

PRODUCTION OF HYPERIMMUNE SERUM AGAINST INFECTIOUS BURSAL DISEASE VIRUS IN RABBITS

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ABSTRACT

The early and accurate diagnosis of infectious bursal disease requires known hyperimmune serum against the disease. In this study, an attempt was made to raise anti-infectious bursal disease virus hyperimmune serum in rabbits. The infectious bursal disease (IBD) virus was isolated from field outbreaks, identified, purified, titrated and its infectivity titre was determined in embryonated eggs. Three different semi purified and purified doses were prepared for inoculation in rabbits: (i) chloroform treated IBD virus; (ii) ultra centrifuged and resuspended virus using sucrose as gradients and (iii) pelleted and resuspended virus with addition of incomplete Freund's adjuvant. Killed IBD virus vaccine (Cevac IBDK) was used as 4th inoculum. It was observed that the serum collected after series of inoculation of 1st and 2nd inoculum provided maximum antibody titre upto log₂6 and Log₂9, respectively. However, the serum collected after series of injection of 3rd and 4th inoculum gave maximum indirect haemagglutination (IHA) titer (log₂10). This study suggested that live antigen containing incomplete Freund's adjuvant provided better immune response and may be a good choice for raising a hyperimmune serum against IBD virus in rabbits.

Key words: Hyperimmune serum, infectious bursal disease, incomplete Freund's adjuvant, rabbits

INTRODUCTION

Infectious bursal disease (IBD) has been considered as one of the important viral diseases threatening the poultry industry worldwide. It is an acute, highly contagious viral infection of young chickens and has lymphoid tissue as its primary target with special predilection of bursa of Fabricius. The disease is characterized by sudden onset, short course, extensive destruction of lymphocytes and profuse watery diarrhoea, followed by death or rapid recovery (Lukert and Saif, 2003). The economic importance of the disease is manifested in two ways; firstly some virus strains may cause upto 20-30% mortality in three weeks and older chickens. The second and most important manifestation is severe, prolonged immunosuppression of chickens infected at an early age (Van-den-Berg, 2000).

The infectious bursal disease virus (IBDV) is a dsRNA virus, which is a member of genus Avibirnavirus of family birnaviridae. This virus exists in two antigenically distinct serotypes i.e. I and II. The serotype I strains are pathogenic to chickens and vary in their virulence, whereas serotype II strains are present only in turkeys (Lukert and Saif, 2003).

Due to its worldwide occurrence, IBD drew the

attention of research workers for an effective control. There is no effective control of IBD except vaccination. The diagnosis of any disease is the first and foremost requirement for its effective control. Routine diagnosis of IBD has been conducted by using immunodiffusion, immunofluorescence, virus neutralization, enzyme linked immunosorbent assay, electron microscopy and monoclonal antibodies (Lukert and Saif, 2003). Most of these tests require known serum against this disease. The specific hyperimmune serum is also very helpful in the differential diagnosis of disease during an outbreak. Locally, the diagnostic serum is not being produced commercially and the imported one is very expensive.

Therefore, the present project was aimed to standardize the best possible method to raise hyperimmune serum against IBDV in rabbits. It will not only serve as diagnostic tool but will also save the precious foreign exchange.

MATERIALS AND METHODS

Source of virus

Infected bursae were collected from an outbreak and homogenized as a 10% W/V suspension in phosphate buffered saline. The suspension was clarified by centrifugation at 3000 rpm for 10 minutes. Supernatant was collected as virus. It was further

purified by mixing with chloroform in 1:1 ratio and centrifuged at 5000 rpm for 20 minutes (Reddy *et al.*, 1997). Three layers were formed and the top layer containing virus was collected. The presence of virus in supernatant was checked by agar gel precipitation test (Cullen and Wyeth, 1975). Sucrose gradient centrifugation method was used for the concentration of IBDV (Nagy and Lominiczi, 1984). The chloroform treated and concentrated IBD virus was titrated by reverse passive haemagglutination test (Rajeswar and Dorairajan, 1999). The infectivity titre of the collected virus was also determined, as described by Reed and Muench (1938).

Raising of hyperimmune serum

A total of 25 healthy adult male rabbits were used for raising hyperimmune serum. All the rabbits were de-wormed with albendazole at the dose rate of 5 mg per kg body weight and their serum was checked by indirect haemagglutination (IHA) test (Hussain *et al.*, 2003) for antibodies against IBD, which was found to be zero. The study was conducted in two phases

Phase I

A total of 13 rabbits out of 25 were divided into three groups i.e. A1, B1 and C1, having 5, 5 and 3

rabbits, respectively. The rabbits of group A1 were inoculated with chloroform treated IBDV, group B1 with pelleted IBD virus, while rabbits of group C1 were kept as un-inoculated control (Table 1). The rabbits were bled fortnightly and their antibody titre against IBD was determined through IHA test.

Phase II

The pelleted IBD virus obtained through sucrose gradient centrifugation was suspended in 2 ml PBS and mixed with equal volume of incomplete Freund's adjuvant (Sigma Co. Ltd). The remaining 12 rabbits were divided into three groups i.e. A2, B2 and C2, having 5, 5 and 2 rabbits, respectively. The live adjuvanted virus inoculum prepared was injected subcutaneously @ 0.2 ml per rabbit in rabbits of group A2 (Jurd and Hansen, 1990). The rabbits of group B2 were injected with commercial killed oil based IBD vaccine (Cevac IBDK) subcutaneously @ 0.2 ml per rabbit. Five injections were given fortnightly in both groups, while the sixth injection was given after a gap of one month. The 2 rabbits of group C2 were kept as un-inoculated control (Table 2). The rabbits were bled periodically to determine the antibody titer in their serum against IBD, using IHA test (Hussain *et al.*, 2003).

Table 1: Inoculation schedule of chloroform treated and concentrated IBD virus in different groups of rabbits (Phase 1)

Inoculation day	Quantity of inoculum (ml)		
	Chloroform treated virus (A-1) (n=5)	Concentrated pelleted virus (B-1) (n=5)	Uninoculated control (C-1) (n=2)
Zero day	0.5	0.5	Nil
7 th	1.0	1.0	Nil
15 th	1.0	1.0	Nil
30 th	1.5	1.5	Nil
45 th	1.5	1.5	Nil
75 th	2.0	2.0	Nil

Table 2: Inoculation schedule of live adjuvanted and killed oil based IBD virus in rabbits of different groups (Phase II)

Inoculation day	Quantity of inoculum		
	Live adjuvanted (A-2) (n=5)	Killed adjuvanted (B-2) (n=5)	Uninoculated control (C-2) (n=2)
Zero day	0.2	0.2	Nil
15 th	0.2	0.2	Nil
30 th	0.2	0.2	Nil
45 th	0.2	0.2	Nil
60 th	0.2	0.2	Nil
90 th	0.2	0.2	Nil

RESULTS

Virus

Infectious bursal disease virus (IBDV) was isolated from infected bursae collected from an outbreak of IBD. The clear precipitation lines in agar gel precipitation test (AGPT) confirmed the presence of IBD virus in supernatant collected after chloroform treatment. The virus was further purified and concentrated by using sucrose gradient centrifugation method. The virus titer was $\log_2 7$ in chloroform treated virus and $\log_2 9$ in concentrated IBDV, as determined through RPHA test. The infectivity titer of the collected virus was found to be $10^{5.4}$ in 11-day-old embryonating eggs.

Phase I

In phase I, the IHA titer was higher in concentrated (ultra centrifuged) IBDV as compared to chloroform treated virus and it increased progressively from $\log_2 6$ to $\log_2 9$ (Table 3).

Phase II

In phase II, IHA titer against concentrated virus adjuvanted with Freund's incomplete adjuvant was higher as compared to non-adjuvanted concentrated virus of Phase I. Killed adjuvanted IBDV vaccine also

showed higher antibody titer but after 3rd inoculation (Table 4).

DISCUSSION

The production of antibodies is a complex biological phenomenon. It is not always possible to follow any recommendations and guidelines outlined in literature, as procedures and protocols have to be modified depending on the antigen. For some purposes, a single injection may be sufficient but in general higher antibody yields are obtained by administering a series of injections (Cruickshank *et al.*, 1968).

In choosing a species for raising polyclonal antibodies, one has to consider the quantity of antiserum needed, the evolutionary relationship between the recipient and the donor of the antigen and the character of the antibody made by recipient. A number of vertebrate species, ranging from farm animal to rabbits, small laboratory rodent and chickens have been used over the years (Carpenter, 1975). Rabbits are the single most used species because they are of convenient size, easy to bleed and handle, have relatively long lifespan and produce adequate volumes of antisera. Moreover, they are also free from antibodies against avian viruses.

Table 3: Antibody titer in rabbits against chloroform treated and concentrated IBD virus during Phase I

Sampling day	Indirect Haemagglutination titer		
	Chloroform treated (A-1) (n=5)	Concentrated/ Pelleted (B-1) (n=5)	Uninoculated control (C-1) (n=3)
Zero day	Nil	Nil	Nil
07	Nil	Nil	Nil
15	\log_2^7	\log_2^7	Nil
45	\log_2^6	\log_2^7	Nil
75	\log_2^6	\log_2^8	Nil
90	\log_2^6	\log_2^9	Nil

Table 4: Antibody titer in rabbits against live adjuvanted and killed oil based IBD virus during Phase II

Sampling day	Indirect Haemagglutination titer		
	Live djuvanted virus (A-2) (n=5)	Killed oil based vaccine (B-2) (n=5)	Uninoculated control (C-2) (n=2)
Zero day	Nil	Nil	Nil
15	\log_2^8	\log_2^6	Nil
30	\log_2^8	\log_2^6	Nil
45	\log_2^8	\log_2^6	Nil
60	\log_2^9	\log_2^8	Nil
75	\log_2^9	\log_2^8	Nil
90	\log_2^{10}	\log_2^8	Nil
105	\log_2^{10}	\log_2^9	Nil
120	\log_2^{10}	\log_2^{10}	Nil

In the first phase of this study, the comparison of chloroform treated and concentrated IBDV showed that the IHA titer against the concentrated virus was higher as compared to chloroform treated virus (Table 3). Hanley *et al.* (1995) have observed that too much or too little antigen may induce tolerance than the active immune response for the given antigen. Ideally, one follows the serum antibody titer in a hyper immunized animal and gives a booster injection of antigen only after the antibody titer has begun to decline. However, when an animal has responded less than maximally, which is more usual situation for small doses of antigen, booster dose of antigen given at 3 to 6 weeks after the first antigen dose will usually increase the serum antibody titer (Cooper *et al.*, 1991).

Antibody formation is enhanced by the use of different adjuvants. They are supposed to prolong the exposure of the antigen to immune system, protect it from degradation and stimulate the immune system (Jennings, 1995). Incomplete Freund's adjuvant (IFA) is a water-in-oil emulsion of mineral oil and surfactant and has been strongly advocated by Jurd and Hansen (1990). As the infectivity titer also plays an important role in raising hyperimmune serum, therefore, possibilities may be that live adjuvanted virus inoculum of group A2 was stronger than the IBD vaccine of group B2.

In phase II, 1:1 suspension of adjuvant with antigen in aqueous solution was prepared until a stable water-in-oil emulsion was achieved. Clinically it appeared that live adjuvanted and killed adjuvanted vaccines did not cause considerable irritation as no untoward reaction or granuloma was observed after injection. The IHA antibody titer was higher in rabbits inoculated with live adjuvanted virus compared to plain viruses of Phase I. The IHA antibody titer in killed oil based vaccinated group started to increase after 3rd to 4th inoculation. Kuby (1994) has reported that the immunogenicity of live attenuated vaccines increases by their transient growth. Killed vaccines, on the other hand, tend to require repeated booster to maintain the immune status of the host.

Our findings are also supported by Kaeberle (1986), who observed that antigen plus an adjuvant generally permitted the use of a much smaller quantity of antigen and greatly enhanced the antibody titer compared with the antigen without adjuvant. The IHA antibody titer in the rabbits of control groups of both

phases was found zero throughout the study. The antiserum collected after the last sample bleeding was heat inactivated in water bath at 56°C for 30 minutes and stored.

The overall results of this study suggested that concentrated IBDV adjuvanted with incomplete Freund's adjuvant provided best immune response in terms of highest antibody titers. This combination can be used for raising of hyperimmune serum in rabbits for the early and accurate diagnosis of IBD.

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