The Effectiveness of *Scrophularia striata* on Newcastle Disease

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Abstract: In this study pigeons were infected with field isolate of Newcastle disease virus (NDV) made in to 0.1 ml dilution. The experimental groups consisted of 75 racing pigeon were divided into three groups. Group 1 remained as control; Group 2 was infected with the field isolated of NDV Newcastle disease virus. After experimental infection group 2 was treated with combination of Ox tetracycline and Doxocycline. In group 3. Group 3 after experimental infection was treated with ethanol plant extract of *Scrophularia striata*. Clinical signs and severe depression were observed by day 4th post infection in all experimental pigeons. By necropsy analysis, hemorrhagic lesions were indicated in heart, brain, with patches of necrotic epithelial cells and multifocal areas of lymphocytic infiltrates in the mucosal patches cranial trachea. Kidney, liver, pancreas, brain, gastrointestinal tract, spleens had extensive zones of necrosis. Lymphoid depletion with necrosis was observed in all lymphoid tissue in infected pigeons. The carcasses of the birds were thin and dehydrated. After therapeutic trial group 3 birds treated with plant extraction of *Scrophularia striata*. Out of pigeons, 9 survived. The activity of *Scrophularia striata* on bacteria inhibition was measured by zone inhibition range (39 - 8 mg/ml *Stphylococcus aureus*; 36 - 9 mg/ml *Enterococcus faecalis*; 16 - 34 mg/ml, control Gentamicine). These findings suggest that *Scrophularia striata* plant extract, had 36% effectiveness on Newcastle disease in racing pigeon. This available traditional herb extract can be considered as a novel therapeutic approach against NDV.

Key words: Paramyxovirus, *Scrophularia striata*, Pigeon, Velogenic Newcastle disease, Iran.

INTRODUCTION

Nature has served as a rich repository of medicinal plant for thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably of plant origin (Cowan, 1999). Iran is well-known for the exuberance and the variety of its mountain plants. Many of these plants are used as traditional natural medicine without any scientific research.

Newcastle disease (ND) is a highly contagious avian disease that causes high mortality in wild and domestic birds. Newcastle disease virus (NDV), also called avian *paramxovirus* type 1, is a member of the *paramyxoviridae* family of viruses (Alexander, 1997). These enveloped viruses are pleomorphic with single-stranded RNA. There are three main pathogenic strains of viruses Lentogenic, typically cause subclinical infection or mild respiratory disease while mesogenic usually results in moderate respiratory disease with occasional nervous signs. Velogenic strain of virus cause extensive hemorrhagic lesions, particularly in the gastrointestinal tract and /or a predominance of nervous signs (Alexander, 2003).

Velogenic NDV are considered exotic to some country poultry industry, exotic vNDV infection outbreak in 2002-2003 resulted in the destruction of 3.3 million birds and cost nearly $200 million dollars to eradicate in California (Pedersen *et al*., 2004; Wise *et al*., 2004). In recent outbreak in Ilam province, west Iran, some poultry farmers during this viral attack had used *Scruphularia striata* plant extraction as a traditional medicine and strongly claimed that could protect pigeons from Newcastle disease. The main aim of this study is to conform this traditional claim by using plant extract of *Scrophularia striata* against NDV.

MATERIALS AND METHODS

Newcastle diseases were diagnosed in a pigeons flock in Southern west of Iran, Ilam province since 2009.

**Virology:**

Selected organs (trachea, lung, liver, pancreas, brain, kidney, spleen, lymph nodes, bursa, thymus, proventriculus, small intestine, ceca) collected from dead birds of different location in area cage, houses and backyard keeping pigeon along with cloacal swabs from dead, sick, healthy birds were processed for attempted virus isolation according to the commercial and economic cooperation (CEC, 1992).

**Pigeons:**

Seventy five racing breed pigeons with three months old ages obtain from animal house school of veterinary medicine, Ilam University, Ilam, Iran were reared under clean isolated conditions until they were free

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of maternally derived antibodies (MDA) to NDV and specific pathogen free. The pigeons were fed and watered at liberty for 3 weeks. Pigeons divided into 3 groups (25/group). Group 1 control negative (G1), group 2 (G2) those pigeons infected with the NDV strain by inoculation intraocular and per-oral with 0.1 ml each 10^5 virus dilution. This group was treated with combination of Ox tetracycline and Doxomycin 50%, 2g m/l liter of water on the next day after infection. Group 3 (G3) pigeons were infected with same strain of NDV strain and treated with ethanol plant extraction of Scrophularia striata on the next day.

**Identification Of Haemagglutinating Agents:**
Allantoic fluids from embryos which died shortly after inoculation were tested for bacterial contamination by using the agar disc diffusion methods. Haemagglutination inhibition test, using a monospecific antiserum against NDV raised in specific pathogen free (SPF) chicken, as described in ED Directive 92/40, (CEC., 1992). The HA and HI assays were conducted by conventional micro titer methods (King, 1996) with serum separated from the blood sample taken at 9 and 15 days post infection. An exception to that sampling schedule involved pigeon inoculated with wtBC which were collected at 8 and 11 day post infection due to the early severe disease onset and subsequent inactivated NDV LaSota was used as test antigen in completing the HI test. Confirmation of NDV-positive fluids and antigenic characterization of virus isolates were conducted by HI using microtiter methods as previously described (King, 1996), four HA units of viral test antigen were used in completing the HI assay with MABs and polyclonal antiserum.

**Herbal Extraction From Scrophularia Striata:**
The plant Scrophularia striata used in this study were collected from Zagros mountain area southwest of Iran, and identified by Herbarium of Institute of Medicinal Plants, ACECR, and Tehran, Iran. The whole part of the plant dried, grinded to powder ,then 200 g powder separated, added with 250 ml of absolute ethanol (Govara chemical Ltd) for 70 h in sterile conical flasks properly covered and kept at +45°C in oven over night and the residue were obtain evaluated method of Abdulrahman et al (2004) . The residue was diluted with deionizer distilled water and daily 2 doses, at morning and evening, 1cc/dose each, were injected intramuscularly (according preliminary studied).

**Antimicrobial Susceptibility Test:**
The ability of the extracts to inhibit growth of clinical bacteria isolate was determined using the agar disc diffusion method (Alade and Irobi,1993). Sterile filter paper discs, 11mm in diameter were impregnated with extract concentration and dried at 35°C in the static incubator. They were then carefully placed aseptically with a forceps on the surface of the nutrient agar plates that were pre inoculated with the 24 h culture of bacteria. The control antibiotic disc containing Gentamycin (40 μg/ml) were placed on the inoculated plates of nutrient agar. The plates were left on the bench undisturbed for 5 minutes, after which the bacterial plates were incubated at 37°C for 24 h. The external diameters of visible zones of growth inhibition were measured after incubation. Microorganisms Staphylococcus aureus g+ and Enterococcus faecalis g- were used in this study obtained from the Microbiology Department School of Veterinary Medicine Ilam University, Ilam, Iran.

**Histopathology:**
Pieces of tissues fixed in 75% alcohol and then 5%formalin was sectioned at 6μm and stained with Haematoxylin and Eosin (H & E) following conventional procedures.

**Results:**

**Serology:**
Following the suspicion of Newcastle disease, samples collected from 30 cages were processed for attempted virus isolation and yielded a haemagglutinating agent on passage. All the samples were reported according to the records in Headquarters of Veterinary Organization of Ilam province. Positive and the haemagglutinating agent were identified as NDV, the frequency of ND in pigeon flocks in the area studies are given in Table 1.

**Table 1:** Frequency of Newcastle disease in pigeon flocks in the area studies.

<table>
<thead>
<tr>
<th>Area</th>
<th>cases</th>
<th>No of death bids</th>
<th>Total No of pigeon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayvan</td>
<td>12</td>
<td>357</td>
<td>321</td>
</tr>
<tr>
<td>Dehuran</td>
<td>15</td>
<td>431</td>
<td>465</td>
</tr>
<tr>
<td>Drahshar</td>
<td>14</td>
<td>534</td>
<td>570</td>
</tr>
<tr>
<td>Abidan</td>
<td>18</td>
<td>654</td>
<td>725</td>
</tr>
<tr>
<td>Ilam</td>
<td>39</td>
<td>985</td>
<td>1100</td>
</tr>
<tr>
<td>Mhran</td>
<td>8</td>
<td>256</td>
<td>312</td>
</tr>
<tr>
<td>Sheevan</td>
<td>11</td>
<td>245</td>
<td>98</td>
</tr>
</tbody>
</table>
**Clinical And Postmortem Investigation Findings:**

The clinical signs were initially characterized by birds were lethargy, feathers were ruffled, conjunctiva was red with edema, all of the pigeon had watery, greenish to white droppings, cyanosis mucus membranes and swelling of the head and neck were observed. In addition, neurological signs including tremors, spasms, paresis and/or paralysis of the wings and legs, torticollis and circling of the birds were observed (Fig 1).

![Fig 1: Torticollis (twisted neck) and circling of the birds.](image)

After treatment the birds in G3 with *Scrophularia striata* ethanol extract lying was decreased with misshapen eggs. One pigeon had permanent twisted neck. Remaining birds in this group were completely healthy and were flying normally and 9 birds (36%) were survived within this group. In G2 there were anorexia; depression, listlessness, and the most neurologic sign torticollis were recorded in one pigeon. The carcasses of all the infected birds were thin and dehydrated. No clinical signs were observed in the non-infected control birds.

**Gross Finding:**

The sampled tissues of all the infected birds were examined microscopically spleen and caecal tonsils tissues of infected pigeons were large, mottled necrotic and there was with extensive necrosis of the follicular lymphoid cell in the bursa of fabricius. In some cases the bursa became very small, thymus in infected pigeon were small, edematous or with petechial hemorrhages. Multifocal hemorrhages were observed in the mucosa of the proventriculus and Small intestine and ceaca. Patches of necrotic epithelial cells and multifocal areas of lymphocytic infiltrates in the mucosa lung of infected pigeon were observed (Fig 2 and Fig 3).

![Fig. 2: Multifocal hemorrhages in the mucosa of the infected Proventriculus of the pigeon. Bar = 30 µm.](image)

![Fig. 3: Patches of necrotic epithelial cells and multifocal areas of lymphocytic infiltrates in the mucosa lung of infected pigeon. Bar =30 µm.](image)
Histological hemorrhagic lesions were seen in infected tissues in the cranial trachea Fig 4.

![Fig. 4: Necrohemorrhagic trachea mucosa of the infected pigeon. Bar = 30µm.](image)

There were patches of necrotic epithelial cells and multifocal areas of lymphocytic infiltrates in the mucosa. At 4 days post infection, the kidney tissue (Fig 5).

![Fig. 5: Gross lesions (urate deposits) and extensive zones of necrosis of kidney in infected pigeon. N = 30µm.](image)

Liver, pancreas, brain, gastro intestinal tract, spleens were examined and had extensive zones of necrosis. The most remarkable histological findings was observed in the brain, lesions with severe, multifocal infiltrations of mononuclear cells, mostly lymphocytes and plasma cells, were observed surrounding the small capillaries of the molecular layer of the cerebellum (Fig 6).

![Fig. 6: Brain tissue of infected pigeon shows intracytoplasmic eosinophilic inclusion bodies. Bar=30µm.](image)

The gastrointestinal tract was empty in all infected birds. Lymphoid depletion with necrosis was observed in all lymphoid tissue in infected pigeons. Oedema and petechial hemorrhages were seen in the eyelids. Control pigeon did not had any gross lesions.

**Mortality Rate Between The Groups:**

There was not any mortality rate in control G1. Out of 25 pigeons in the G2 only 1 bird could survive up to the end of experimental studies. In the group 3rd pigeons infected with field collected NDV and then being treated with *Scrophularia striata* herbal extract, 9 of 25 birds (36%) could survive.
**Bacteriological Investigation:**
Routine bacteriological investigations of selected organs consistently gave negative results in all the birds examined.

**Antimicrobial Activity:**
The antimicrobial activity of the ethanolic extracts of the plant *Scrophularia striata* was observed by zone of inhibition ranging from 39 to 8 mg/ml for *Staphylococcus aureus*, 36 to 9 mg/ml for *Enterococcus faecalis* and for control Gentamicine were 16 to 34 mg/ml (Fig 7).

![Fig. 7: Sensitivity of S. Aureus and E. Faecalis to Different Concentration of Scorophularia Striata.](image)

**Discussion:**
From the gross histopathological lesion, mortality and clinical sign data were collected before and during the study period. It is apparent that pigeon are highly susceptible to ND. The most remarkable and consistent histopathological changes were observed in the brain, similar pathological report in pigeon published by (Kommers et al., 2002). Report of Wilczynski et al., (1977) indicated central nervous system disease developed several days after maximal virus replication had occurred in the brain of infected birds and NDV followed by death, which is consistent with our results. A previous study documented that juvenile and adult pigeon experimentally infected intraconjectivally with viscerotropic velogenic NDV (Fontana 1083, 10<sup>4</sup> ELD50 per bird) developed clinical disease characterized by head tremors, wry neck, opisthotonous, wing droop, and leg paralysis, with higher mortality and morbidity in juveniles relative to adults (Erickson et al., 1980; Wakamatsu et al., 2006). Severe ND clinical signs reported by Kapczynski et al., (2006) is in agreement with our results, in contrast to our results about morbidity and clinical signs, publication of Carrasco et al (2008), is important to compare the variation among NDV strains and virulence ability, which is responsible for outbreak in commercial poultry farms and pigeon in different countries.

In our study, in control G1 no mortality was observed, whereas 24 birds from G2 and 16 birds from G3, were reported dead. In relation to this findings, there are many reported data indication variable mortality rate in NDV infected chicken and turkeys (Wakamatsu et al., 2006; Brown et al., 1999; Hamid et al., 1991; McDaniel and Orsborn., 1973; Panigarhy et al., 1993). In the studies of Alexander, (1997) and Wakamatsu et al., (2006) turkeys and chickens are appeared more resistant to viscerotropic velogenic NDV (GB 97/6 and GB/1) and pigeon were the least susceptible to the California END virus.

Sever clinical disease and mortality were observed in the field before and after inoculated pigeons with this virus, in this study gross and histopathological changes characteristic of highly virulent NDV, shows typical of the velogenic viscerotropic NDV pathotype, same results has been reported previously (Brown et al., 1999; Hamid et al., 1991; McDaniel and Orsborn., 1973; Panigarhy et al., 1993). Extraction of *Scrophularia striata* plant can go for further extensive investigation against ND in pigeons. Out of 25 birds, 8 of them completely recovered from NDV, which is interesting reason to continue this research. Although herbal extraction of *Scrophularia striata* are traditionally used against various infectious diseases in Ilam, and region, farmers of Zagros mountain in southwestern Iran still believe and use this plant for medication against ND. Therefore, more studies are required to complete phytochemical analysis to point out effective chemical material for *in vitro* and *in vivo* trials.

**ACKNOWLEDGEMENTS**
Authors are grateful all our laboratory members for their excellent technical assistance and help. We also thank Ali Razaa Dosti and Reza Houshmandfar for tremendous help in sample collection.
REFERENCES


