The Effect of Supplemental Ascorbic Acid on the Development of Newcastle Disease in Japanese Quail (Coturnix coturnix Japonica) Exposed to High Ambient Temperature

B.A. Usman¹, A.U. Mani², A.D. El - Yuguuda³ and S.S. Diarra⁴

¹Department of Veterinary Medicine, University of Maiduguri, P.M.B. 1069, Borno State, Nigeria
²Department of Animal Health and Production, Mohamet Lawan College of Agriculture, P.M.B. 1427, Maidugun, Nigeria
³Department of Veterinary Microbiology and Parasitology, University of Maiduguri, Maidugur, Nigeria
⁴Department of Animal Health and Production, Yobe College of Agriculture Gujba, P.M.B. 1104, Damaturu, Nigeria

Abstract: In order to study the development of Newcastle Disease (ND) in Japanese quail subjected to high ambient temperature with or without supplementation with ascorbic acid (AA), eighty 3 week-old Japanese quails were randomly allotted to four equal groups. Two of the groups were kept at room temperature (26±0.5°C) with one supplemented with AA (R₂) and the other receiving no AA (R₁). The other two groups were kept at a continuous temperature of 41.8±0.6°C with one of them supplemented with AA (H₂) and other receiving no AA (H₁). The group R₀ served as the control. Ascorbic acid was supplemented at the rate of 1000mg/kg feed. Twelve birds in each of four groups were challenged occlusally with a pigeon isolate of velogenic ND virus and the rest left as in contact. Morbidity rate was 100% in all challenged and in contact birds except in the incontact of the group supplemented with AA which recorded 50%. One hundred percent mortality was recorded in heat stressed groups irrespective of AA supplementation while 0% and 50% mortality was recorded in those kept at room temperature with or without AA supplementation respectively. None of the unchallenged in contact birds died in any of the groups. Lesions of ND in the experimentally infected quails were characterized by pathological lesions. The results of haemagglutination inhibition test performed on blood samples of all birds on days 0, 7, 14 and 21 post inoculations indicated no effect of heat treatment or AA supplementation on the humoral immune response of the Japanese quail. It was concluded that prolonged high ambient temperature has detrimental effects on the survival of Japanese quail with AA supplementation having some ameliorating effects. High ambient temperature may not adversely affect the humoral immune response of Japanese quail to Newcastle disease and AA may not be an important factor in the resistance of quails exposed to prolonged high ambient temperature to ND virus infection.

Key words: Pathology, quail, ascorbic acid, high ambient temperature, Newcastle disease, immune response

Introduction
The Japanese quail (Coturnix coturnix japonica) have tremendous potential for village and backyard production as well as an important laboratory animal (Lombin, 2007). This is because of their small size, the ease with which they can be kept, requiring only simple cages and equipments and little space (Ruskin, 1991), high egg production reaching 300 per hen per year (Phillips, 1948; Banks, 1979) and a short reproductive life cycle (only eight weeks are required to hatch eggs and raise the chicks to sexual maturity (Phillips, 1948). They are also robust, disease resistant and hardy and can easily adapt to many different environments. There is paucity of data on the pathology of Newcastle disease in quail especially in hot environment, even though several reports of outbreaks of Newcastle disease in flocks of Japanese quail have been made (Kalota and Baldauf, 1988; Chandrasekaran and Aziz, 1988; Islam et al., 1994). High ambient temperature has also been shown to have an adverse effect on the development of immunity in birds (Siegal, 1971; Thaxton, 1978; Gross and Siegal, 1993) as well as on resistance to diseases, growth rate and reproduction (Ladewig, 1987). The beneficial effect of ascorbic acid (AA) supplementation on the performance of birds challenged with stressors has been reported (Lin et al., 2006).

In this study the effects of ambient temperature and ascorbic acid (AA) supplementation on the development of ND in quails was investigated.

Materials and Methods
Eighty three-week-old Japanese quail were randomly divided into four groups/treatments containing 20 birds...
each. Two of the groups were exposed to high ambient temperature (41.8±0.6°C), with one of them supplemented with ascorbic acid (AA) and the other receiving no AA (Ho). The other two groups were kept at room temperature (26±0.5°C) again with one of them supplemented with AA (RA) and the other receiving no AA (RO). The group maintained at room temperature with no AA (RO) served as reference /control group. Ascorbic acid (AA) was supplemented at the rate of 1000mg/kg feed.

Twelve birds in each group were challenged oculonasally with 0.05ml of pigeon strain of velogenic Newcastle disease virus and the remaining 8 birds were left unchallenged as in contact. Blood was collected from the wing vein of all birds for serological examination. Birds were observed for clinical signs and postmortem examination was performed on all dead birds.

The tissues were taken from lungs, intestine, proventriculus and brain and fixed in 10% formalin, cut 5mm thick in paraffin wax and stained with H and E (Drory and Wallington, 1967) and observed under light microscope at x 80.

Results

Clinical signs: Clinical signs were first observed in group H2, five days post-infection. The major clinical signs in all affected birds challenged or unchallenged in contact were dullness, ruffled feathers and respiratory signs in the form of gasping (plate I), dyspnea and nasal discharge. All the challenged birds in the four groups except R3 challenged exhibited greenish diarrhoea and nervous signs in the form of torticolis, paralysis of legs and wings (plate II). Group R3 challenged exhibited only respiratory signs.

Morbidity and mortality: The morbidity and mortality of the four groups are shown in Table 1. One hundred percent (100%) morbidity was observed in all the challenged groups irrespective of their environment (room or high ambient temperature) AA supplementation and in contacts with no supplemental AA irrespective of environmental temperature. Fifty percent (50%) morbidity was recorded in contact groups supplemented with AA irrespective of their environmental temperature (H3, and R3).

The vents of the challenged quail that died were soiled with greenish faeces. The trachea of birds in the H3 group were haemorrhagic, those of R3 were mucoid while those of H3 showed no lesion. Three of the dead birds in H3 group had slightly inflamed and haemorrhagic Peyer’s patches. The air sacs were cloudy, the lungs oedematous, the proventriculus parenchymatous and the intestines congested in all the dead birds (Plate III).

| Table 1: Morbidity and mortality of Japanese quail in the different treatment groups after experimental infection with a pigeon isolate of Newcastle disease virus |
|-----------------|-----|-----|-----|-----|
| Parameter (%)   | RO  | RA  | HO  | HA  |
| Morbidity       |     |     |     |     |
| Challenge       | 100 | 100 | 100 | 100 |
| Unchallenged    | 100 | 50  | 100 | 50  |
| Mortality       |     |     |     |     |
| Challenge       | 50  | 0   | 100 | 100 |
| Unchallenged    | 0   | 0   | 0   | 0   |

RO = room temperature with no ascorbic acid, RA = room temperature with ascorbic acid, HO = high temperature with no ascorbic acid, HA = high temperature with ascorbic acid.

| Table 2: Geometric mean titre (GMT) value of HI antibody in Japanese quail in the different treatment groups before and after experimental infection with a pigeon isolate of Newcastle disease virus |
|-----------------|-----|-----|-----|
| Treatments      | 0   | 7   | 14  | 21  |
| RO              |     |     |     |     |
| Challenged      | 5.6 | 4.6 | 8.9 | 80  |
| Unchallenged    | 5.6 | -ve | 10.0| 28.3|
| RA              |     |     |     |     |
| Challenged      | 5.6 | 2.2 | 28.3| 40  |
| Unchallenged    | 5.6 | -ve | -ve | 80  |
| HO              |     |     |     |     |
| Challenged      | 5.6 | 4.0 | 20.0| 160 |
| Unchallenged    | 5.6 | 2.0 | 10.0| 40  |
| HA              |     |     |     |     |
| Challenged      | 5.6 | 12.6| 10.0| NT  |
| Unchallenged    | 5.6 | 10.0| 10.0| 0   |

RO = room temperature with no ascorbic acid, RA = room temperature with ascorbic acid, HO = high temperature with no ascorbic acid, HA = high temperature with ascorbic acid NT. Not tested (birds in the group died before day 21 post challenge).

Histopathological lesions: Lesions were observed in the brain, lungs, proventriculus and intestine of the dead and dying quail in all the groups except group R3, which experienced no mortality.

Brain: Non supplicative encephalitis characterized by hyperaemia of blood vessels with lymphocytic infiltration into the Virchow-Robin’s space (Plate IV) was observed in birds of the three groups that recorded mortality.

Lungs: These exhibited congestion, haemorrhage, oedema and lymphocytic infiltration into the interstitium (Plate V).

Proventriculus: Lesions observed in this organ included necrosis of mucosal lining and infiltration of lymphocytes into lamina propria (Plate VI).

Intestine: Necrosis of mucosal epithelium with lymphocytic infiltration into the lamina propria and submucosa were observed in the intestine (Plate VII).

Geometric mean titre (GMT) value: Table 2 shows The GMT values of Japanese quails challenged oculonasally with the pigeon virulent isolate of ND virus and those in contacts. Quails in all the groups recorded positive sero-
Plate 1: Japanese quail infected with ND virus showing dullness, ruffle feathers and gasping for air.

Plate 2: Japanese quail infected with ND virus showing torticollis and paralysis of legs.

Plate 3: Proventriculus of Japanese quail infected with ND Virus showing focal areas (arrow) of haemorrhagic conversion just before challenge. Positive titre was detected at 7 day post infection (PI) in all groups except in controls at room temperature (R₁ and R₂). The highest titre (40) was recorded in the challenged birds of group H₂.

Plate 4: Nonsuppurative encephalitis in the brain of Japanese quail infected with ND virus showing lymphocytic perivascular cuffing (arrow) and hyperaemia of blood vessels. (H and E) x80.

Plate 5: Lungs of Japanese quail infected with ND virus showing pneumonitis with congestion (arrow) and oedema. (H and E) x 80.

At 14 days PI sero-conversion was also recorded in all the groups except in the unchallenged in contact of H₂ group, the challenged half of which recorded the highest (28.3) titre. By the 21st day after challenge all challenged birds in group H₂ died while all other groups, both challenged and unchallenged in contact, recorded their highest sero-conversion of the experiment.

Discussion
The incubation period of 5 days, clinical signs, gross and histopathological lesions observed in quail infected with the velogenic pigeon strain of ND virus and those in contact are similar to those reported in quail and other avian species elsewhere (Hills et al., 1953; Kurmanan et al., 1990; Cross, 1991; Emmerson, 1994; Lam, 1996; Saldu et al., 2006; Oladele et al., 2008).
Plate 6: Nonsuppurative proventriculitis in Japanese quail infected with ND virus. Note. Mucosal epithelial necrosis (arrow) with mononuclear cell infiltration in the lamina propria and submucosa (H & E) x80.

Plate 7: Intestine of Japanese quail infected with ND virus showing necrosis of villi (arrow) and leucocytic infiltration. (H & E) x80.

The results of this study clearly demonstrated the susceptibility of quail to infection with ND virus challenged or unchallenged and direct contact with infected birds. The higher (100%) mortality recorded in all the challenged groups suggest that the susceptibility of quail to the ND virus appeared to be not influenced by heat stress or AA supplementation. Ascorbic acid (AA) supplementation however, appeared to have beneficial effect on the morbidity of the disease in in-contact birds irrespective of heat stress. The lower mortality rate recorded in non-heat stressed compared with heat stressed quails indicates the detrimental effects of high environmental temperature on the outcome of ND virus infection. This observation supports the view of Thaxton, (1978) that the environment influences birds' ability to withstand infectious diseases.

The absence of any difference in mortality rates between AA supplemented and unsupplemented groups of the heat stressed quails suggests that AA supplementation has no beneficial effects in reducing mortality due to ND virus infection in the heat stressed birds.

The results of geometric mean titre (GMT) determination in this study suggest that heat stress did not interfere with humoral immune response of Japanese quails challenged with ND virus since all the challenged and unchallenged in contact birds developed similar antibody titres. The levels of humoral immune response however were not sufficient to confer protection against virulent challenge by virulent ND virus since 100% morbidity was recorded in all the challenged groups. The lack of significant disparity in immune response between heat-stressed and non-heat-stressed quail could be associated with the inherent ability of the Japanese quail to regulate ambient temperatures.

Similar studies in chickens have also shown that circulating antibody concentrations were not affected by high temperature when chickens were exposed to chronic heat treatment (Thaxton et al., 1968; Thaxton, 1973). Thaxton (1978) however, demonstrated that established antibody levels could be reduced within a relatively short period of time following an acute heat treatment. This effect was defined by Thaxton and Siegel (1973) as high environment temperature-mediated immunodepression (HTD).

The ability of quail to survive the infection with this virulent ND virus especially in in-contact groups despite similar humoral response with those that did suggest the possibility that there are other mechanisms for conferring immunity in infected birds.

It was concluded that ascorbic acid is not an important factor in the resistance of quail exposed to high ambient temperature to Newcastle disease virus infection. However, in quail that are not exposed to elevated temperature, the presence of AA in their diet enhances their resistance to infection with Newcastle disease virus.

References