A Serological Study of Newcastle Disease in Pre- and Post-Vaccinated Village Chickens in North of Iran

M. Hassanzadeh* and M.H. Bozorgmeri Fard
Department of Poultry Diseases, Faculty of Veterinary Medicine,
University of Tehran, P.O. Box 14155-6453, Tehran, Iran
E-mail: Mhzadeh@ut.ac.ir

Abstract: Newcastle disease is one of the major problems for village chicken production, which is an important item in the economy of villages in Iran. In order to investigate the Newcastle disease status in village flocks of Iran, a serological study was performed on the prevalence of Newcastle disease in pre- and post-vaccinated village chickens by means a haemagglutination inhibition (HI) test. In the first experiment, a serological survey was carried out to detect antibodies against Newcastle disease virus in unvaccinated chickens of four villages. In a second study, conventional vaccines, routinely considered for the control of Newcastle disease, were evaluated. Immune responses in unvaccinated chickens indicated a previous exposure of the birds to a natural infection of Newcastle disease virus. No significantly difference was found between the antibody titres of HB, vaccinated birds and the unvaccinated control birds. However, birds that received an inactivated vaccine, had significantly higher antibody titres compared with the live vaccinated birds. Furthermore, the highest antibody titres were detected in the group of birds that was only given the inactivated vaccine or booster vaccine by veterinary technicians (TI, THI). Our results provide strong evidence for the presence of Newcastle disease virus in village poultry populations of Iran. Due to the high infection potential, vaccination campaigns by inactivated conventional vaccines will have more benefit if they can be applied by the personal who has suitable technical experience.

Key words: Newcastle disease, village chickens, vaccine, HI

Introduction
Newcastle disease (ND) is worldwide regarded as one of the most important diseases of poultry and other birds, because of the devastating consequences of ND virus infections on infected birds, with flock mortality rating up to 100%, as well as the economic impact of trading restrictions and embargoes placed on areas and countries where outbreaks have occurred (Alders and Spradbrow, 2001). ND is enzootic in some areas of the world, however, especially where rural chicken breeding is dominant, ND has become endemic. Control is possible, but requires an efficient application of vaccines and rigorous biosecurity (Spradbrow, 1990, 1993/4).

The strains of ND virus infecting industrialized poultry in Iran are velogenic and viscerotrophic (Bozorgmeri, 1998) and existing vaccination programmes, if applied under controlled conditions, protect chickens from death and severe symptoms. However, in spite of vaccination, cases of the disease continue to be observed on industrialized poultry farms. One factor that could contribute to this is the presence of small traditional flocks which are either not vaccinated or which chickens have a weak immune response due to failed vaccination against the ND virus (Veterinary Organization of Iran, personal communication).

As in many tropical and subtropical countries in Asia, Africa and South America, a large population of small traditional chicken flocks exists alongside of large industrialized poultry farms. The flocks are small and multi-aged. During the day, the birds roam around the village in search of feed but return to home for laying for the night. At night, the chickens will congregate in smaller household groups, either in houses, under houses or in trees. All chickens are in direct or indirect contact each other.

As in many countries (Spradbrow, 2004), Newcastle disease is the most important constraint to productivity of village chicken flocks in Iran and the use of conventional vaccines has been considered for the control of ND in these chickens. Such conventional vaccinations are limited to areas where village chickens have reasonable housing. Even then, there remains a risk of low level protection in vaccinated birds due to an unsuitable vaccination technique. Hence, it is supposed that conventional vaccination is not suitable for control of ND in village chickens in many areas (Veterinary Organization of Iran, personal communication). In view of this situation, the present experiments were initiated to investigate the ND in village flocks of the north Iran, Gillan province. In the first experiment a serological survey was undertaken to detect antibodies against ND in unvaccinated village chickens by means of a haemaggululation inhibition (HI) test. The presence of antibodies could indicate a previous natural infection with ND virus. Second experiment was carried out to
evaluate the conventional vaccines, routinely used for the control of ND in village chickens of Iran. In this study, different regimes of vaccination were employed and antibody titres in the serum against ND were compared.

**Materials and Methods**

**Experiment 1**: This study was carried out in the north of Iran, in the west of Gillan province, a region in which no ND vaccination of village birds had previously been performed. Four villages, that each village had 75-100 households with 25-75 scavenging and backyard birds per household, were randomly selected. A total of six hundred eggs of these chickens collected. The Eggs were transported to a laboratory incubator, at the faculty of veterinary medicine, university of Tehran, and were incubated in a standard incubator until chicks hatched. Half of the newly-hatched chicks were kept under normal conditions in a clean laboratory room (HL) in the Tehran university, while the second half chicks were transported and distributed between households of four villages (HV), in the same area and under the same conditions as scavenging and backyard birds. Additionally, in each village approximately 100 newly-hatched chicks which hatched naturally in the villages (NV), were randomly selected. All birds were marked but remained unvaccinated. Blood samples were taken from the wing veins of 15 chickens of each group on days 30, 60, 90 and 120 of age. Blood samples were allowed to clot, sera was separated, transported to the laboratory in a refrigerated box and subsequently stored at -20°C until titre determination for antibodies against ND virus by a HI test using the method of Allan and Gough (1974).

**Experiment 2**: Six villages were chosen within a radius of 20 km of a mountain area of Gillan province, north of Iran. The specific region was chosen because all population (human and poultry) is located in clearly separated villages, thus providing discrete groups for experimentation, and furthermore, no vaccination of poultry had previously been carried out in any of these villages. In each village, all chickens, turkeys, guinea-fowl, in all ages, were vaccinated according to the programmes given in Table 1. Thirty-day-old (approximately) chickens were marked for the collection of blood samples. The live vaccine, Hitchner B, (HB) (Razi Institute of Iran), containing one dose according to the manufactures recommendations, was given as a single eye-drop, by government veterinary technicians in village 1 (TH) or by villagers in village 2 (VH). Inactivated vaccine (Intervet) was given, by intra-muscular injection of 0.5 ml for adult chickens and 0.1 ml for small chicks. The inactivated vaccine was applied by government veterinary technicians in village 3 (TI) or by villagers in village 4 (VI). In the 5th village, birds received two vaccines, HB, at day 1 of experiment and inactivated vaccine 25 days later, by veterinary technicians (THI). In the 6th village all birds received an eye-drop of distilled water as a control (TC) group.

Blood samples were taken from the wing veins of 40 randomly selected marked-chickens, per group (village), at day one before vaccination and at 30, 60, 90 and 120 days postvaccination. Sera was separated and titrated for antibodies against ND virus. Statistical analysis was performed using the “General linear model procedure” (SAS, 1986). If a significant overall effect (P<0.05) was found, treatment means were compared by using the Scheffe test.

**Results**

**Experiment 1**: Antibody titres against ND virus were decreased with age in chickens that were kept in the laboratory room at the university (Fig. 1), while the antibody titres of HV and NV birds, that were naturally reared in the households of villages, increased significantly (P<0.0001) during the experimental period. Furthermore, the mean antibody titres were significantly
Hassanzadeh and Bozorgmeri: Newcastle disease in rural birds of Iran

Table 2: Mean haemagglutination inhibition (HI) antibody titres against Newcastle disease virus in different groups of vaccinated rural chickens that were reared traditionally in the villages

<table>
<thead>
<tr>
<th>Groups / Post vaccination</th>
<th>TH</th>
<th>VH</th>
<th>TI</th>
<th>VI</th>
<th>THI</th>
<th>TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 days</td>
<td>1.9 ± 0.6</td>
<td>2.6 ± 0.6</td>
<td>2.1 ± 0.3</td>
<td>1.9 ± 0.6</td>
<td>2.8 ± 0.6</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>30 days</td>
<td>4.3 ± 0.6</td>
<td>3.8 ± 0.6</td>
<td>9.2 ± 0.4</td>
<td>6.5 ± 0.8</td>
<td>8.5 ± 0.8</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>60 days</td>
<td>4.8 ± 0.8</td>
<td>5.4 ± 0.4</td>
<td>8.9 ± 0.8</td>
<td>6.6 ± 0.7</td>
<td>9.9 ± 0.1</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>90 days</td>
<td>4.7 ± 0.6</td>
<td>4.3 ± 0.3</td>
<td>8.6 ± 0.3</td>
<td>4.9 ± 0.5</td>
<td>9.5 ± 0.2</td>
<td>4.3 ± 0.5</td>
</tr>
<tr>
<td>120 days</td>
<td>5.9 ± 0.8</td>
<td>6.4 ± 0.4</td>
<td>7.9 ± 0.3</td>
<td>4.2 ± 0.5</td>
<td>9.3 ± 0.4</td>
<td>4.4 ± 0.5</td>
</tr>
</tbody>
</table>

TH: A single dose of HB vaccine was applied by veterinary technicians.
VH: A single dose of HB vaccine was applied by villagers.
TI: Inactivated vaccine was applied by veterinary technicians.
VI: Inactivated vaccine was applied by villagers.
THI: A single dose of HB, vaccine plus inactivated vaccines were applied by veterinary technicians.
TC: An eye-drop of distilled water was applied by veterinary technicians.

* * * Within rows, means with different superscripts are significantly different (P<0.05).

higher in chickens of HV and NV groups compared with the birds of HL group at the 60, 90 and 120 days of the experiment. No significant difference was seen between the antibody titres of HV and NV birds.

Experiment 2: Results of antibody titres against ND virus in differently vaccinated chickens are presented in Table 2. No significant difference was found between the antibody titres of the different groups before vaccination (0 days post vaccination) in contrast, the antibody titres increased from 30 days post vaccination onwards in all experimental groups. The antibody titre was not significantly different among the control (TC) birds and TH and VH vaccinated birds, which received the HB vaccine either by government veterinary technicians or by villagers. However, the titres of these three groups were significantly lower compared with the TI, VI and THI vaccinated birds.

The HI titres were significantly higher in TI vaccinated birds at 30, 60, 90 and 120 days post vaccination compared with the VI vaccinated birds. The antibody titres did not differ between chickens that were vaccinated with a single dose of inactivated vaccine (TI) or received booster vaccine (THI) by veterinary technicians. In contrast the difference was significant between the THI and VI vaccinated birds. Furthermore, the antibody titres of VI vaccinated chickens significantly decreased from 90 days onwards compared with the TI and THI vaccinated birds.

Discussion

In experiment 1, the distribution of ND virus antibodies in the unvaccinated birds showed an obvious difference between the birds, which were reared in a clean laboratory room and the birds that were naturally reared in households of the villages. The antibody titres (maternal antibody) decreased rapidly in unvaccinated HL birds with age which indicates that the HL birds were not infected with ND virus during experiment. In contrast, the wider range of antibody titres against ND virus observed in unvaccinated birds from the HV and NV groups, that were reared naturally in households of the villages, indicate a natural infection and a higher potential for infection of the these regions to ND virus as observed in the studies of Bell et al. (1990) and Vui et al. (2002).

Birds vaccinated only once with a single dose of eye-drop vaccine either by government veterinary technicians (TH) or by villagers (VH) had moderate responses to the HB1 vaccine compared with the birds vaccinated intramuscularly by the inactivated vaccine as shown in Table 2. A similar increase in antibody titres could be observed in the TH, VH and TC groups. This increase was also similar to that of HV and NV unvaccinated birds in the experiment 1. The similar immune responses among the unvaccinated (TC, HV and NV) and vaccinated birds indicate an interference between the virus vaccine strain and a naturally challenge by the field strain virus in those regions.

The significantly lower immune responses in the vaccination programme applying a single dose of inactivated vaccine by villagers (VI) compared with the veterinary technician vaccinated birds, (TI and THI groups), could be due to a lack of technical experience of the villagers. Since the level of antibody titres between the birds of TI and THI groups, vaccinated by veterinary technicians, was almost similar, the efficiency of HB, vaccination in THI birds should be interpreted with caution. Moreover, given the increase in antibody titres in the control group pointing a natural infection, so it can not be excluded that a natural infection took place in the other groups. Such increasing antibody titres might be correlated with the natural challenge of birds to the wild-type virus as observed in the unvaccinated chickens.

The presence of a pathogenic strain of ND virus, which is endemic in many tropical and subtropical countries, is one factor which is necessary for the disease to develop, but in itself is not sufficient (Spradbrow, 1990). Our results provide strong evidence for the presence of ND virus in village poultry populations of Iran. Up till now, the use of conventional vaccines has been considered for the control of ND in village chickens. Conventional
Hassanzadeh and Bozorgmeri: Newcastle disease in rural birds of Iran

Acknowledgments

This research was funded by the Research Committee of Tehran University, Faculty of Veterinary Medicine. F. Vandemaele, Dr. E. Hossieni is appreciated for their help and Dr. F. A. Mostagim from the Veterinary Organization of Iran, Gilan province, for providing facilities.

References


Spradbrow, P.B., 2004. Appropriate vaccination and therapies for rural poultry flocks in developing countries and their relevance to developed countries. 22rd World’s Poultry Congress, June 8-13, Istanbul, Turkey.