Vaccination of Chicks with an Experimental Oil-emulsion Vaccine against Infectious Bursal Disease Virus

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Received 5 Mar 2000; accepted 7 Jul 2001

Summary

To prevent against recent virulent strains of infectious bursal disease virus (IBDV), a local isolate of the virus was propagated in bursal tissue, formalized and used for vaccine production. An experimental vaccine adjuvanted by oil ISA-70 was prepared and compared with a commercial IBDV-NDV inactivated vaccine. A single injection of the two vaccines protected chickens against mortality but the oil adjuvanted bursal derived vaccine conferred a higher percent of bursal protection.

Key words: Chickens, oil-emulsion vaccine, Infectious Bursal Disease virus, vaccination

Introduction

Infectious bursal disease (IBD) is an important viral disease of broilers and pullets, which cause morbidity and mortality or immunosuppression and poor growth rate. IBD virus (IBDV) belongs to the family Birnaviridae and possesses two serotypes, of which, viruses of type 1 are pathogenic for chickens (McFerran *et al* 1980). Since 1988, very virulent strains of IBDV type 1 have been reported in Europe (van den Berg *et al* 1991) and subsequently in Africa and Asia. While the classic strains of IBDV type 1 cause about 5% mortality in susceptible flocks, the new virus strains cause higher mortality up to 30% in broilers and 70% in pullets (Lasher & Shane

immunosuppression (Nakamura *et al* 1992), can establish infection in the presence of high levels of maternal antibodies (van den Berg & Meulemans 1991). Therefore, to protect chickens, live attenuated vaccines have been used at the first days of chickens' life (Lasher & Shane 1994). But, due to neutralization of mild and intermediate live vaccines by maternal antibodies and the need to measure these antibodies before vaccination there has also been some interests in the use of inactivated vaccines (Gaudry 1993). In Iran, as in many other parts of the world, despite an excessive use of live attenuated vaccines, IBD causes great economic losses to the poultry industry. Therefore, the present study was undertaken to prepare and evaluate an inactivated vaccine for immunizing chickens against IBD.

Materials and Methods

Virus isolation. A local isolate of IBDV (AZ-98) was obtained from the bursa of broiler chickens with clinical IBD and about 25% mortality. Isolation of the virus was performed by intra bursally inoculation of a homogenate of infected bursa to a group of specific antibody negative (SAN) broiler chickens. To decrease the probable contamination of IBDV with other agents such as adenovirus and reovirus, the infected bursal homogenate was subjected to two passages in the bursa of SAN chickens at a 1/100 dilution. An agar gel immunodiffusion (AGID) test using an IBDV-specific polyclonal antibody (KPL) was carried out for detecting of the viral antigen in bursal tissue (Rosenberger 1989).

Antigen preparation. A 10% suspension of AZ-98 propagated in the bursa of SAN chickens was made in normal saline and titered for infectivity. 10-fold serial dilutions of the virus were inoculated to groups of five 3-week-old SAN chickens by oral route. The titer of virus was estimated to be at least 10^4 Chicken Infectious Dose (CID_{50})/0.1 ml. Subsequently, 50 SAN chickens of 3-week-old were injected intra bursally with approximately 10^3 CID_{50}/0.1 ml of AZ-98. Three days after inoculation bursa were harvested, homogenized and made as a 40% suspension in normal saline. After extraction with an equal amount of chloroform, bursal extract was inactivated by adding formaldehyde to a final concentration of 0.2% followed by incubation at

37°C for 48 h. The antigen was then inoculated to SAN chickens to assess its inactivation and it was finally titered by AGID.

Vaccine preparation. Oil adjuvant ISA-70 (SEPPIC, Cosmetics-Pharmacy Division, Paris, France) was mixed with the antigen (having a titer of 2 in AGID) at a ratio of 7:3 and homogenized in a mixer through 20 cycles of 5 seconds each.

Experimental design. Sixty SAN chickens of 21 day-old were divided into 3 groups of twenty birds each. In the group 1, chickens were inoculated by 0.5 ml of the experimental vaccine subcutaneously, at the back of the neck. For comparison the commercial IBDV-NDV inactivated vaccine (Merioux) was injected to the chickens of group 2 as per the manufacturer recommendation. Group 3 was considered as control. In each group, 10 chickens were randomly bled at 1, 2 and 3 weeks postvaccination. After the third bleeding, half of the chickens in each group were challenged orally with approximately 10^3 CID_{50}/0.1 ml of AZ-98 and observed up to one week. Then they were weighted individually and killed humanely, their bursa was removed and weighted. Bursal atrophy was determined by calculation of bursa:body weight (B:B) ratio as described by Lucio & Hitchner (1979). Sera were tested with AGID, for the presence of IBDV-specific antibody, in a single dilution.

Results

The serum reactions of each group of chickens by AGID, following a single subcutaneous injection of the vaccines are presented in table 1.

Table 1. *Serological response of chickens in AGID[1] after vaccination*

Group	Vaccine	Reactivity of sera at the days			
		0	7	14	21
1	Experi.	-	1/10[2]	10/10	10/10
2	IBDV-NDV	-	-	2/10	2/10
3	-	-	-	-	-

[1] Sera were tested in AGID as undiluted.

[2] Number of positives per 10 sera.

The AGID was positive in some of chickens that injected with the experimental vaccine within 1st week after vaccination. All of them were positive on the second

week postvaccination. Among chickens receiving the commercial vaccine, only 20% showed antibody at the second and third weeks after vaccination. After challenge of ten chickens from each of the immunized and control groups, three chickens in the unvaccinated control group were died from IBD but no mortality or significant clinical signs were observed in other groups.

Means B:B ratio and percents of bursal protection for each group of chickens, are shown in table 2.

Table 2. *Effects of vaccines on the B:B ratio and bursal protection after challenge*

Group	Vaccine	Mean B:B ratio		% of protection
		Unchallenged	challenged	
1	Experi.	1.12±0.41	0.96±041	60
2	IBDV-NDV	1.18±0.45	0.68±0.25	33
3	-	0.92±0.45	0.53±0.13	-

After challenge, group 1 of chickens, injected with the experimental vaccine had significantly a higher means B: B ratio. On the other hand, in this group mean B:B ratio in challenged chickens was not significantly different from that of the unchallenged chickens. Whereas in group 2, immunized with the commercial vaccine and in the group 3, means B:B ratio in challenged chickens were significantly ($P<0.05$) lower than those of the unchallenged chickens. The experimental vaccine conferred the highest percent of bursal protection (60%).

Discussion

The aim of the present study was to prepare an efficient inactivated vaccine for immunizing chickens against IBD. The antigenic nature of IBDV in Iran, has not yet adequately defined, therefore a recent local isolate of the virus was used for vaccine preparation. The vaccine was made with oil ISA-70 as adjuvant and compared with a commercial imported vaccine in SAN chickens. A single injection of both experimental and commercial vaccines protected chickens against mortality. Nevertheless, chickens vaccinated with the experimental vaccine showed a higher immune response by AGID and were significantly protected to a higher degree

against bursal atrophy. This indicates that the bursal derived inactivated vaccines, as prepared in this work, is more efficient vaccine for preventing against the local isolate of IBDV than commercially available inactivated vaccine. Whether the difference between the experimental and commercial vaccines is related to their antigenic contents or this is due to some antigenic variations in our local IBDV isolates, remained to be determined. In a preliminary work, conducted on one of the highly virluent isolates of IBDV in Iran (Aghakhan *et al* 1996), it has been shown that the virus can still be regarded as belonging to the standard type 1. But, further studies and characterization of several local isolates by monoclonal antibodies are necessary to elucidate the antigenic nature of IBDV in Iran.

The higher efficiency of bursal derived vaccine may also be related to the system in which the virus was propagated. The differences in structural proteins between a population of viruses grown in bursal tissue compared to viruses grown in chicken embryo fibroblasts have been reported (Muller *et al* 1986). It appeared that cellular factors such as proteases are important in the maturation of IBDV particles and differences in the micro-environment among different host cells could account for the development of defective virus particles.

Immunogenicity and potency of bursal derived inactivated vaccines have also been studied in some other works. Lue *et al* (1996) showed that a formolized bursal derived vaccine could efficiently protect chickens against IBD. Wyeth & Chettle (1982) compared a killed bursal derived vaccine with three embryonated egg origin inactivated vaccines in pullets, previously immunized with a live attenuated vaccine. The results indicated that the bursal derived vaccine induced a more prolong maternal immunity in progenies. Despite a higher efficiency of bursal origin inactivated vaccines, currently, the most available IBDV inactivated vaccines are those prepared in embryonated egg or cell cultures. This is mostly due to the fact that the production of bursal vaccines is more difficult and more expensive. However, these problems could be overcome, if the virus is cultivated in bursal tissue, in an organ culture system and presently we are standardizing this technique in our laboratory.

Acknowledgements

This research was supported by grant No.319 of Shahid Chamran University of Ahwaz, Iran.

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