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VISCEROTROPIC VELOGENIC NEWCASTLE DISEASE IN QUAILS (COTURNIX COTURNIX)

(With 3 Table and 3 Figuers)

By

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(Received at 3/1/1993)

مرض النيوكاسل شديد الضراوه للأحشاء في السمان (كوترنكس كوترنكس)

كمال الزناتى ، طلبه عبد المطلب

فى يونيه ١٩٩٢ حدث مرض النيوكاسل فى قطعان السمان (تسمين - تربيه) فى مزرعة كلية الزراعه. جامعة أسيوط. تم تسجيل الاعراض الاكلينيكيه والصفه التشريحيه. تم عزل عترات لفيروس النيوكاسل فى أجنة البيض وتم التعرف عليها بواسطة اختبار تلازن الدم وتلازن الدم المضاد. تم توصيف عترتين من هذه العترات توصيفا كاملا بأستخدام التجارب الفيزوكيميائيه والبيولوجيه وتبين أنها من العترات الشديدة الضراوه للأحشاء. العدوى الصناعيه لهاتين العترتين فى الدجاج والسمان أتضح أن هذه العترات أكثر احداثا للمرض فى الدجاج عن السمان وذلك لاحداثها وفيات عاليه وشيوع الصفه التشريحيه المرضيه فى الأحشاء الداخليه للدجاج أكثر من السمان.

SUMMARY

A natural outbreak of Newcastle disease (ND) in june, 1992 occured in breeding and growing quail flocks in College of agriculture farm, Assiut University. The clinical signs and post mortem lesions were recorded. Hamagglutinating viral agents were isolated in embryonated chicken eggs (ECE) and further identified as ND viruses by hemagglutination inhibition (HI) test. Two virus isolates (AG_1 & AG_2) were characterized by physicochemical and biological tests as viscerotropic velogenic Newcastle disease (VVND) viruses. Experimental infection of virus isolates (AG_1&AG_2) in chikens and quails revealed that the viruses were more pathogenic for chickens than quails as indicated by high mortality and prevalence of viscerotropic lesions in chickens than quails.

INTRODUCTION

ND virus has very common, world wide distribution, causing very severe to inapparent disease and has been reported to infect a wide variety of birds from over 240 distinct species *(Alexander, 1989)*. Different species and ages of birds, human and other mammals are susceptible to Viscerotropic Velogenic Newcastle disease virus (VVNDV). VVNDV has been reported in more than 150 countries *(DWIGHT SCHWARTZ, 1988)*. Quails as carrier and disseminator of NDV was reported by *ZARZUELO and GUTIERREZ GALIANO, 1969. CARRADO, (1970)* failed to infect Japanese quails (coturnix coturnix japonca) with NDV. In addition to chickens and turkeys NDV produce mild and sometimes fatal infection in other bird species including quails *(LANCASSTER and ALEXANDER, 1975)*.

The present study describes natural outbreak of NDV in breeding and growing quail flocks including isolation, identification and characterization. Experimental infection of virus isolates in chickens and quails was also reported.

MATERIAL AND METHODS

Specimens: Twenty (10 from 3-week-old quails and 10 from adult ones) pooled samples (5 birds/sample) of brain, spleen, liver, kidneys from freshly dead quails were finely minced, placed in antibiotic saline (10,000 IU penicillin & 10 mg

streptomyocin/ml) as 10 % weight/volume. The samples were left for 2 hours at 22°C, centrifuged at 2000 rpm for 10 min. The supernatant fluids were kept at -20°C till used for chicken embryo inoculation. Samples from liver, brain and heart blood were also taken at the same time from the same cases for the bacteriological examination.

Embryonated chicken eggs (E.C.E.) and one day old chicken: For virus isolation, titration and characterization were supplied from native farm. One day old chicks were reared in strict isolation without vaccination till used. All chicks were checked for freedom from HI-ND antibodies before the experiment.

Reference virus: TAD NDV vaccinal strain (LaSota) was prepared in ECE (virus titre $10^{8.3}$ ELD_{50}/ml. Frozen at -20 °C till used.

Reference ND antiserum: A locally prepared ND hyperimmune serum (HI titer =1024) was used.

Pigeon squabs and adult quails: 4-6 weeks old squabs and adult quails were obtained from local market. All birds were checked for freedom from HI-ND antibodies before used in the experiments.

Hemagglutination (HA) test : A rapid and micro-slow HA test was employed after *ANON, 1971* using 10 % and 0.75 % washed erythrocytes, respectively.

Hemagglutination inhibition (HI) test : The microtechnique HI test was done after *ALLAN and GOUGH (1974)* using 4 HA units.

Virus isolation and identification : 0.1 ml volumes of the sample supernatant was inoculated into the allantoic cavity of five 9 to 10 day old ECE. Inoculated embryos were incubated at 37°C and candled twice daily. Embryos died within 24 hours after inoculation were discaded as nonspecific deaths. Allantoic/amniotic fluids from embryos died after 24 hours were tested by rapid HA and then slow HA test. All positive fluids were further identified in HI test using reference ND-antiserum. Positive HI virus isolates were tested for freedom from bacteria.

Virus characterization and pathotyping : Two ND virus isolates desingated AG_1 & AG_2 (2 nd egg passage) from young and adult quails respectively were subjected to further characterization and pathotyping.

Virus titration in ECE: The embryo lethal dose-50 (ELD_{50}) per ml for the two virus isolates (AG_1 & AG_2) as well as LaSota ND virus was estimated after *REED and MUENCH (1938).*

Virus Characterization :

a- Sensitivity to chloroform :

Two virus isolates were subjected to 10 % chloroform sensitivity at 22 C for 30 minutes after *LUCKERT (1989).* A loss of infectivity of Log_{10} or greater indicates sensitivity to chloroform(*FELDMAN and WANG, 1961).*

b- HA-activity for avian and mammalian erythrocytes:

The quantitative micro-HA test for two virus islates (AG_1 & AG_2) for chicken, quail, turkey, sheep cattle and donkey erythrocytes was performed after *WINSLOW* et al. (1950) using 0.75 % washed erythrocytes.

c- Elution activity rate:

Rate of elution of agglutinated red blood cells for virus isolates (AG_1 & AG_2) was done after *SPALATIN* et al. (1970).

d- Hemagglutinin thermostability:

The thermostability of the hemagglutinin virus-isolates at 56 C for different periods was determined (*HANSON and SPALATIN, 1978)* using chicken erythrocytes.

Pathotyping of virus-isolates :

a- Mean death time (MDT) in ECE :

Chicken embryo mean death time of the minimum lethal dose of the two virus isolates (2nd egg passage) and LaSota ND virus was determined after *HANSON (1975).*

b- Intracerebral (IC) pathogenicity test in susceptible pigeon squabs :

The test was carried out according to *AHMED* et al. (1980) Ten 4-6-week old squabs/isolate were inoculated into one cerebral hemisphere with 0.05 ml of each virus isolate containing $10^{5.7}$ ELD_{50}. Two control squabs were inoculated IC with 0.05 ml sterile normal saline and kept as control. The birds were observed daily for 10 days. (IC-PI) pathogenicity index was calculated for isolates according to the scoring system adopted by *AHMED* et al. (1980), giving death a numerical value of 4, illness 2 and no ill effect zero, and intracerebral mean death time (IC-MDT) was calculated after *LANCASTER and ALEXANDER, (1975).*

c- Intracloacal pathogenicity (ICL-P) test in 6-week-old susceptible chickens:

The test was done after *ALEXANDER (1989).* Swabbing the cloaca of five chickens/virus isolate with undiluted virus suspension (2nd egg passage) to distinguish VVNDV. Obsevation period was 10 days. An isolate was considered to be of velogenic viscerotropic pathotype when hemorrhagic gastrointestinal lesions were prevalent in the majority of

inoculated chickens. Intracloacal mean death time (ICL-MDT) was calculated after *LANCASTER and ALEXANDER (1975)* . The intracloacal pathogenicity index (ICL-PI) was determined in adopted manner similar to *ANON (1963).*

d- Intramuscular (IM) pathogenicity test in chickens and quails:

To compare the clinical and pathological manifestations of the virus isolates (AG_1 & AG_2) in 8-week-old chickens and quails (8-week-old). Ten susceptible chicken and ten susceptible quails per virus isolate were inoculated IM by 0.1 ml containing $10^{6.2}$ per bird of each virus suspension (2nd egg passage). Two birds of each species were inoculated 0.1 ml/bird with sterile saline as control. The birds were observed for 10 days and the clinical signs, mortalities and post mortem findings were recorded. The IM-pathogenicity index was determined after *ANON (1963)* and also IM-mean death time (*LANCASTER and ALEXANDER, 1975).*

specifity of deaths in experiments b, c and d was confirmed by virus reisolation.

RESULTS

Clinical signs of natural outbreak:

The first clinical signs observed in 4790 three week old and adult 1075 breeding quails was depression, diarrhea, listlessness, incoordination, fine tremors, ataxia, paresis and deaths. In young quails death was usually prompt, occurring 2-3 days after clinical signs appear. Edema of head and face and paralysis of legs were noticed in some birds. The mortality rate in adult and young quails was 24..5 % and 44.2 %, respectively. In breedig quails, drop in egg productin and hatchability rate was decreased from 92 % to 16 % and 42 % to 19 %, respectively.

Post mortem findings :

The most prominant lesions were congestion in subcutaneous blood vessels, petechial or ecchymotic hemorrhages on the liver, spleen, heart ,severe congesion of the brain with hemorrhages (Fig., 1). Numerous hemorrhages were also seen in lining of proventriculus, ventriculus and small intestine particulary duodenum in young quails (Fig., 2). Liver was dark congested and swollen in some birds.

Bacteriological examination :

Samples from liver, brain, and heart blood revealed negative results in bacteriological examination.

Virus isolation and identification :

Nine hemagglutinating agents (three from adult and six from young quails) were isolated in ECE out of twenty pooled samples in the first passage by rapid HA. All inoculated embryos died within 2-5 days post inoculation. Lesions observed in dead embryos were mostly subcutaneous hemorrhages, severe congestion of the visceral organs with petechial or echymotic hemorrhages. Bacteriological examination of allantoic fluids revealed negative results. Virus isolates were identified as NDV by HA and HI tests. Two virus isolatees designated AG1 from young quails and AG2 from breeeding ones were subjected for further studies.

Virus titration in ECE :

ELD_{50} for the two virus isolates (AG1 & AG2) were $10^{9.2}$ and $10^{8.2}$, respectively and LaSota NOV was $10^{8.9}$.

Characterization and pathotyping of virus-isolates :

I- Characterization

a- Sensitivity to chloroform: The two virus isolates (AG1 & AG2) were sensitive to chloroform treatment.

b- HA-activity for mammalian and avian erythrocytes: HA-activity of the two virus isolates are illustrated in Table (2).

c- Elution activity rate: The elution rate of virus isolates from erythrocytes are shown in Table (2).

d- Hemagglutinin thermostability: Results of hemagglutinin thermostability are shown in Table (1).

II- Pathotyping of virus isolates (AG1 & AG2):

a- **MDT in ECE:** MDT/MLD of two virus isolates and LaSota ND are shown in Table (3).

b- **IC-pathogenicity test in susceptible pigeon squabs:** The two virus isolates were pathogenic to squabs which induce nervous signs (incoordination and paresis of leg) and deaths. IC-PI and IC-MDT for the virus isolates are shown in Table (3).

c- **Intracloacal pathogenicity test in 6-week-old susceptible chickens :** All chickens intracloacal swabbed with (AG1 & AG2) virus isolates revealed severe hemorrhagic lesions in cloaca area, petechial to ecchymotic hemorrhages in gastrointestinal tract and deaths occured on 5th to 8th day post infection. Intracloacal pathogenicity indicies and ICL-MDT are shown in Table (3).

d- Intramuscular pathogenicity test of the virus isolates in 8-week-old chickens and quails:

Clinical signs appeared 3 days post infection in chickens and 4 days in quails. No respiratory signs were recorded in both species. Chickens showed depression, inappetance and diarrhea 3 days post infection. Infected quails showed clinical signs identical to that described in natural outbreak (list lessness, incoordination, parasis and paralysis of legs, fine tremors of the head and ataxia). Diarrhea was also observed in infected quails. Eight of ten inoculated chickens died between 4-7 days post infection while only 5 quails were died (5-9 days post infection). The main post mortem findings in quails were severe congestion of the brain with haemorrhages (Fig. 3) and the haemorrhagic visceral lesions were restricted to proventriculus, proventricular junction and the small intestine (Fig. 3). In chickens, the haemorrhagic visceral lesions were more severe, predominant and numerous hemorrhages of gastrointestinal tract were usually seen in the proventriculus, ventriculus and in upper and lower intestinal tract. Regularly necrosis of gastrointestinal tract was observed in two protracted dead chickens. The IM-MDT and IM-PI for the two virus isolates in chickens and quails were illustrated in Table (3).

All deaths were considered specific through virus reisolation. All control birds in all experiments remained quite healthy through observation period.

DISCUSSION

Although there are extensive literatures on ND in different bird species, there is little available reports on ND in quails. This study describes for the first time the natural outbreak of NDV in quailsin Egypt. The young quails are more susceptible to natural outbreak of ND infection than adult ones as indicated by high mortalities (53.5 %) and severity of clinical signs and post mortem lesions. According to the results of pathogenicity tests, MDT in ECE for the two isolates (AG_1 & AG_2) was < 60 (54.8 & 58.4 hours), IC-application in squabs revealed IC-MDT of 3.9 & 4.1 days and IC-PI of 2.48 & 2.24, the two isolates had IM-MDT in chickens of 4.6 & 5.2 days and IM-PI of 1.90 & 1.88, the two isolates could thus be categorized as velogenic strains. ICL-application in chickens revealed deaths with hemorrhagic lesions in the gastrointestinal tract and the two isolates had ICL-MDT of 5.4 & 5.6 days and ICL-PI of 1.80 & 1.76, respectively, the two isolates could be considered as velogenic strains with viscerotropic character. Similar results of NDV strains

pathotyping were reported by *OLAH and PALATKA (1963), AHMED* et al. *(1980).* Concerning the pathogenicity of the virus isolates (AG_1 & AG_2) in chickens and quails, it was clear that chickens are more susceptible than quails to NDV infection as indicated by short course and high mortalities in chickens than quails. *CORRADO (1970)* failed to infect Japanesse quails with NDV. Prevalence of nervous signs and predominance of viscerotropic lesions in infected quails and chickens, in addition to absence of respiratory signs in both species indicated the pantropic character of velogenic NDV strains. *CHEVILLE* et al.*(1972) and TAKEHARA* et al. *(1986)* reported that velogenic ND viruses are pantropic in birds and can produce signs and lesions in the digestive, nervous and respirtaory systems but the degree of predominance in one system over the two others may be a function of several epidemiological variable of ND viruses. From the present study, it is concluded that quails can be naturally infected with NDV and play an important role in epidemiological aspects of ND virus.

Vaccination of commercial breeding quails flocks at 7th day-old with Hitchner B_1 and followed by LaSota NDV strain in drinking water at 5th week of age was recommended to control the disease. Quails given an amount of virus equivalent to a single dose recommended by the manufacturers for chickens. Vaccination protected these flocks against the natural Newcastle disease.

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Table (1):Thermostability of hemagglutinins of the two virus isolates and LaSota NDV at 56°C.

Exposure time (minutes)	Virus HA-titer AG_1	AG_2	LaSota
0	1024*	512	1024
5	1024	512	16
15	1024	128	0
30	512	16	-
60	32	0	-
120	0	-	-

* The highest virus dilution giving Complete hemagglutination.

Table (2):HA-activity for avian and mammalian erythrocytes and elution activity rate from chicken erythrocytes of the two virus-isolates.

Test	AG_1	AG_2
HA-activity		
Chichen	1024*	512
Quail	512	512
Duck	128	256
Sheep	64	128
Cattle	256	128
Donkey	128	32
Elution activity rate	slow	slow

* The highest virus dilution giving complete hemagglutination.

Table (3):Pathogenicity of virus isoates (AG_1&AG_2) and Lasota NDV.

Virus	MDT/MLD[a] in ECE (hours)	IC-Pathogenicity in pigeons		ICL-Pathogenicity in chickens		IM-Pathogenicity in chickens		IM-Pathogenicity in quails	
		MDT[b] (days)	PI[c]	MDT[d] (days)	PI[e]	MDT[f] (days)	PI[g]	MDT[k] (days)	PI[l]
AG_1	54.8	3.90	2.48	5.40	1.80	4.60	1.90	5.20	1.60
AG_2	58.4	4.10	2.24	5.60	1.76	5.20	1.88	6.00	1.59
LaSota	118.0	-	--	-	-	-	-	-	-

a MDT/MLD = Mean death time of the minimal lethal dose in ECE (hours).
b IC-MDT = Intracerebral mean death time in 4-6-week old pigeon squabs.
c IC-PI = Intracerebral pathogenicity index in 4-6-weeks old pigeon squabs.
d ICL-MDT = Intracloacal mean death time in 8-week old chickens.
e ICL-PI = Intracloacal pathogenicity index in 8-week old chickens.
f IM-MDT = Intramuscular mean death time in 8-week old chickens.
g IM-PI = Intramuscular pathogenicity index in 8-week old chickens.
k IM-MDT = Intramuscular mean death time in adult quails.
l IM-PI = Intramuscular pathogenicity index in adult quails.
- = Not determined.

Fig.1: Congestion and hemorrhages in the brain in naturallyinfected adult quails .

Fig.2: Hemorrhages in the gastrointestinal tract in naturally infected quails .

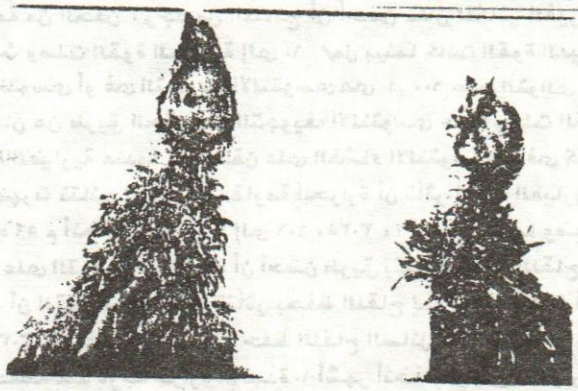

Fig.3: Congestion and hemorrhages in the brain in experimentally infected quails .