

## **SEROLOGICAL STUDY FOR DETECTION OF NEWCASTLE DISEASE VIRUS IN JAPANESE QUAILS IN SOME STATE OF DIYALA PROVINCE, IRAQ**

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### **ABSTRACT**

Newcastle disease (ND) is a highly contagious viral disease all over the world that could cause several losses for the poultry industry. The current study was conducted to clarify the real evidence that the quails play important role in the epidemiology source of NDV. Two groups of quail were used at 50 and 150 day old for identification of the NDV. Antigen Rapid Test Kit (Bionote, South Korea) used to identify the positive cases, all positive cases by this kit were tested by Enzyme- Linked Immunosorbent Assay (ELISA) to determine the antibody titers against NDV in all study groups. The results of this study revealed that antibodies against NDV were detected with the mean titer of  $2904.39 \pm 119.238$  and  $4664.36 \pm 136.659$  to each age groups respectively, in spite of no clinical signs of NDV were observed in the two groups. The results referred that quail were carrier for the NDV and may be transmission the pathogen virus to other poultry that housed together, and that calls the attention to the importance of the quail from the epidemiological point of view as a potential source of NDV to commercial poultry (broiler or layer) that housed with or near quails.

**Key words:** Newcastle disease, Quail, ELISA, Diyala.

### **INTRODUCTION**

Newcastle disease virus (NDV) is a member of the genus Avulavirus of the Paramyxoviridae family of enveloped negative-stranded RNA viruses. It causes high mortality, hemorrhagic intestinal lesions, severe respiratory distress, nervous disorders, and decreased egg production (Hanson, 1980). The virus is widespread among wild and domestic birds and it is capable to infect all birds species and some other vertebrates, including humans (mild transient conjunctivitis) (Leighton and Heckert, 2007; Alexander, 2000). The disease has been widely studied among poultry species (Chickens, turkeys, etc) and also among some wild birds but there is a lack of studies about the role of exotic and ornamental birds like quails in the epidemiology of the ND which were considered by many research as an important carrier for the ND virus (Silva et al., 2004).

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The Japanese quail belongs to the order Galliformes, family phasianidae , genus *Coturnix* and species *japonica*, which were firstly domesticated around the 18<sup>th</sup> century in Japan. The plumage color of the wild type is predominately cinnamon brown. However, adult female have pale breast feathers that are spotted with red feathers on the breast and cheek and may lay eggs when only 35 days old (Mizutani, 2003).

Many studies showed that, the commercial production of Japanese quails is extensively distributed in several countries around the world due to highly resistant for many infectious agents which included viral and bacterial diseases and can easily adapt to reared in many different environment (Merino *et al.*, 2009; Mizutani, 2003; Islam *et al.*, 1994).

Generally, the domestic chicken is a common host bird that is highly susceptible to NDV. Infections with highly virulent strain of NDV can cause a broad range of symptoms varying from asymptomatic enteric infections to systemic infections which cause more than 90% mortality and the virus able to infect all avian species all over the world (Gary *et al.*, 1993). Other researchers showed that other domestic and semidomestic species of birds, such as quails, pigeons, ducks, turkey and geese are do not tend to develop severe signs to NDV infection, hence acting as asymptomatic carriers for NDV and could be an important role for epidemiology of the ND on regions of extensive poultry production led to highly mortality with severe economic implication (Lima *et al.*, 2004; Gary *et al.*, 1993).

In most Iraqi villages, it is common to find a combination of different poultry species and breeds being kept in the same area, including chickens, turkey, ducks and quail, in this point we noticed that the virulence of the NDV increased and led to high mortality in chickens.

However, there is little information available dealing with serological assays in quail sera and manage programs in this species. Thus, the present study aimed to detect the antibodies against NDV in quail flock in Diyala Governorate by using serological assays (Rapid test kit and ELISA) for the rapid screening of antibody activity titers against Newcastle disease virus (NDV).

## **MATERIALS AND METHODS**

### **1-Site of the Experiment**

The serological detection for this experiment was performed in the Virology Laboratory, College of Veterinary Medicine, University of Diyala, Diyala, Iraq. Whereas the practical experiment carried out in Buhri's Town, Diyala province (Figure, 1).



**Figure1:Groups of apparently healthy quails reared in cages and used for identification of NDV.**



**Figure 2:Shows quails feeding formulated as balanced ration.**

## **2-Experimental Birds**

A total number of 72 domesticated Japanese quails (*Coturnixcoturnix japonica*) at,50 and 150 days- old were distributed into two groups of 36birds for each group. The 18 quails from each group were used for the serological identification.All the birds were housed in cages supplied with water and feed offered *ad Libium* (Figure, 2). The diet based on corn and soybean meal and formulated as balanced ration according to (NRC, 1994) and also all groups were not vaccinated against NDV.

## **3- Serological Testing**

From each two groups, ten birds were selected to detect NDV antigens by using the Rapid Test Kit (Rivetz, *et al.*,1985). The kit use for qualitative detection of NDV antigen in avian oropharynx, spleen, kidney and feces.A purple test line will be visible in the result window if there are enough Newcastle Disease virus antigens.

For the determination of NDV antibodies in quails by sing ELISA, 18 serum samples were collected from each group and Indirect ELISA was used. The test was performed according to the manufacture (ProFLOK® NDV ELISA Kit (Synbiotics–USA).

### **Blood Sampling**

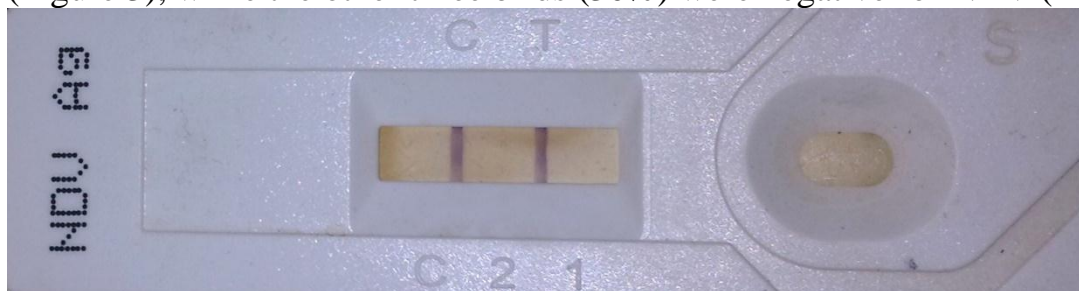
From each quail group, 3-5 ml blood samples were collected from 18 birds from the wing by vein puncture by using disposable syringe (Terumo, Japan). The blood was collected from the two groups of quails at different age into sterile tubes without anticoagulants.

The blood was allowed to clot at room temperature for 45 minutes then centrifugation at 3000rpm for 5 minutes (Hettich Centrifuge, Germany), after that the serum wastransferred into new tubes, and stored frozen in plastic vials at -20<sup>0</sup>C until use.

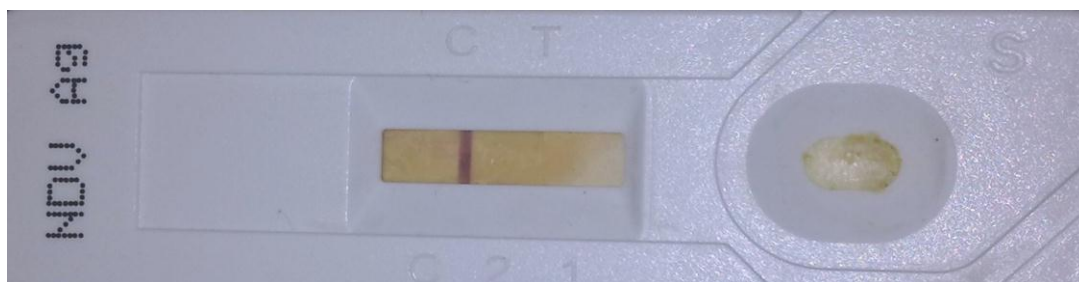
## **RESULTS AND DISCUSSION**

### **1-Rapid NDV Ag test kit (One step Newcastle disease virus antigen test)**

By using rapid test kit, for identification the NDV. Ten quails from each flocks of 50 and 150 days old were choose randomly, all of these birds were apparently healthy. The result showed that seven birds were positive result (70%) for NDV (Figure 3), while the other three birds (30%) were negative for NDV (Figure 4).



**Figure 3: NDV rapid test kit, positive results with two bands.**



**Figure 4: Rapid test kit showed negative result, only one band.**

### **2- Serological Testing**

The results of antibody titers to NDV measured by Elisa test at 50and 150 days old are shown in table, 1and 2.

The measurement of antibodies against NDV that collected from 18 quails (as indicated by ELISA test) at 50 days old for the group 2, showed a marked elevation 2969.78 with 716.613 and 168.907 for St. Deviation and Std.Error respectively, for the first collected serum when it was compared to the findings in the first group of the same age 2839.00 with 728.826 and 171.786 for St. Deviation and Std.Error respectively (Tables, 1).

On the other hand, the measurement of antibodies titers against NDV on day 150 showed also non-significant elevation in the mean antibody titer of 4692.94 with 854.875 and 201.496 for St. Deviation and Std.Error respectively for the first collected serum at 150 days old when it was compared to the second group of the same age 4635.78 with 807.254 and 190.272 for St. Deviation and Std.Error respectively (Table, 1). Table-2 included the mean titer of the two groups at 50 days old with 2904.39 and 4664.36 at 150 days old.

**Table-1: Means of antibody titer against NDV on 50 days and 150 days old quails as measured by ELISA test.**

Age	Mean	Std.Deviation	Std.Error	No.of birds	No.of group
50	2839.00	728.826	171.786	18	1
50	2969.78	716.613	168.907	18	2
150	4692.94	854.875	201.496	18	1
150	4635.78	807.254	190.272	18	2

**Table 2: Means of antibody titer against NDV on 50 and 150 days old quails as measured by ELISA test.**

Age	Mean	Std.Deviation	Std.Error	No.of birds
50	2904.39	715.426	119.238	36
150	4664.36	819.955	136.659	36

Newcastle disease virus (NDV) is one of the most serious infectious diseases and can affected both domestic and wild birds. The disease currently has a worldwide distribution and affected several birds species including quails (Alexander,2004).

The cellular and humeral response have been suggested to play important roles in the poultry host defense against NDV, whereas the severity of the ND determines by the increased the level of immunity titer(Musa *et al.*, 2013; Beard and Brugh, 1975).

The current study showed that rapid NDV Ag test kit is a chromatographic immunoassay which used for the qualitative detection with a high degree of

accuracy of NDV in oropharynx, spleen, Kidney or feces under field conditions. The kit may be helpful in detection of NDV if post-exposure prophylaxis is needed in birds reared together with chicken. This was consistent with the observation work of Rivetz *et al.*, 1985.

Various approaches have been used for identification the specific mechanism of immune system involved in protection against NDV and a considerable amount of laboratory investigation has been directed at the development of ELISAs for the seroepidemiological studies on NDV and some other viruses. Although a majority of this research has been concerned with the development aspects of ELISA for used it as a regular diagnostics protocols for NDV (AbdelRhman *et al.*, 2013; Richtzenhain *et al.*, 1993; Brown *et al.*, 1990; Adair, *et al.*, 1989; Snyder *et al.*, 1983).

For the serological survey from quails reared together with chickens, this study considers the first work conducted locally to screen NDV antibodies in quails. The study found that most quails had been exposed to NDV regardless their age and gender, and also there were no any clinical signs appeared on this birds. The work is consistent with findings reported by other researches worldwide (Musa *et al.*, 2013; Oladele *et al.*, 2008; Lima *et al.*, 2004).

The present study revealed that the titer of antibodies against NDV increased with the increased in age of quails in 50, 150 days old respectively, which means that most the quails group showed positive antibodies, regardless of the direct or lack of direct contact to ND virus. The increasing in the antibodies titer lead to suspected the severe infection with virulent NDV and may lead to highly mortality rate in these flocks, whereas all the flock showed good standing with no mortality and all seemed healthy. Similar findings were observed by Lima *et al.*, 2004 and Higgins and Wong, 1968, who found that, following challenge of flocks of Japanese quails (vaccinated or not vaccinated) with viscerotropic velogenic NDV strain, the flock did not showed any clinical lesions indicative of Newcastle disease.

Whereas other works by Musa *et al.*, 2013; Oladele *et al.*, 2008; Islam *et al.*, 1994 found that quails were susceptible to several pathogens affecting chickens, and they exhibited similar signs and developed similar gross lesions for chickens reared on the same farm.

The results of current study inconsistent with some published data (El-Tarabili *et al.*, 2009; Alexander *et al.*, 1998; Alexander, 1997) who detected that NDV can infected a variety of domesticated and wild birds led to induce enormous variations in the severity of the disease and lesion.

The current study pointed that the combination breeding of poultry (layers or broilers) and quails in the same houses which has been observed in recent years



in Iraq could be a way of exposing chickens to the threat of NDV outbreaks that led to possessing more virulent NDV strains from quail to chicken that lead up to high mortality to all poultry species, on the other hand-quails showed high resistance to infection.

The present study was in agreement with previous study of El-Tarabili *et al.*, 2009 and Alexander *et al.*, 1999, who observed that quails played the real role in the epidemiological plan under the perspective as infection source of NDV that led to infected poultry at the same location.

In conclusion, this study provide evidence that Japanese quails can carry NDV after infection lead to possessing more virulent character through the replication processes of the virus in the quail tissue, which is very important role in the spread and epidemiology of NDV to domesticated birds that sensitive to this virus. For this reason quails can play important risk factor of the dissemination of the NDV to broiler, layer and turkeys that may be raised close to the quail habitat.

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### دراسة سيروولوجية للتحقق من وجود مرض النيوكاسل في طيور السمان في بعض مناطق محافظة ديالى، العراق

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### المستخلص

مرض النيوكاسل هو أحد الأمراض الفايروسية المهمة عالمياً والمسبب لخسائر اقتصادية هائلة في مجال تربية الدواجن. الغرض من هذه الدراسة للتأكد من أن طيور السمان لها دور في نشر مرض النيوكاسل في الدجاج. استخدمت في هذه الدراسة مجموعتان من طيور السمان بأعمار (50، 150) يوماً، تم التأكد من وجود الاجسام المضادة في دم تلك الطيور من خلال استخدام عدة تشخيصية خاصة من شركة بايونوتي الكورية الجنوبية، وقد وجد ان جميع الطيور المفحوصة من كل مجموعة تحمل اجساماً مضادة لهذا المرض وتم التأكد وبصورة قاطعة باستعمال تقنية الاليزا. أظهرت نتائج قياس المعدل العام للاجسام المضادة في دم تلك الطيور وكما يلي (2904.39 - 4664.36) تزامناً مع اعمار الطيور اعلاه وبدون وجود اعراض خاصة للمرض. دلت النتائج على ان هذا النوع من الطيور يحمل الاجسام المضادة للمرض بدون ان تظهر عليه الاعراض وعليه يستطيع هذا النوع من الطيور من نشر المرض للدواجن المرباة في بيئة مشتركة واحدة مؤدياً الى خسائر اقتصادية كبيرة في مجال تربية الدواجن.

الكلمات المفتاحية: مرض النيوكاسل، السمان، الاليزا، ديالى.