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Serological Survey of Newcastle Disease Virus in Chukar Partridges by Vaccination with Inactivated Oil Adjuvant Vaccine Following Use of Live Vaccines

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Abstract: Newcastle Disease (ND) is an acute, highly contagious viral disease in poultry. Despite the intensive vaccination programs to control Newcastle Disease Virus (NDV), the recent infection of NDV in Iran has led to severe morbidity and mortality in Chukar Partridges. The aim of this study was to investigate the ability of Inactivated Oil Adjuvant Vaccine (IOAV) following use of live vaccine to have better protection in Chukar Partridges against ND. Three hundred Chukar Partridges were divided in to 5 groups. The groups were vaccinated according to following program: I-vaccinated with B1 strain of NDV via drinking water at five weeks of age; group II-vaccinated in the same mode as group I with B1 live vaccine and revaccinated at seven weeks of age with la Sota strain of NDV via drinking water; group III-vaccinated at five weeks of age with strain B1 live vaccine given by drinking water in combination with IOAV vaccine administered subcutaneously at the nape of each bird; group IV-vaccinated at five weeks of age with B1 by eye drop in combination with IOAV vaccine also administered as in group III; and group V-not vaccinated. All groups were reared in the same conditions. At 17 weeks of age, all Chukar Partridges were challenged with Hertz 33/56 strain of NDV. All the Chukar Partridges in group V died, indicating that there was no disease resistance of this unvaccinated control group of Chukar Partridges. The disease resistance of chukar Partridges in the vaccine groups (100.00) was significantly different from that of the control group (0.00) ($p<0.001$). The mean antibody titers between all groups were significantly different ($p<0.001$). In the groups that vaccinated simultaneously with B1 live vaccine and IOAV, the enhancement of titers was meaningful in comparison to the groups that received only lived vaccines.

Keywords: Chukar partridges, inactivated ND vaccine, live ND vaccine, newcastle disease, serology

INTRODUCTION

Newcastle Disease (ND) is one of the most serious infectious diseases of poultry (Alexander, 2003). The epizootic nature of the disease has caused severe economic losses in the poultry industry worldwide since the 1920s. NDV designated as Avian Paramyxovirus serotype 1 (APMV-1) and belongs to the Avulavirus genus of the Paramyxoviridae family (Mayo, 2002). The virus has a single stranded RNA like other members of the Paramyxoviridae family. NDV possesses two surface proteins: the Haemagglutinin-Neuraminidase (HN) protein and Fusion protein (F), which are important in the identification and biological characteristics of the virus (Alexander, 1997). NDV Strains have been grouped into five pathotypes (viscerotropic velogenic, neurotropic velogenic, mesogenic, letogenic and asymptomatic enteric) on the basis of the clinical sings seen in infected chickens (Beard and Hanson, 1984). The first cases reported of the disease in poultry from Java, Indonesia, since the 1920s. In Iran for the first time after the 2nd World War in Khuzestan, ND has been reported. It was arrived to the vicinity of Tehran very soon. NDV has been confirmed by huge casualties of foreign races of poultry industries in Iran, which took place since the 1951 (Hadipour *et al*., 2011). The strains of NDV infecting industrialized poultry in Iran are velogenic and viscerotropic velogenic (Hassanzadeh and Bozorgmeri, 2004). The disease normally affects the respiratory, gastrointestinal and nervous systems. In outbreaks in poultry due to the NDV, clinical sings often begin with listlessness, increased respiration and weakness, ending with prostration and death. Morbidity and mortality rates of infected poultry vary from 1-100% (Alexander, 1997). Therefore, vaccination and biosafety measures are needed to control this disease. Today there are commercial live and inactivated oil adjuvant vaccines which are very effective as immunization antigens (Bennejean *et al*.,

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1978). The live ND vaccines are produced from lentogenic and mesogenic virus strains (Lancaster, 1981). Various NDV strains used in the production inactivated vaccines. Observation and reports declared that undesirable vaccination reaction following administration of live ND vaccines are incapable to produce sufficient immunity in poultry. On the other hand inactivated ND vaccines dose not provoke undesirable long immunization. Therefore to promote proper immunological protection, vaccination programs should be carried out first by live vaccine and then inactivated vaccine. Recent reports indicated that NDV has been produced hyperacute fatal disease in Chukar Partridges.

The Chukar Partridge or Chukar (Alectoris chukar) is a Eurasian upland gamebird with 32-35 cm long in the pheasant family Phasianidae. According to the reports, at present time this is the major problem of Chukar Partridges industry in Iran. There was no information reported for correct vaccines and also vaccination program that cause protective immunity in Iranian Chukar Partridges. In this study, we investigated the possible induction of protective immunity in Chukar Partridges by vaccination with IOAV following use of live vaccine and then challenge trial because of importance efflorescence Chukar breeding industries.

MATERIALS AND METHODS

This research was carried out during Jan. 2011 to Sep. 2011 in the Laboratories of the Department of Poultry Diseases Research and Diagnosis of Razi Vaccine and Serum Research Institute (RVSRI), Shiraz Branch, Iran.

Chukar partridges: Three hundred one-day-old unvaccinated Chukar Partridges were maintained in the temperature controlled rooms under the same conditions in Razi Vaccine and Serum Research Institute (RVSRI), Shiraz, Iran. They were reared until the maternal antibodies against NDV were decreased to negligible amount, and became undetectable by standard HI test. Before vaccination they were divided into five similar groups and also breeding continued in the same conditions.

ND vaccines: The La Sota and B1 live vaccine (Lentogenic strains of NDV) were reconstituted from freeze-dried vials and also NDV-V4 strain inactivated vaccine (Asymptomatic strain of NDV), as oil-adjuvant inactivated vaccines were used in the experiment. Live and IOAV vaccines were supplied by Razi Vaccine and Serum Research Institute-IRAN (RVSRI), according to the manufacturers recommendations, each vaccine is in 1000 doses vials, one dose being at least $10^{5.7}$ and $10^{6.5}$ 50% Embryo Infective Dose (EID_{50})/bird, respectively.

Vaccination program: The Chukar Partridges were divided in separate groups named as I, II, III, IV and V (60 Chukar Partridges per each group). All were completely healthy in experimental examinations before vaccination. Group I, the Chukar Partridges were vaccinated at 5 weeks of age with strain B1 live vaccine given by drinking water. Group II, the Chukar Partridges received B1 live vaccine in the same way with group I then boostered at 7 weeks of age with La Sota strain of NDV vaccine again via drinking water. Group III, vaccinated at 5 weeks with strain B1 live vaccine given by drinking water in combination with IOAV vaccine administered subcutaneous. Group IV, the Chukar Partridges were vaccinated at 5 weeks of age with strain B1 live vaccine given by eye drop route in combination with IOAV as in group III. Chukar Partridges in group V were kept as unvaccinated controls.

Samples collection: Blood samples of five groups were collected from the wing veins, from 1-17 weeks of age, at regular 3-4 weeks intervals. All blood samples were taken under sterile conditions and remained to be clotted. Then the samples centrifuged at 3000 rpm for 5 min. Separated sera were stored at -20ºC until to use in Haemagglutination Inhibition (HI) test. Isolation test was carried out to determine that which group may shed the challenge virus after inoculation. Therefore oropharyngeal and chloacal swab samples were gathered before and after challenge test and kept in -70ºC for isolation test.

Haemagglutination test (HA): HA test was performed as described in the Office International des Epizooties (OIE, 2009) manual, using reference antigen for B1 strain of NDV (Razi Institute, Shiraz, Iran). A V-bottomed 96 wells microtitre plate was used for virus titration. The antigen was added to well NO.1 and as two fold dilution in each well up to well NO.10. It was prepared in phosphate buffer saline (PBS, PH 7.4). Two other wells 11 and 12 were used for Chukar partridge RBC and antigen control.

Haemagglutination Inhibition test (HI): Serum titers to NDV were detected using the HI test as described in the OIE (2009). Two-fold dilutions of the Chukar Partridges serum in PBS were mixed with the same volume of B1 antigen (4 HA units). The mixture was incubated at room temperature for 30 min. After this 0.025 mL of 1% Chukar Partridge RBC was added to each of the mixtures in the wells and allowed to settle for 40 min at room temperature. The serum titer then read, as the highest dilution of serum inhibiting haemagglutination by the virus.

Challenge study: At 17 weeks of age, Chukar Partridges in each vaccine group were challenged with Hertz-33/56 ($10^{6.0}$ EID50/100 μL) via the oral drop and intranasal routes. Unvaccinated Chukar Partridges were also challenged with Hertz-33/56 as described above. The challenged groups were observed daily for clinical signs (morbidity), and mortality Oropharyngeal and cloacal swabs were collected from Chukar Partridges for a period of 14 days after challenge.

Virus isolation: Swab samples were placed in transport medium, Brain-Heart Infusion broth (BHI), containing antibiotics, penicillin (10000 units/mL) and gentamycin (250 μg/mL) and stored at -70ºC until use. For viral isolation, all of the swabs centrifuged at 1000 g for 10 min. Following centrifugation, 0.2 mL of supernatant inoculated into the allantoic cavity of 9-11 days embryonated eggs. The inoculated eggs were incubated at 36-37ºC for 2-3 days. The HA assay was performed on the amnio-allantoic fluids using 10% chicken RBCs. NDV virus was confirmed by the use of specific antiserum (RVSRI-Iran) in HI test.

Statistical analysis: Variation in the antibody titers was analyzed by using student t-test. Variation between or within groups was considered to be significant at $p<0.001$.

RESULTS

The effect of NDV vaccine on serum antibody response: The results of antibody titer changes of Chukar Partridges in the vaccinated groups are presented in Table 1. As the control group (V) was not vaccinated, its HI antibody titers were null during all the experimental period. No significant differences were found between the antibody titers of the different groups before vaccination. At 8 weeks of age, antibody titers against NDV were detected in vaccinated groups. The student t-test test demonstrated significant differences among groups vaccinated with inactivated vaccine following use of different route of vaccination by live vaccines. The mean antibody titers of the Chukar Partridges in the vaccinated groups III and IV was significantly higher than those of groups I and II ($p<0.001$) which were vaccinated just with live B1 & La Sota vaccines, especially at 11 weeks of age (Fig. 1).

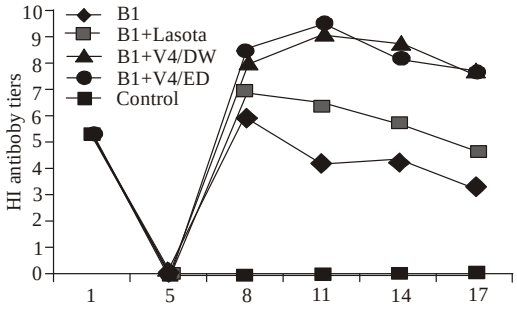

Fig. 1: Haemagglutination Inhibition (HI) antibody titers against newcastle disease in the different 5 groups of chukar partridges

Protection of vaccinated chukar partridges against challenge: The results of the challenge with velogenic NDV in Chukar Partridges and the virus isolation are presented in Table 2. None of the Chukar Partridges in the vaccinated groups showed clinical signs after challenge with Hertz 33/56 however, just group 1 shed the challenge virus during the vaccinated groups. In contrast, 100% of the unvaccinated Chukar Partridges that had been

Table 1: Mean antibody titers by HI test (log_2) of chukar partridges detected after the different vaccination programs against ND

	Vaccination		Chukar partridges ages (weeks)					
Group	vaccine type	route *	1.0	5.0	8.0	11	14	17
I	IB	DW	5.4	0.0	6.0	4.2	4.3	3.3
II	B1+LaSota	DW	5.4	0.0	7.0	6.5	5.7	4.7
III	B1+V4 (oil)	DW/SC	5.4	0.0	8.0	9.2	8.7	7.6
IV	B1+V4 (oil)	ED/SC	5.4	0.0	8.5	9.5	8.2	7.8
V	contro		5.4	0.0	0.0	0.0	0.0	0.0

*DW: Drinking water; ED: Eye drop; SC: Subcutaneous

Table 2: Results of challenge with velogenic NDV in the 5 test groups and virus isolation in chukar partridges

		Number of challenged chukars		Viral isolation	
Group	vaccine type	challenge route	total protection* (%)	Op	C
I	B1	IN/ED[20]	20/20(100.0)	+	+
II	B1+LaSota	IN/ED[20]	20/20(100.0)	-	-
III	B1+V4 (oil)	IN/ED[20]	20/20(100.0)	-	-
IV	B1+V4 (oil)	IN/ED[20]	20/20(100.0)	-	-
V	Control	IN/ED[20]	0/20(0.0)	+	+

*: The data are the numbers of Chukar Partridges survived/number of challenge test Chukar Partridges; IN: Intranasal; ED: Eye drop; OP: Oro-pharyngeal sample; C: Cloacal sample; +: Isolation positive; -: Isolation negative

challenged with Hertz 33/56 died within 5 days post-challenge and all of them showed viral shedding before to die. The clinical signs in affected Chukar Partridges included depression, anorexia, respiratory problems and green diarrhea. NDV was isolated from theses Chukar Partridges, indicating that the virus utilized in the challenge had the potential to cause disease. The mortality rate of Chukar Partridges in the vaccinated groups were significantly different ($p<0.001$) in compare with Chukar Partridges in the control group. The protection percentage of Chukar Partridges in the vaccinated groups (100.00) was not significantly different ($p<0.001$).

DISCUSSION

It is well known in chickens that a combination of live and inactivated oil adjuvant NDV vaccines could elicit the protection against NDV (Bennejean *et al*., 1978). But, there is little information available on health control programs in the Chukar Partridges. Analysis of the NDV that were recently caused high losses to Chukar Partridges intensive breeding farms in Iran has led major problem to Chukar breeding industries that the absence of enough scientific information within subject may be responsible for the continuing distribution of ND in Iranian Chukar partridge farms.

Here, we assessed whether a correct vaccination program can overcome the infection of NDV induced by the currently available ND vaccines. In this study it was found that all Chukar Partridges in the non-vaccinated group V died after challenge. Chukar Partridges in groups I (vaccinated with B1 strain live vaccine) and II (vaccinated with B1 and La Sota strains live vaccine) and III & IV (vaccinated with IOAV and B1 strain live vaccine) exhibited resistance after challenge as observed for chickens in the studies published by other scientists (Chansiripornchai and Sasipreeyajan, 2006). The Chukar Partridges in groups III and IV revealed the antibody titers against NDV higher than the Chukar Partridges in groups I and II as reported by Paulillo *et al*. (2008), for another race of Partridges like Rhynchotus rufescens. The reason for the high antibody response obtained with inactivated oil-emulsion following use of live virus vaccines given simultaneously could be due to make a stable emulsion in which the antigen is slowly released, thus a prolonged immune stimulus is observed (Warden *et al*., 1975). As it mentioned in comparison with those group that received live virus vaccines, groups III and IV showed higher HI titres which probably induce better level of protection when more number of viruses attacks or more pathogenic virus invade the body.

On the other hand, the low invasion capacity of the B1 strain (Hofstad, 1951) and the high production adverse effects potential of the La Sota strain (Allan and Borland, 1979) are not compatible with the high antibody titers detected by HI in vaccinated Chukar Partridges. Also it should be declare that in spite of chickens, bursa of fabricious remains in Chukar Partridges and does not regress like chickens.

We also found that all Chukar Partridges only vaccinated through live ND vaccine, the antibody titers were decreased during the time and serum antibody levels reach to a risk levels after 12 weeks of vaccination. Thus, Chukar Partridges with lower antibody titer induced immunity may continue to be susceptible to NDV infection and losses, despite those that vaccinated with inactivated ND vaccine that behave as a booster dose. The present study also showed that all vaccine groups had a full protection rate (100.00) in comparison to the control group (0.00) when they were challenged with Hertz 33/56 strain velogenic NDV. The Chukar Partridges in the control group had a null level of antibodies and failed to protect against the challenge. This agrees with Allan *et al*. (1978), who reported that 100% mortality on challenge when the HI titers were 2 $\log_2$ or less and no mortality when the HI titers fell between 4 and 6 $\log_2$ with a mean HI titer of 5.2 $\log_2$. This finding indicates a breakdown of immunity or inadequate vaccination with poor vaccines can causes outbreaks of NDV in Chukar partridge farms. Alternatively, it is considerable to more prevent of NDV spread to offer to use of those vaccines that may act as line of defense against NDV.

CONCLUSION

Our study has shown that commercially available ND vaccines for chickens induced a high antibody response in Iranian Chukar Partridges, without any clinical sings of post-vaccinal reaction. These finding indicate that vaccination, dependent to the vaccination program used including live NDV vaccine and or IOAV in combination with live NDV vaccine, can efficiently protect Chukar partridge against this virus in the farms.

ABBREVIATIONS

ND : Newcastle Disease
NDV : Newcastle Disease Virus
IOAV : Inactivated Oil Adjuvant Vaccine
RVSRI : Razi Vaccine and Serum Research Institute
HI : Heamagglutination Inhibition
HA : Heamagglutination Activity
OIE : Office International des Epizooties
PBS : Phosphate Buffer Saline
BHI : Brain-Heart Infusion

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