NATURAL AND EXPERIMENTAL INFECTIONS OF QUAILS (COUTURNIX COUTURNIX JAPONICA) WITH NEWCASTLE DISEASE VIRUS

El-Tarabili M. M.*, El-Shahiedy M. S.*, Hammouda M. S.**, Fetaih H. A.***, Abdel-Wahab Shahira A.* and Ramzy Neven M. ****


ABSTRACT

This study was carried out to clarify the susceptibility of quails to the infection with Newcastle disease (ND) virus and its possible role in the epidemiology of the disease. Also to locate the virus in tissues and organs of naturally and experimentally infected quails. Isolation and characterization of the ND virus quail strain from naturally infected quails was done. Twenty diseased quails aged 3 weeks-old, showing mild respiratory and nervous manifestations were obtained from the farm of the Faculty of Agriculture, Suez Canal University that was suffering 10% morbidity and 1.6% mortalities, were investigated. NDV was isolated from 3 birds out of them. The isolated strain was mesogenic and used for experimental study. The experimental infection caused deaths in 25% of the experimented quails after one week. The serological tests including HI, HA, AGPT and inoculation in specific pathogen free (SPF) embryonated chicken eggs (ECE) were used for diagnosis and virus isolation along this study. In addition, gross and histopathological investigations of different organs were carried out. All changes in the HI titres, and changes in the body tissues were recorded.

INTRODUCTION

Newcastle disease (ND) is a major disease problem of poultry in many countries of the world, especially in Africa and Asia (Spradbrow 1992, Awan et al. 1994 and Oladele et al 2005). Over 200 species of birds have been also reported to be susceptible to natural and/or experimental infection with ND virus. Birds other than domestic chickens have been known to be sources of the spread of ND virus (Lancaster, 1963, Roy et al 1998). ND is complicated so that different isolates and strains of the virus may induce enormous variations in the severity of
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disease even in a given host such as the chickens (Beard and Hanson 1984). Concerning quails, Lima et al (2004) considered them as important carriers for ND virus. On the other hand, Higgins and Wong (1968) and Higgins (1971) stated that quails are rather resistant to NDV infection; but they may become infected under stress conditions. In other studies, quails were found to acquire the natural infection with a velogenic strain of ND virus (CzirJac et al 2007, Lima et al 2004 and Sa'idy 2004). In Egypt, (Assiut province) El-Zanty and Abd-El-Motelb (1993) recorded a viscerotropic velogenic ND in quails (Coturnix coturnix japonica). A natural infection occurred in young and adult quails. The clinical, postmortem changes and characterization of the virus were described. Oladele et al (2008) recorded the histopathological and HI antibody titer in Japanese quails experimentally infected with NDV. The infected quails developed ND with classical clinical signs and lesions which included focal necrosis in most of the organs, mononuclear cells infiltration and depletion of lymphoid tissues. There was a rise in HI titer from zero to maximum mean antibody titer of 10 log₂. Sa'idy (2004) studied the serological evidence of ND in quails in Nigeria and detected infection in 12% on examined birds with low mean HI titre (0.4 log₂). Czirjak et al. (2007) recorded 100% morbidity and mortality in an outbreak of ND in quails with severe clinical symptoms and pathological lesions in both digestive and nervous systems. Ucan and Catatoluk (2002) concluded that the breeding of quails and chickens together in the same house could be a way of exposing chickens to the threat of ND virus outbreaks possessing more virulent character. Peroulis and O'Riley (2004) isolated and characterized avian paramyxoviruses in quails based on their haemagglutination and -haemagglutination-inhibition activity using a panel of antisera. PMV-1 was isolated from examined quails. There is a contradiction in the results of previous studies and still scarce information present about the disease in quails. Therefore, the present work was designed to determine the clinical picture and tissue changes due to ND virus infection in quails, changes in the HI antibody titre in the natural and experimental infections as well as isolation and characterization of the virus.

MATERIAL & METHODS

1- Field study:

1- a- Quails:

A farm belonging to Faculty of Agriculture, Suez Canal University, containing about 5000 quails (Coturnix coturnix japonica) was kept under observation for 2 weeks. There were 10% morbidity and 1.6% mortality, and the initial diagnosis was directed towards the
Newcastle disease. Twenty diseased quails, 3 weeks-old, showing mild respiratory and nervous symptoms were submitted to the laboratory for serum samples and tissue specimens collection.

1- b- Serum Samples:
A total of 40 serum samples were collected, including 20 blood samples from quails at acute stage and 20 samples of the same quails 2 weeks later. The samples were taken for serological screening of antibodies against ND virus in acute and convalescent stages.

1- c- Serological Screening of ND virus antibodies in serum:
Antibodies against ND virus were screened in the collected serum samples using haemagglutination inhibition test (HI).

1- d- Postmortem examination and tissue specimens for virus isolation:
After postmortem examination, pooled samples of liver, spleen, lung, kidneys and cecal tonsils of each bird of the 20 quails were finely minced, frozen and thawed 3 cycles. The inoculums were prepared as usual and kept for weeks or days at -70°C until used for egg inoculation.

1- e- Inoculation of SPF embryonated chicken eggs (ECE):
Twenty SPF embryonated chicken eggs were used for each sample. The eggs were supplied by "Abbasia Laboratories for Serum and Vaccine Production". The eggs of 3 days old embryos were candled to exclude the infertile eggs and dead in shell. At 9 days old embryos, 10 eggs were inoculated with 0.2 ml/egg by allantoic sac route and another 10 eggs were inoculated onto chorioallantoic membrane.

1- f- ND virus identification:
1- f- 1- Agar gel precipitation test:
Agar gel precipitation test was used for identification of ND virus quail isolates and detection of ND antibodies in serum of experimentally infected quails. The test proper is done according to Woerle (1963).

1- f- 2- Haemagglutination test:
Allantoic fluid of inoculated eggs that suspected to contain ND virus is subjected for detection of haemagglutinating property. The test was carried out by 2 means, rapid slide agglutination test and microtitre plate method.

1- g- NDV antigen preparation:
1- g- 1- Agar gel precipitating antigen:
was prepared from harvested chorioallantoic membranes that showed hemorrhagic spots and inflammation with early embryonic death Woerle (1963).

1- g- 2- Haemagglutinating antigen preparation:
Allantoic fluid of inoculated eggs were used as haem-
agglutinating antigen Allan and Go- ugh (1974).

1- h- Titeration of NDV quail strain and reference NDV in SPF – ECE: ELD50 was calculated according to formula of Reed and Munch (1938).

1- i- Strain variability and charac- terization:
The obtained ND virus quail strains were tested to distinguish the patho-type of the isolates. Mean death time of the minimum lethal dose, thermo-stability of haemagglutinin and agglutination of mammalian erythrocytes were used to determine ND virus pathotypes according to the method of Rosenberger et al (1975) and Islam et al (1994).

2- Experimental study: This was performed after the isolation and ch-aracterization the virus from the nat-urally infected quails, to study the pathogenicity of the isolated ND vi-rus quail strain in quails.

2- a- Experimental design:

Thirty six healthy native quails (Coturnix coturnix japonica), aged 3 weeks-old were randomly divided into 4 groups: The first group (gp I): 12 quails received 0.2 ml of NDV quail strain of titer $10^7$ ELD50 /ml, intraperitoneally. The second group (gp II): 12 quails received the same dose, intraocular. The third group (gp III): 6 quails, received the same dose but of VVND virus reference chicken strain, intraocular for comp-arison. The fourth group (gp IV): contained 6 quails as non infected control group, injected intraperitoneally only with sterile saline.

Quails in each group were kept in a separate room and separate cages. All quails were kept at restricted hy-gienic measures and supplied water ad libitum and non drug suppleme-nted ration for 5 weeks. All quails were observed daily for presence of clinical signs or death. Serum and tissue samples were collected wee-ly. Serum was used for mean HI antibody titre determination of all birds. Part of the tissue samples was kept frozen at -20 °C for ND virus reisolation, while the other part was taken for histopathological examin-ation.

2- b- Pathological examination:

Four quails from gps (I and II) were taken every week for pathological investigations. The other 2 groups were subjected to the same inves-tigations at the end of the experi-ment.

Specimens from lungs, heart, liver, kidneys, spleen, brain, intestine and proventriculus of each quails were taken at necropsy and immediately fixed in 10% neutral buffered form-alin for 24 hours. The specimens were then processed by the conven-tional methods, embedded in para-fin wax, sectioned at 4-5 microns and stained with the routine stain
H&E, according to Drury and Wallington (1980).

RESULTS

**Clinical signs of naturally infected quails:** The affected birds showed loss of appetite, abnormal thirst, huddling and weakness. Some birds showed mild respiratory dyspnea and gasping with coughing and sneezing. The morbidity occurred in 10% of quails. The affected quails started to die after 1 week from onset of the disease. Mortality rate was 80/5000 with a percentage of 1.6%.

**Postmortem lesions of naturally infected quail:** Postmortem examination of dead quails showed inflammatory and focal hemorrhagic lesions in the respiratory system. Liver, spleen, kidneys and heart showed occasionally hemorrhagic spots on the serosal surface, enlargement in size and pale discolouration. No obvious postmortem changes were observed in alimentary tract.

**Results of Serological screening of naturally infected quails:** Forty serum samples were collected during the acute phase of the disease and another 40 samples were collected during the convalescent phase 2 weeks later. As shown in table (1), seroconversion of NDV antibodies were observed between the two stages.

**Table (1): HI antibody titer and agar gel precipitating antibodies to NDV in the quail's farm:**

<table>
<thead>
<tr>
<th></th>
<th>Antibodies in acute stage</th>
<th>Antibodies in convalescent stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean HI antibody titer</td>
<td>2(^{\circ}) (1/32)</td>
<td>2(^{\circ}) (1/256)</td>
</tr>
<tr>
<td>*AGP antibodies</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

* AGP = agar gel precipitating antibodies.

**Isolation and characterization of NDV from the quails:** After the third passage of 20 inoculums prepared from tissues of naturally infected birds, in SPF-ECE, 15 samples gave haemagglutination results for chicken RBCs with HA titer ranged from 1/8 to 1/2048 dilutions and the embryo death rate was 35/100. Six samples were positive with the agar gel precipitation test, but 3 only of them were strong positive. So, ND virus was successfully isolated from 3 quails (Srtains N0. 8, 18 & 19).

**Titration of NDV quail strains and reference NDV strains:** After three passages of the virus in SPF embryonated chicken eggs, the obtained results are shown in table (2).
Table (2): Titers of NDV quail and reference strains in embryonated chicken eggs:

<table>
<thead>
<tr>
<th>ND virus strain</th>
<th>First Passage</th>
<th>Second Passage</th>
<th>Third Passage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELD 50/ml</td>
<td>HA titer</td>
<td>ELD 50/ml</td>
</tr>
<tr>
<td>New Jersey LaSota strain</td>
<td>*10⁹</td>
<td>1/1024</td>
<td>10⁷</td>
</tr>
<tr>
<td>VVND strain</td>
<td>10⁴</td>
<td>1/2048</td>
<td>10⁶</td>
</tr>
<tr>
<td>NDV quail strain 18</td>
<td>10³</td>
<td>1/512</td>
<td>10⁵</td>
</tr>
<tr>
<td>NDV quail strain 19</td>
<td>10⁵</td>
<td>1/1024</td>
<td>10⁷</td>
</tr>
<tr>
<td>NDV quail strain 8</td>
<td>10⁵</td>
<td>1/1024</td>
<td>10⁶</td>
</tr>
</tbody>
</table>

* Titer are expressed log₁₀ ELD50/ml.

**Determination of NDV virulence quail strains:** The mean death times of minimal lethal dose of the 3 strains were 70, 80, and 75 hours, the haemagglutinin was not stable at 56 °C for 5 minutes and haemagglutination of mammalian erythrocytes (equine and bovine RBCs) was negative. Therefore, the isolated NDV quail strains were found to be mesogenic strains, according to the known parameters used for determination of NDV virulence.

**Pathogenicity of NDV quail strain in experimentally infected quails:**

**Clinical signs and mortalities:** Clinical signs were observed only in quails inoculated intraperitoneally (gp I). The signs included: general weakness, loss of appetite, unthriftiness, dropped wings (Fig.1) and mild respiratory manifestations as coughing and gasping with respiratory rales. There were no obvious digestive symptoms. The morbidity rate was the same like the mortality rate. Mortalities occurred only in the same group, 3 quails in the 1st week PI (25%) and one quail in the 2nd week PI (8.3%). In gp (II) that was inoculated intraocular, no deaths occurred. Five days post infection of gp (III) with VVND virus chicken strain, only one bird died.

**Mean HI antibody titer and virus reisolation:** Mean HI antibody titer and virus reisolation from tissues and organs of quails experimentally infected with ND virus quail strain in gp (I) and VVND virus reference virus in gp (III) were shown in table (3). Results of the gp (II) inoculated intraocular, were negative.
Table (3): mean HI titre and virus isolation of intraperitonially infected group with NDV quail strain and infected group with VVNDV chicken strain:

<table>
<thead>
<tr>
<th>Times postinfection &amp; post Challenge</th>
<th>Intraperitoneal infection group (gp I)</th>
<th>Group infected with VVND chicken strain (gp III)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean HI Titer Log₂</td>
<td>Virus Isolation</td>
</tr>
<tr>
<td>1st week PI</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>2nd week PI</td>
<td>1/128</td>
<td>+</td>
</tr>
<tr>
<td>3rd week PI</td>
<td>1/128</td>
<td>-</td>
</tr>
</tbody>
</table>

Pathological changes: Postmortem examination of all groups revealed pathological lesions only in quails of groups I & II (gp I & gp II) and only in the first two weeks PI. After the 3rd week PI the remaining quails appeared normal on gross examination. The detected pathological lesions were severe to moderate in dead or moribund quails of the group inoculated intraperitoneally (gpI), while they were mild to non significant in gp (II) inoculated intraocular. Grossly, there were generalized congestion of the internal organs, petechial hemorrhages on the serosal surface of digestive tract, particularly proventriculus, also on pancreas and on trachea. Dark red pneumatic areas (Fig. 2) and occasionally nodular lesions were seen in the lung. Enlargement of liver, spleen, kidneys with pale discoloration were also observed. Heart was, sometimes, suffering from enlargement and exhibiting a wrinkled surface. Histopathological examination of the lung revealed severe to moderate pneumonia in the affected lungs where the bronchioles and parabronchi were filled with inflammatory exudate, mostly lymphocytes and macrophages (Fig. 3), with necrosis of the lining epithelium and underlying smooth muscles. In the nodular pneumonic lesions there was heavy infiltration of the lung tissue and all air spaces with the same types of cells (Fig. 4), beside presence of some necrotic areas. Large blood vessels exhibited severe congestion, vasculitis and perivascular edema. The digestive tract showed only mild catarrhal inflammation with necrosis of lymphoid aggregations. Proventriculus showed necrosis and loss of surface epithelium, sloughing of the glandular epithelium in the glandular lumens that mixed with inflammatory cells and aggregations of lymphocytes in the subepithelial and interglandular connective tissue septa. Liver and kidneys showed degenerative changes with focal necrosis and infiltration with mononuclear cells. In some cases, glomerulonephritis was diagnosed. The heart muscles suffered from dege-
neration and focal necrotic areas that was infiltrated with mononuclear cells (Fig. 5). Marked depletion of lymphocytes was observed in the spleen (Fig. 6). Brain of most cases showed neuronal degeneration and perivascular and pericellular edema. Pathological examination of groups (III) & (IV) did not show significant changes.
Legend:
Pathological figures of quails intraperitoneally infected with NDV quail strain, 1 week PI showing: 1) Ruffled feathers, dropped wings, unthriftness and opened mouth. 2) Severe congestion and dark pneumonic areas in the lung. 3) Pneumonia with severe congestion and inflammatory exudate filling the parabronchi and other air spaces, in addition to vasculitis and perivascular edema. H&E. X60. 4) Severe pneumonia due to heavy infiltration of the lung tissue with mononuclear cells. H&E. X180. 5) Focal area of cardiac necrosis and infiltration with mononuclear cells. H&E. X180. 6) Severe depletion of the lymphoid tissue with necrotic changes in the white pulp of spleen. H&E. X60.

DISCUSSION

Newcastle disease is defined as a "list A" disease by OIE (1996), as it is highly contagious causes severe economic losses in domestic and wild bird species specially chickens (Kaleta, 1992 and Alexander and Jones, 2001). The virus is capable to infect all bird species and some other vertebrates (Leighton and Heckert, 2007). Concerning quails, Silva Lima et al (2004) considered quails as important carrier for ND virus. In this study it was proved that quails can be infected either naturally or experimentally but with low mortality and morbidity rates. This is in agreement with the findings of Sa'idu et al. (2004) and Tawfik Hoda et al (2004) but they recorded higher rates of mortality and morbidity. This also was in partial agreement with the findings of Islam et al (1994) who considered stress is an important factor for getting the infection. The isolated quail strain in this work was mesogenic and the infection resulted in signs, mainly of respiratory and nervous illness, as recorded previously by Czerjak et al. (2007). In this aspect, Calnek et al (1997) mentioned that the clinical signs, gross lesions and the organs affected are dependent on the strain and the pathotype of ND virus, in addition to the host and other factors like the rout of infection.

The main diagnostic tests used in this study were HI, HA and AGPT. These techniques are applied for many virus infections in chickens (Calnek et al 1997). For confirmation of ND, the OIE standards commission prescribes ND virus isolation in embryonated chicken eggs and identification using HA and HI test with a NDV monospecific antiserum (OIE, 1996). The HI test had proved the seroconversion in the natural infection study and clarified the immune status in experimental infection. In this aspect, it is worth of mentioning that the low HI antibody titer of naturally infected quails recorded in our study is below the protective level, as said by Lancaster (1963).

Accordingly, Czirjak et al (2007) stated that any outbreak of ND in
The other tests, in addition to RT-PCR, were used for isolation and characterization of the ND virus quail strain. Increase in HA titres after 3\textsuperscript{rd} passage of inoculums from natural cases in SPF is in accordance with the explanation of Ismail et al. (1979) and Sa'idu et al. (2004). Concerning the mean HI titers and virus isolation of NDV from experimentally infected and challenged quail with VVND virus are indicating that the mean HI titer appear to begin in the 2\textsuperscript{nd} weeks post infection and 2 weeks post challenge, in other hand ND virus strains could be isolated only 1 week and 2 weeks post infection and post challenge. The same results obtained by Czirjak et al. (2007) and Oladele et al. (2008).

Quails experimentally infected by intraperitoneal route showed signs and gross lesions one and two weeks post infection. These results are mostly in agreement with those reported in quails and other avian species by Cross (1991), Emmerson (1994), Lam (1996) and Oladel et al. (2008). In this study, the infection with ND virus quail strain intraperitoneally induced 25\% mortalities in the first week post infection, and no deaths occurred after challenge with VVND virus chicken strain. This may be due to that the quails evoked anti NDV immune response that protected them. In this point, these results disagree with that of Usman et al. (2008) who recorded high mortalities (100\%) in a challenged group.

The pathological changes seen in this study indicated the pantropism of the virus. Most of the body organs were involved. The detected histopathological changes were in accordance with that of Usman et al. (2008) and that of Oladel et al. (2008) in two studies on quails in Nigeria. In chickens nearly similar changes were found in studies done by Alexander (1991), Okoye et al. (2000) and Oladel et al. (2005). Some differences with that of the other birds were recorded. The lungs in some quails showed a nodular form of pneumonia. The route of infection might play a role in the lesion distribution and form. The blood vessels exhibited necrotizing vasculitis and perivascular edema. Changes in the proventriculus were detected mainly on microscopical examination, and changes in the intestinal tract seemed to be of low significance in this type of birds. Concerning the involvement the heart, Brown et al. (1999) suggested that the compromise of the myocardium may predispose mesogen and lentogen infected birds to secondary infection.

In a conclusion this study has demonstrated that ND virus quail strain of intermediate virulence can infect quails, naturally or experime-
ntally and can cause changes in body parameters as HI titres and injury in different organs. The virus causes the disease with low morbidity and mortality rates. The picture of the disease in quails is slightly different from that in chickens.

REFERENCES


Peroulis, I. and O'Riley, K. (2004): Detection of avian paramyxoviruses and influenza viruses amongst wild


الملخص العربي

العدوى الطبيعية والتجريبية فى السمان بفيروس مرض النيوكاسل

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أجريت هذه الدراسة لأيضاح مدى إمكانية حدوث مرض النيوكاسيل بالعدوى الطبيعية والتجريبية في طائر السمان والدور المحتمل له في وفاته المرض، وكذلك دراسة الأعراض والتغيرات المرضية في عصافين امتصاصية الطائر في حالة العدوى، ولهذا الغرض تم تحضير عزل فيروس النيوكاسيل من 02 طائر السمان التي كانت قد أظهرت اعراضاعراض تفضلية وعصبية في مزرعة كلية الزراعة بجامعة قناة السويس والتي ظهر بها اعراض مرضية بنسبة 10% وفيات بنسبة 14% وامكن عزل الفيروس من 3 حالات واستخدم الفيروس المعزول بعد ذلك لأحداث عدوى تجريبية لمرض النيوكاسيل على طيور ذوات ريش والفصوص لطيور ذوات ريش المطكل عنها في هذه التحويلة بعد اسبوع من العدوى. ولعزل الفيروس وإجراء التشخيص والفحص للفيروس في هذه الدراسة استخدمت الاختبارات المناعية المختلفة: اختبار التلزن الدموي واختبار التلزن الدموي واختبار التشخيص في الأجارة وكذلك الحقن في البيض المخصب خالى من الميكروبات، بالإضافة إلى الفحص بالتشخيص المرضى والنتسجوباثولوجي، هذا وقد سجلت كل التغييرات التي حدثت في السمان الذي استخدم في الدراسة. ولقد تم تصنيف عدوى فيروس النيوكاسيل المعزولة من السمان وتحديد ضرايتها من الدرجة المتوسطة.