ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 3 (10): 658-661, 2004

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_1 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 devastative 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 worlds, 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 viscerotropic (Bozorgmeri, 1998) and existing vaccination programmes, if applied under controlled conditions, protect chickens from death and sever 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 haemagglutination inhibition (HI) test. The presence of antibodies could indicate a previous natural infection with ND virus. Second experiment was carried out to

Table 1: Vaccination programmes in rural chickens of six different villages

Groups	Vaccination programme		Vaccinators
	Primo vaccine*	Booster vaccine**	
TH	HB_1***	-	Government veterinary technicians
VH	HB_1	-	Villagers
TI	Inactivated vaccine	-	Government veterinary technicians
VI	Inactivated vaccine	-	Villagers
THI	HB_1	Inactivated vaccine	Government veterinary technicians
TC	Distilled water	-	Government veterinary technicians

*Applied at day 1 of the experiment, **Applied at 25 days post primo vaccination, *** Hitchner B_1

TH: A single dose of HB_1 vaccine was applied by veterinary technicians.

VH: A single dose of HB_1 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_1 vaccine plus inactivated vaccines were applied by veterinary technicians.

TC: An eye-drop of distilled water was applied by veterinary technicians.

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_1 (HB_1) (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_1 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

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

Groups / Post vaccination	TH	VH	TI	VI	THI	TC
0 days	1.9 ± 0.6	2.6 ± 0.5	2.1 ± 0.3	1.9 ± 0.6	2.8 ± 0.6	2.1 ± 0.4
30 days	4.8 ± 0.6^{c}	3.8 ± 0.6^{c}	9.2 ± 0.4^{a}	6.5 ± 0.8^{b}	8.5 ± 0.8^{a}	3.2 ± 0.3^{c}
60 days	4.8 ± 0.8^{c}	5.4 ± 0.4^{bc}	8.9 ± 0.6^{a}	6.6 ± 0.7^{b}	9.9 ± 0.1^{a}	3.8 ± 0.5^{c}
90 days	4.7 ± 0.6^{b}	4.3 ± 0.3^{b}	8.8 ± 0.3^{a}	4.9 ± 0.5^{b}	9.5 ± 0.2^{a}	4.3 ± 0.5^{b}
120 days	5.9 ± 0.8^{b}	6.4 ± 0.4^{b}	7.9 ± 0.3^{a}	4.2 ± 0.5^{c}	9.3 ± 0.4^{a}	4.4 ± 0.5^{c}

TH: A single dose of HB_1 vaccine was applied by veterinary technicians.
VH: A single dose of HB_1 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_1 vaccine plus inactivated vaccines were applied by veterinary technicians.
TC: An eye-drop of distilled water was applied by veterinary technicians.
$^{a, b, c}$ 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_1 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_1 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

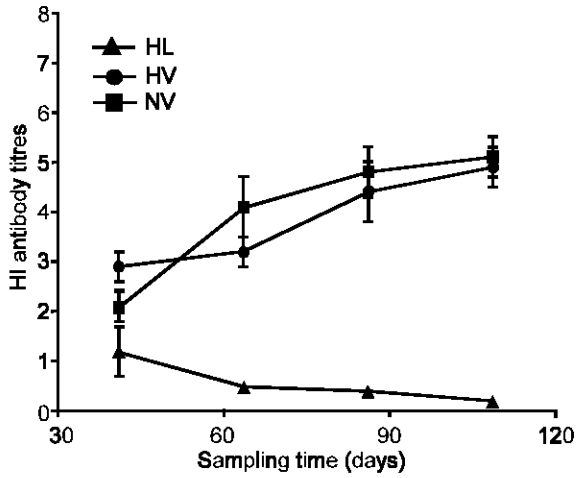

Fig. 1: Haemagglutination inhibition (HI) antibody titres against Newcastle disease in 3 groups of unvaccinated rural chickens.

HL = Chickens that were hatched in hatchery and reared in the clean room at the university

HV = Chickens that were hatched in hatchery of university but reared naturally in households of villages

NV = Chickens that were hatched and reared naturally in the villages

methods are majority based on live vaccines that are mostly applied by the villagers. It would be a significant advantage if villagers could perform vaccination themselves but this is too cumbersome to sustain and mostly the immunity of such vaccination would be negligible as was argued by Spradbrow, (1993/4). The government veterinary service is however unable to provide such great number of personal and cost-effective services for routine vaccination campaigns in small and dispersed backyard flocks, as is not economically feasible for the villagers as well. Live vaccines have traditionally been heat-sensitive and require -expensive- cold chains, which are important limiting factors for the control of Newcastle disease and consequently the productivity of village poultry. Since a large proportion of the poultry is kept under village conditions, these flocks could act as a reservoir of virus for industrial poultry, which might want to attempt a controlled trail experiment under village conditions. To overcome these problems, a low cost vaccine delivery system, which could be operated by village people, such as thermostable vaccines is highly needed as reported (Copland, 1987; Spradbrow, 1993/4; Spradbrow, 2004).

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, Gillan province, for providing facilities.

References

Alders, R.G. and P.B. Spradbrow, 2001. Controlling of Newcastle disease in village chickens. A field manual. ACIAR %Monograph No. 82, 112pp. Canberra: Australian Centre for International Agricultural Research (ACIAR).

Allan, W.H. and R.E. Gough, 1974. A standard haemagglutination inhibition test for Newcastle disease. (1) A comparison of macro and micro methods. Vet. Rec., 95: 120-123.

Bell, J.G., D. Ait Belarbi and A. Amara, 1990. A controlled Vaccination trial for Newcastle disease under village conditions. Prev. Vet. Med., 9: 295-300.

Bozorgmeri Fard, M.H., 1986. Newcastle Diseases. In: Poultry Diseases. pp: 170-185.University Tehran Press.

Copland, J., 1987. Newcastle disease in poultry. In Newcastle Disease in poultry. A New Food Pellet Vaccine, ed. J. W. Copland. Monograph No. 5, Canberra: Australian Centre for International Agricultural Research (ACIAR).

SAS Institute Inc., 1986. Language Guide for Personal Computers. Version 6. Cary NC: SAS Institute Inc.

Spradbrow, P.B., 1990. Village poultry and preventive veterinary medicine. Prev. Vet. Med., 8: 305-307.

Spradbrow, P.B., 1993/4. Newcastle Disease in Village Chickens. Poult. Sci. Rev., 5: 57-96.

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

Vui, T.Q., J.E. Lohr, M.N. Kyule, K.H. Zessin and P.O. Baumann, 2002. Antibody levels against Newcastle disease virus, infectious bursal disease virus and avian influenza virus in rural chickens in Viet Nam. Int. J. Poult. Sci., 1: 127-132.