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Research Article

Evaluation of Three Different Vaccination Regimes Against Newcastle Disease in Central Anatolia

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Abstract: A change in the vaccination strategy used in the Central Anatolia region of Turkey to combat the effects of infections with endemic virulent Newcastle disease virus (NDV) in maternally immune birds is described in this retrospective work. A novel vaccination schedule was applied to approximately 2,230,000 chickens from different breeds. The alterations were based on the use of an inactivated NDV + infectious bursal disease virus (IBDV) and the application of aerosol vaccines with different timings compared to traditional programmes. In a district in which Newcastle disease (ND) epidemics had been occurring, a satisfactory humoral protection (mHI titre >log 2^7) was achieved in birds up to 9-11 weeks of age by the early administration of inactivated NDV + IBDV vaccine. It is suggested that inactivated NDV + IBDV vaccination at 1 week of age be recommended as an alternative procedure to prevent both ND and infectious bursal disease (IBD), although the data presented here on immunity to IBD was essentially restricted to clinical observations.

Key Words: Newcastle disease, vaccination, retrospective, chicken.

İç Anadolu Bölgesi'nde Newcastle Hastalığına Karşı Uygulanan 3 Farklı Aşılama Programının Değerlendirilmesi

Özet: Bu retrospektif çalışmada, Orta Anadolu Bölgesi'nde Newcastle Hastalığı'na karşı uygulanmakta olan aşılama programında yapılan değişiklikler ve bunların, maternal olarak hastalığa immüniteleri bulunan tavuklardaki etkileri incelendi. Yeni bir program, farklı yetiştirme tiplerinden yaklaşık 2.230.000 tavuğa uygulandı. Aşılama programındaki değişiklikler, esas itibariyle, inaktif Newcastle Hastalığı Virusu + Infeksiyöz Bursal Hastalık (NDV + IBDV)'ın kullanılması ve geleneksel aşılama programlarındaki uygulama zamanlarından farklı olarak uygulanan aerosol aşıların kullanımından ibarettir. Newcastle Hastalığı'nın görülmekte olduğu bir bölgede, inaktif NDV + IBDV ile aşılanan 9-11 haftalık yaştaki tavuklarda yeterli sıvısal korumanın (mHI titresi >log 2^7) geliştiği tespit edildi. Her ne kadar İnfeksiyöz Bursa Hastalığı ile ilgili gözlemler klinik gözlemlerle sınırlı da olsa, 1 haftalık yaştan itibaren uygulanabilecek inaktif NDV + IBDV aşılaması, hem Newcastle Hastalığı ve hem de İnfeksiyöz Bursa Hastalığının önlenmesinde mevcut aşılama programına alternatif bir uygulama olabilecektir.

Anahtar Sözcükler: Newcastle hastalığı, aşılama, retrospektif, tavuk.

Introduction

The economic threat of mortality and loss of production due to Newcastle disease (ND) has resulted in continuous efforts being made to develop vaccination programmes that are effective and cheap. It has been reported that perhaps one of the least expected uses of oil emulsified Newcastle disease virus (NDV) vaccines is to actively immunise maternally immune chicks at risk to early infection of NDV (1). In countries with a fairly modernised poultry industry, this problem (i.e. early infection caused by endemic infection) seldom arises, but in less modernised situations, the problem is common and without a potent killed-vaccine it is difficult to deal with.

The latest outbreak of ND in Turkey started to appear as an epidemic in chicks (both layers and broilers) and chickens during the second half of 1996. In order to prevent both ND and infectious bursal disease (IBD), inactivated NDV plus IBD virus (IBDV) vaccines have been in use (2-4), which led to a significant decrease in the incidence of both infections (3,4). However, despite the

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higher antibody titres for ND (log 2^{9-12}) the presence of symptoms like torticollis and difficulty in breathing in chicks, respiratory signs and decrease in egg production (10-25%) in layer flocks were found to be common. Therefore, alterations to present vaccination strategies were needed. The occurrence of ND, despite the presence of higher antibody titres, brought atomic vaccination into application as a further practice.

This study was primarily conducted to evaluate the effects of alterations made to the ND vaccination regime in 2,230,000 chickens in Central Anatolia.

Materials and Methods

The poultry studied were kept in well-built, ventilated, light- and temperature-controlled houses throughout the study.

The selection of poultry farms for the study was based on the presence of symptoms like torticollis and respiratory signs, and decrease in egg yield reported in the previous production period. Of these, approximately half of the pullet flocks and a few of the parent stock (PS) flocks (2 layers; 1 broiler PS) were known to have overcome the disease. Flocks from PS and commercial layer hens were monitored during the study for egg production.

A total of 1,080,000 Babcock B300, B380 and ISA White pullets were assigned randomly to pens. These commercial layers were included in the study over a period of 17 weeks. About 1,000,000 hens were also used in this study. In addition, the study comprised 50,000 PS broilers (Avian Farms and Shaver) and 100,000 PS layers. All the animals were monitored from day 1 until month 9. An application of inactivated NDV + IBDV vaccination to 1-week-old chicks was used. The atomic vaccination was also in use from the second half of 1997 (Table).

Blood samples were collected from chicks and chickens at 2-3 week and 4-week intervals, respectively. Antibody titres to NDV from all flocks were monitored by using a micro haemagglutination-inhibition (mHI) test. A standardised microtitre HI test, using 4 haemagglutinating units and 2 serial dilutions of serum, was used. The results were noted after 60 min as the $\log_2$ of the reciprocal of the serum dilution, giving the 50% inhibition of haemagglutination as described by Erganis et al. (3).

In the study, IBD was monitored by recording clinical and necropsy findings from infections.

Table. Vaccination schedules used in Central Anatolia, Turkey.

Until 1996		Between 1996 and 1997		Since 1997	
days	Vaccine (route)	days	Vaccine (route)	days	Vaccine (route)
1-3	ND + IB	3-5	HB_1 (cs)	1	ND (cs)
5	HB_1 (dw)	7-10	HB_1 (dw)	7-10	Inactivated NDV + IBDV (sc) Gumboro (bd)
13	Gumboro (dw)	8-10	Inactivated NDV + IBDV (sc)	17-19	Gumboro (dw)
21	La Sota (dw)	12-13	Gumboro (dw)	23-27	Cloned ND*
25-26	Gumboro (dw)	16-19	Gumboro (dw)	50-60	La Sota (dw)
60	La Sota (dw)	21-24	La Sota (dw)	85-90	Cloned ND*
		63-67	La Sota (dw)	112	Inactivated ND (sc)
		112	Inactivated ND	133-135	Cloned ND*
				180-185	Cloned ND*

* Atomist (Cloned) ND vaccine (1000 dose / 250 ml water added with 1 g milk powder / litre demineralised water) was applied as particles in 20 µm diameter by atomist method using a Hurricane atomiser at H level. at: atomist. In: Inactivated. cs: coarse spray. dw: drinking water. bd: beak deeping. sc: subcutaneous.

Results

The serum anti-ND titres of the flocks from the representatives of different types of production are shown in Figure 1a. The usual ND vaccination schedule which had been used until 1996 in the flocks produced a serum level of anti-ND titres of log 2^{5-10} measured by HI test. This schedule, which is broadly used by local veterinarians, showed uneven serum anti-ND levels when a production period of 90 days was considered. On the other hand, the schedule used in this particular study made a consistent serum antibody level for NDV in comparison with the previous schedule (Figs. 1 b, c and d).

Discussion

To prevent clinical ND in layers and breeders, we sought to create a uniform antibody level as high as possible for as long as possible. It was suggested that this should be 7 $\log_2$ or more (1). However, this study revealed that a high HI titre (>log 2^7) may not totally correlate with protective immunity. On the other hand, by using inactivated viruses, it was possible to ensure that each bird within a given treatment was responding to a similar antigen dose. Figure 1 shows antibody titres to ND in the serum samples obtained from different types and numbers of flocks. The flocks represented in Figure 1 have a titre of antibody more than log 2^7 for their whole life. Because of the retrospective nature of the work, there were 2 groups of flocks to be compared. Flocks vaccinated by inactivated vaccines were compared with those vaccinated by live vaccines since similar timings between these 2 regimes existed. A La Sota vaccination given to 9-11-week-old birds protected the birds until 16-18 weeks of age, although a very low occurrence of torticollis symptoms (<0.1% of all the chicks in the flock) in some individuals was seen. Nedelciu and Edu (5) showed that inactivated (both oil emulsion ND and

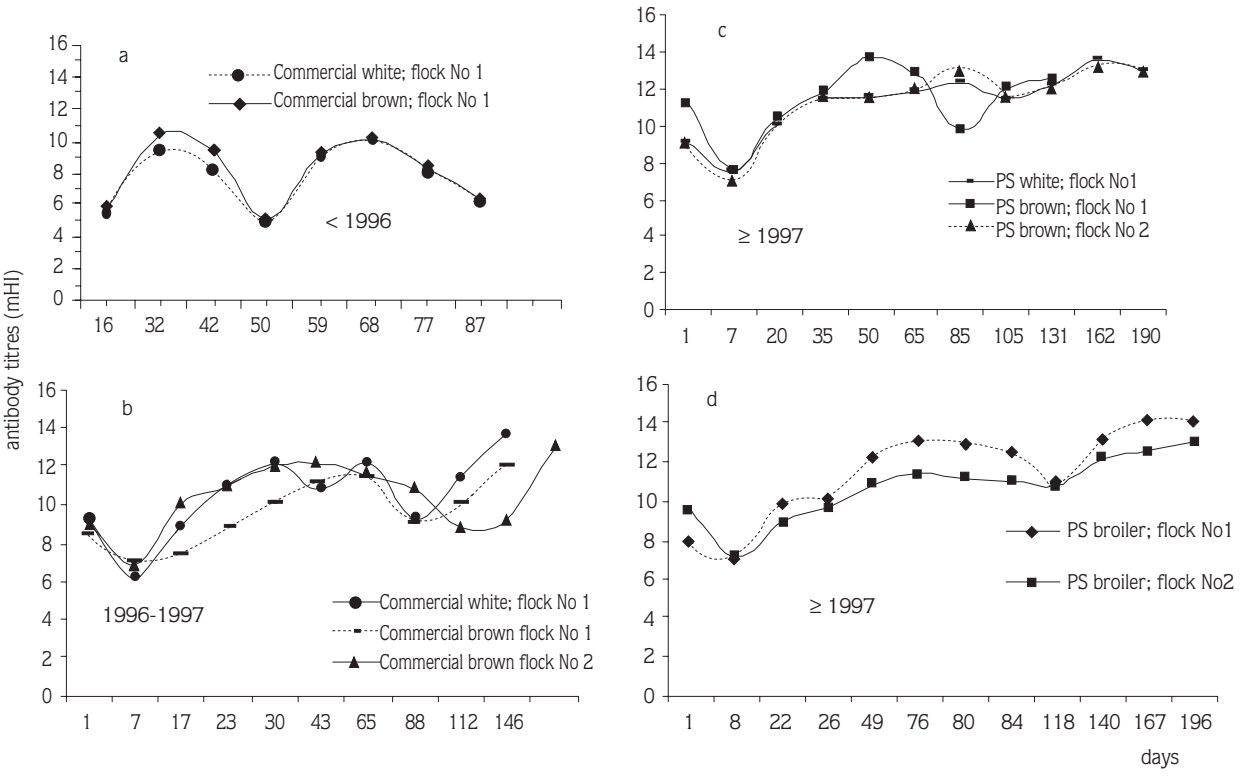

Figure 1. Monitoring of anti-ND antibody titres in flocks of different breeds.
Serum anti-ND titres of the flocks. Each line shows data from a corresponding flock as follows: a. Commercial flocks vaccinated until 1996 and with only live vaccines throughout their lives. All the flocks in b, c and d are those vaccinated according to trial schedule; b. Commercial layer flocks. The flocks were vaccinated with the schedule given in the column of "between 1996 and 1997" in the Table; c. Layer parent flocks, a vaccinated group of "since 1997"; and d. Antibody titres of broiler PS flocks. X and Y axes represent days and mean antibody titres to ND, respectively. Each point represents mean data from at least 25 individuals.

aluminium hydroxide ND) vaccines gave the best (100%) protection against challenges in laboratory conditions in chicks. It is accepted almost as a rule that because of the inadequate physiological capacity of chicks to respond to immunogenic stimulation, to obtain protective immunity against ND more than one form of vaccination is necessary. The efficacy of immunisation is closely related to the type of vaccine used as well as to the intervals between and route of vaccinations. The present work is one of the few reports from the Middle East on a regime suggesting inactivated NDV + IBDV vaccine application for 1-week-old chicks. In the vaccination regime used in the present study, a coarse spray vaccination was proceeded by an inactivated ND oil emulsion vaccine at 7-10 days of age so as to stimulate the immune system by different pathways. For this purpose, on day 23-27 another aerosol vaccination using NDV was also carried out. Although it is not common practice to vaccinate the chicks with an inactivated oil emulsion vaccine at 7 days of age, a good state of protection was obtained since in pullets or PS chickens which had already been monitored (until days 112 and 270 for pullets and PS, respectively), neither IBD nor ND clinically existed. The clinical signs such as torticollis, difficulty in breathing and even digestive system symptoms typically seen in previous production cycles, were not noted.

Furthermore, after application of the cloned NDV vaccine by the atomic method, no decrease in egg production due to ND was observed in any of the commercial laying hen, young chicken or laying adult flocks. Erganiş et al. (3) showed that the antibody titres to NDV in a flock vaccinated with live vaccines decreased more rapidly than those of flocks vaccinated by live and inactivated strains. Data from the present work was seen to be in parallel with that study since the timing of the vaccinations was almost the same in both studies (Figure 1a).

Following inactivated NDV + IBDV vaccination, 2 live Gumboro vaccines (first, just after; second, 7-9 days after inactivated vaccine administration) were applied earlier than normal and they provided satisfactory protection as determined by clinical observations. Although no laboratory evaluation was made to determine the degree of protection against Gumboro, if the sizes of the populations observed are taken into consideration, the results could be considered promising; however, more detailed research is needed to understand the actual mechanism(s).

In one study (6), in which antibodies in the air passage washings and in the tears of chickens immunised by aerosol or im NDV vaccine (attenuated or inactive) were compared to serum antibodies produced by similar methods of administration, HI antibodies in secretions reached higher levels after aerosol vaccination than after im administration, whereas in serum the situation was reversed. The authors also stated that the antibody activity in airway washings and in tears is associated with IgA, while in serum the main antibody is IgG. The results from the present study suggested that aerosol vaccination could stimulate the mucosal immune system in an effective manner. Similar stimulation results have also been reported by others (6,7). However, the predominant form of the disease seen previously in the flocks included in this study was visceral. It appears likely that inactivated vaccination through parenteral route primarily induced a systemic immune mechanism which provided good protection.

The term “hypervirulent strain” has been used to describe both very virulent European strains and variant American strains causing less than 5% mortality (8). Wyeth et al. (2) suggested that controlling Gumboro caused by very virulent (vv) IBDV in England seems to be possible by employing vaccines prepared from intermediate strains of the virus. In addition, the Gumboro vaccines in question could be neutralised by maternal antibodies. The authors, thus, emphasised the necessity of using IBDV-inactivated vaccines. It has been reported that (9) mitogenic inhibition occurs early, during the first 3-5 days of IBDV exposure, and then the mitogenic response of T cells returns to normal levels. The authors suggested that intrabursal T cells and T cell-mediated responses may be important in viral clearance and promoting recovery from infection. Sharma et al. (10) reported that IBDV did not affect normal natural killer (NK) cell levels in chickens. Furthermore, methods to measure the progress of antibody titres and vaccination to Gumboro in layer chicks have been reported by Erganiş and Orhan (11). First, the authors detected the average level of maternal antibodies of a flock of 45,000 laying hens (Babcock). Titres to IBDV were about 5,000 as determined by ELISA test. Second, applications of 1 inactive and 3 subsequent live Gumboro vaccines between the ages of days 9 and 26 were reported to produce serum antibody levels of 70 and 4264 on days 32 and 52, respectively. Although the

serum levels of anti-IBDV were low, the chicks were not infected with IBDV between days 28 and 35, throughout which the chicks were much more susceptible to Gumboro infection. The authors' explanation for this was that cellular immunity might play a dominant role. In parallel with all the reports given above, in the present study it may be considered that sufficient protection for Gumboro could be achieved by early vaccination of the birds through a different stimulation process since no clinical sign of Gumboro was determined. However, the success of the protection against Gumboro should not be expected only from early vaccinations as suggested in a similar work (4). The authors suggested that all the requirements of maintaining good management, especially uniformity of the flock, are also crucial in the Gumboro protection. Although immunomodulation caused by different pathways in hens is ill-defined and more basic studies are needed to provide answers (12), we speculate that the application of both inactivated viruses together interfere with immune suppression or produce immune stimulation or do both somehow. The triggering of immune response by attenuated or dead IBDV with or without the ND antigen would be worth investigating when they are administered simultaneously since the actual sequence within VP2 responsible for the induction of the apoptosis of B cells has not been identified (12). Thus, the absence of this sequence cannot be essential for viral replication. If this is the case in the present study, the lack of such sequence could be speculated to be mimicked by inactivated IBDV.

Neither of the infections were detected in the commercial layer and PS flocks. Furthermore, no reductions were observed in egg production during the study. Thus, this vaccination protocol appears to be effective enough to prevent these diseases. Therefore, in any type of chicken breeding, inactivated NDV + IBDV vaccination at 1 week of age and coarse spray and/or atomist vaccination for chickens of all ages are recommended as an alternative procedure to prevent both ND and may be IBD.

In conclusion, since the birds failed to show signs of ND by application of this vaccination schedule, the oil emulsion vaccine could be used primarily in laying and breeding stocks especially at the early stage of life despite the higher cost of the vaccine itself and individual vaccination. The results presented here were obtained from field data. Thus, experimental work needs to be planned to assess this changed vaccination schedule or to determine the effects of individual changes.

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