.u penicillin and 10 mg streptomycin per ml.

Organs and tissues from each kind were collected in three types of specimens, 1- respiratory (larynx , trachea and lungs) . 2- gastrointestinal tract ; proventriculus , samll intestine (duodenum, jejunum and ilium), ceaca and large intestine . 3- Other organs, heart, liver, kidneys , brain m, spleen. Table (1) demonstrate the number of birds , localities and type of specimens collected during the study.

Fertile chicken egg were obtained from the Faculty of Agriculture Farm, Suez Canal University, Ismailaia . Sterile phosphate buffer saline , pH 7.2 was used as diluent for preparation of specimens and washing of embryos and membranes. Sand acid washed, medium fine from M & B, Ltd, Bagenham, England was used for preparing emulsion from organs. Difco Nobel agar was used for agar gel precipitation test. Brain heart infusion broth was used for detecting any bacterial contamination during isolation process as a sterility test.

For serological screening precipitating antigens of Avian Adenovirus (CELO) , eovirus and IBDV were used in agar gel precipitation test . Lasota strain vaccine was used as a haemaggultinating antigen (NVD) in HI test. These antigens were kindly supplied by diagnostic Lab. of Ismailia , MISR Investment poultry company, Ismailia.

Precipitating antigens of Avian Adenovirus (CELO), Reovirus (S-1133) and IBDV (D78) were used as positive control antigens in identification trials of viral isolates . These antigens were provided by Dr.Metwally, S.Hamouda who prepared it in SPF eggs in Fayettville, Poultry Health lab., Faculty of Agriculture, University Of Arkansas, USA.

IBDV (D-78) , Reovirus (S-1133) and Adenovirus antisera from SPAFAS, USA were provided by Dr. Metwally , S.Hamouda, NDV antiserum, chosen from vaccinated chickens and proved to have HI titers of 2^8 when examined against 4 HI units of Lasota vaccine strain .

Methods :

1- Birds were subjected to clinical and postmortum examination.

2- For detection of antibodies against NDV ; HI test was carried out using the micro-technique according **to Beared and Wilkes (1973)** using 4 HA units.

3- Precipitating antibodies against IB, CELO , IBD and REO viruses were detected by Immunidiffusion test according to the method described by Woernle (1963) using 1.5% Difco Noble agar dissolved in phosphate buffered saline (Pbs), 8.5% sodium chloride and adjusted to 7.2 pH. The medium was poured in Petridishes. After solidification , wells of approximatly 4 mm in diameter and as near as 3 mm a part arround central one were cut. The known reference antigen was put in the central well surrounded by the tested antisera. The Petridishes were put in a humid chamber at room temperature and periodically examined during 4 days for the presence of specific lines.

1- Trials for virus isolation :

Tissues and organs were finely minced and suspended in Pbs. Ph 7.2 containing 10,000 i.u penicillin and 10 mg streptomycin per ml to made 10-20% suspension. Tracheal and cloacal swabs were suspended in Hank's solution containing the same concentration of antibodies . Suspensions were left at room temperature for about 1 hour , then centrifuged at 1000 rpm for 20 minutes.

Supernates of every 5 samples were pooled together to prepare pooled samples from internal organs , gastrointestinal tract and respiratory tract. 0.2 ml volume of each sample was inoculated into four 9-11 day old chicks embryos via allantoic sac route (AS), four 11-13 day old chickens embryos via chorioallantoic membrane (CAM).

Inoculated eggs were candled once daily, dead embryos detected 24 hours post inoculation were discarded . Dead embryos detected till the end of the 6th day were examined by collecting the amniollantoic fluids for the presence of haemagglutinating agent and embryos were examined to detect any P.M.lesions-chorioallantoic membrane (CAMS) were examined for the presence of pock or any other lesions.

5- Viral identification :

Haemagglutination test was carried out according to standard procedure reported by **Anon (1963).** Haemagglutination test was carried out on each haemaggluting isolant with convalescent NDV-specific according to **Beard and Wilkes (1973).** Agar gel precipitation test was carried out to identify suspected isolates of reovirus, Adenovirus and IBDV according to **Woernle (1963).**

RESULTS

Clinical and P.M.examination revealed that the 4 species of birds were in a good health condition. HI antibodies against NDV were negative in all samples. Agar gel precipitating antibodies against IB, Adeno Reo and IBD viruses were summmarized in Table (2) .

Haemagglutinating agents were ioslated and all isolates were identified as NDV strains using HI tested summarized in Table (3).

Results of isolation trials of Avian Adenovirus from different species detected by agar gel precipitation (AGP) were summarized in Table (4) .

Results of isolation bursal disease (IBD) virus detected by agar gel (AGP) test were summarized in Table (5). Results of isolation of Reo virus tested with a agr gel were negative.

DISCIUSSION

The role of the migratory birds in initial introduction of many diseases were recorded in different countries throughout the world. In Egypt, few workers discussed the ioslation of viral agents from these migration birds **(El-Dahaby, 1978 ; Mostafa, 1979 ; Hosny et al.,1980 ; Hadia, 1987 and Hanan, 1991) .**

In the present work , trials had been made to isolate and detect antibodies against a group of viruses including NDV, Influenza viruses, Adeno viruses , reo viruses, infectious bronchitis virus and IBDV.

In this study , as recorded in Table (4), among 169 apparently healthy migratory birds were trapped in Sinai , Port Said and Domitta, during 1993 and 1994 migration season , 12 hemagglutinating agents were ioslated from most species in different sites : respiratory tract (6 isolates), gastrointestinal tract (5 isolates) and one from internal organs.

HA activity of all isolates were inhibited by NDV antiserum using HI test. The isolates were classified according to killing time of chicken embryos. All isolates from pochard and coot, and one from quail characterized as Lentogenic strains of NDV. Whereas 4 isolates from quail were typed as mesogenic strains. These results were in agreement with the finding **of Rosenberger and Krauss, 1974 ; Pearson and McCann, 1975 ; Webster et al.,1976 ; Abenes et al.,1982 ; Ottis and Bachmann, 1983 ; Lipkind et al.,1993** who found NDV in different species of migratory birds. Also our results coincide with those obtained by **El-Dahaby (1978)** who

detected NDV antibodies and **Mostafa , 1991 ; Hadia, 1987 and Hanan, 1991** who isolated NDV from migratory birds in Egypt.

Using the agar gel precipitation test, Adenoviruses were isolated from 5 pooled quail, 4 coot, 3 pochard and one from teal. These results nearly agreed with **McFerran et al.,1976 ; King et al., 1981 and Gough et al.,1990 and with Risso et al.,1985** for their serological study and by **Hanan , 1991**in Egypt. These isolation need further investigation especially for their pathogenicity to domestic fowls.

In this study, the isolation of IBDV from migratory birds seemed to be the first time in Egypt. There is no svailable information about the isolation of such viruses from migratory birds and seens to be the time to isolate. Such results give furtehr evidence to the fact that birds are more frequent reservoirs of viral and bacterial agents than it was previously supposed. The results in Table (5) showed that IBDV was isolated from 8 pooled quail samples, 2 pochard and 2 from coot. The isolation of IBDV from ducks is in partial agreement with **Yamada et al.,1980 and Christopher, 1982 .** Also , **Mcferran et al.,1980** reported that ducks can acquire natural infection with IBDV. Isolation of IBDV from quail in the present work is in agreement and supported by the finding of **Mcferran (1993)** who isolated it from domestic quail, meanwhile disagree with **Weisman and Hitchner (1978) .**

Resulting of detecting antibodies against IBDV from some species of migratory birds are in agreement with those of hanan (1991) who detected IBDV antibodies in sera of such migratory birds (quail, coot and pochard).

The results of IBDV added a probable factor of epidemiological importance in the recently appeared IBD outbreaks among our domestic flocks.

From the above mentioned results we can conclude that NDV, Adenovirus and IBDV are widely distributed among migratory birds, and some of these viruses are known to cause or exacerbate diseases among domestic flocks. Further investigation is needed for identification and characterization of isolated IBDV.

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Table (1) Number of birds, locality and type of specimen collected.

Species	Locality	Number of birds tested			Specimens collected		
		1993	1994	Total	Blood (serum)	Whole carc-ass	Trac-heal/ cloa-cal swabs
Quail	Medi-terranean cost of Sinai	35	15	50	50	50	0
		30	0	30	30	0	0
Coot	Manzala lake	15	0	15	15	15	0
		15	0	15	15	0	15
		20	0	20	20	0	0
Pochard	Manzala lake	15	0	15	15	15	0
		15	0	15	0	0	15
		04	0	4	4	0	0
Teal	Manzala lake	05	0	5	0	0	5
Total		154	15	169	149	80	35

Table (2) Serological investigation on collected sera against IB, Adeno-, Reo- and IBD viruses.

Species	Total No. of tested sera	No. & % of positive reactors to different viral antigens.							
		IB		Adeno		Reo		IBD	
		No.	%	No.	%	No.	%	No.	%
Pochard	19	0	0	3(2,3,8)	15.8	0	0	1(5)	5.26
Coot	50	0	0	1(6)	2.0	0	0	2(8,34)	4.0
Quail	30	0	0	4(10,13, 34,40)	5.0	4(14,16, 20,69)	5.0	0	0

Table (3) Results of isolation trials of NDV from different species inoculated through allantoic sac route.

Species	Pooled sample numbers	Source of samples		
		Respiratory tract	Gastro-intestinal tract	Internal organs
Quail	6-10	-	+	+
	11-15	+	+	-
	36-40	+	-	-
Pochard	11-15	-	+	-
	16-20	+	-	-
	21-25	+	+	-
	26-30	+	+	-
Coot	16-20	+	-	-

+ = positive for virus isolation.

- = negative for virus isolation.

NDV was identified by HA and HI tests.

Table (4) Results of isolation trials of Avian Adenovirus from different species as tested by agar gel precipitation test.

Species	Pooled sample numbers	Source of samples					
		Respiratory tact		Gastro-intestinal tract		Internal organs	
		A.S	CAM	A.S	CAM	A.S	CAM
Quail	1-5	-	-	-	+	-	+
	11-15	-	-	-	+	-	-
	26-30	-	+	-	-	-	-
	36-40	-	-	+	-	-	-
	46-50	-	-	+	-	-	-
Pochard	6-10	+	-	+	-	+	+
	11-15	+	-	-	-	-	+
	25-30 a	-	-	-	+	ND	ND
Teal	1-5	-	-	-	+	-	-
Coot	1-5	-	-	+	-	-	-
	6-10	+	+	-	-	+	-
	11-15	-	+	+	+	+	+
	21-25 a	-	-	-	+	ND	ND

A.S = Allantoic sac route.
CAM = Chorioallantoic membrane route.
+ = Positive for virus isolation.
- = Negative for virus isolation.
ND = not done

Table (5) Results of isolation trials of infectious bursal disease (IBD) virus as tested by the agar gel precipitation test.

Species	Polled sample numbers	Source of isolation					
		Respiratory tract		Gastro-intestinal tract		internal organs	
		A.S	CAM	A.S	CAM	A.S	CAM
Quail	1-5	-	-	-	-	-	+
	6-10	-	+	-	-	-	+
	11-15	-	-	-	+	-	+
	16-20	+	+	+	+	-	+
	21-25	+	-	+	-	-	-
	31-35	+	-	-	-	-	+
	36-40	-	+	-	-	-	-
	46-50	-	+	-	-	-	-
Pochard	6-10	+	-	+	-	-	-
	11-15	-	-	-	-	+	-
Coot	6-10	+	-	+	-	-	-
	11-15	-	-	-	-		-

A.s = Allantoic sac route.
CAM = Chorioallantoic sac route.
+ = Positive for virus isolation.
- = Negative for virus isolation.

الملخص العربى

دور الطيور المهاجرة فى نقل بعض الأمراض الفيروسية

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أجريت هذه الدراسة لإستبيان دور بعض هذه الطيور فى نقل بعض الأمراض الفيروسية إلى قطعان الطيور المختلفة بجمهورية مصر العربية ، وقد اختص بالدراسة طيور السمان والغر وبط الحمراى وبط الشرشير . وقد تم أخذ عينات من ١٦٩ طائر مهاجر (ثمانون من السمان - وخمسون من الغر - وأربعة وثلاثون من البط الحمراى وخمسة من البط الشرشير).

وباجراء المسح السيرولوجى لعينات السيرم لهذه لطيور ثبت وجود أجسام مناعية لفيروس الأدينو فى أربع عينات سمان ، ثلاث بط حمارى وعينة واحدة من الغر كما وجد أجسام مناعية ضد فيروس الربو فى أربعة عينات فقط من السمان . ووجد أيضاً أجسام مناعية ضد فيروس الجمبورو فى عينة واحدة فى كل من الغر والبط الحمارى . ولم توجد أجسام مناعية لكل من أمراض النيوكاسل والإلتهاب الشعبى المعدى .

وقد تم أخذ عينات من الأعضاء المختلفة للطيور المستخدمة وتقسيمها الى عينات من القناة الهضمية والجهاز التنفسى وكذلك الأعضاء الداخلية ثم وضعت فى عينات مجمعة كل منها يتكون من خمسة طيور وذلك للعزل الفيرولوجى . وأظهرت نتائج هذه الدراسة عزل فيروس النيوكاسل من عدد خمسة عينات سمان وعينة واحدة من الغر وخمسة عينات من البط الحمارى . وتم عزل فيروس الأدينو من خمسة عينات سمان وأربعة عينات غر وثلاث عينات من بط حمراى وعينة واحدة من البط الشرشير . وكذلك تم عزل فيروس الجمبورو من ثمانية عينات سمان وعينتين من كل من الغر والبط الحمارى . وقد تم المسح السيرولوجى والتشخيص الفيرولوجى للعترات المعزولة باستخدام اختبارى منع التلازن الدموى والترسيب المناعى فى الأجار .

من نتائج هذه الدراسة يتضح الدور الخطير الذى يمكن أن تلعبه الطيور المهاجرة فى وبائية بعض الأمراض الفيروسية مثل النيوكاسل والجمبورو والأدينو . لذلك تتضح الأهمية القصوى لعدم تسرب هذه الطيور الى أماكن تربية قطعان الدواجن واتخاذ الإجراءات اللازمة للحد من هذا الخطر .

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