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NEUTRALISING ANTIBODY AND CHALLENGE RESPONSE TO LIVE AND INACTIVATED AVIAN ADENOVIRUS-1 IN BROILERS

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SUMMARY

Immune responses to live and inactivated avian adenovirus-1 were evaluated in broilers by neutralising antibody response and challenge reaction. The neutralising antibody titre was 1 : 256, 1 : 64 and 1 : 32 in live virus, inactivated virus and uninoculated control birds respectively at 3 weeks post inoculation when they were challenged. At one week post challenge the antibody titres were 1 : 941, 1 : 247 and 1 : 375 in live virus, inactivated virus and control birds, respectively. There was a booster effect of challenge in the live virus inoculated group up to 3 weeks post challenge. The post challenge histopathological evaluation of liver, kidneys, lungs and bursa revealed less severe changes in the live virus inoculated group as compared to other groups.

INTRODUCTION

Inclusion body hepatitis (IBH) of chickens is an important disease condition caused by avian adenovirus (Helmboldt and Frazier, 1963; Fadly and Winterfield, 1973). Several workers have suggested that the significance of avian adenoviruses in causing disease alone is doubtful except for the Australian strain of fowl adenovirus which can cause acute IBH alone (Erny *et al.*, 1991). In India IBH is caused predominantly by avian adenovirus-1 (AAV-1) in broilers (Grewal *et al.*, 1981; Nagal *et al.*, 1990; Singh *et al.*, 1996) and in Japanese quail (Singh *et al.*, 1995). AAV-1 has also been isolated from cases of IBH-hydropericardium syndrome in broiler chicks experiencing heavy mortality (Oberoi *et al.*, 1996). Keeping in view the heavy economic losses caused by AAV-1 infections in broilers by way of IBH and allied conditions, the present study was conducted to evaluate the immune and challenge response to live and inactivated AAV-1, as a preliminary trial to develop a suitable vaccine.

MATERIALS AND METHODS

Virus

The AAV-1 (PL-1 isolate) used in this study was isolated from field outbreaks of IBH in chicken embryo liver cell culture. The plaque purified virus when inoculated into experimental chicks by intraabdominal route produced moderate to severe lesions of IBH (Singh and Oberoi, 1994). The virus was titrated in chicken embryo liver cell culture before experimental inoculation in broiler chicks.

Inactivation of virus

The cell culture propagated virus was inactivated by 0·01% v/v formalin at 4°C for 24 hrs. The concentration of formalin was determined after conducting viability studies in 10 day old embryonated chicken eggs.

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TABLE I

Neutralising antibody titre of experimentally inoculated and challenged broilers

	Reciprocal of 50% neutralisation end points		
	Control group (I)	Inactivated virus group (II)	Live virus group (III)
Weeks post inoculation			
0	20	20	49
1	32	186	266
3[1]	32	64	256
Weeks post challenge			
1	375	247	941
2	124	160	944
3	32	160	971
4	30	123	473

[1]Day of challenge.

Birds

Day-old broiler chicks were purchased and housed in cages. The birds were given a broiler starter ration, tested to be free of mycotoxins (Singh *et al.*, 1996), and water *ad libitum*. All the birds were screened for the presence of antibodies to AAV (Woernle, 1966) and for excretion of AAV (Khanna *et al.*, 1992).

Experimental design

Broiler chicks which were free of antibodies and not excreting AAV were divided into 3 groups of 20 birds each.

Group I: birds were kept as uninoculated controls.
Group II: birds were inoculated at one week of age with formalin inactivated virus (10,000 $TCID_{50}$/0·2 ml) mixed with equal volume of Freund's incomplete adjuvant (Sigma chemicals), at a dose rate of 0·5 ml per chick, intramuscularly.
Group III: birds were inoculated at one week of age with live virus orally containing 1,000 $TCID_{50}$/0·2 ml at a dose rate of 1 ml per chick.

Monitoring of neutralising antibody (NA) response

Serum samples were taken at weekly intervals from one week post inoculation (WPI) to 7 WPI from birds of all groups. The NA response was tested in 10-day old embryonated chicken eggs. Two fold serial dilution of serum samples (1 : 10 to 1 : 1280) were tested against 100 EID_{50}/0·1 ml of virus.

Challenge test

All 20 birds from each group were subjected to challenge test with homologous virus (PL-1) at 4 weeks of age (3 WPI). Each bird received 0·5 ml virus containing 1,000,000 $TCID_{50}$/0·2 ml via the intraabdominal route. From each challenged group 5, 4, 4 and 2 birds were necropsied at 5th, 7th, 9th and 11th days post challenge (DPC) respectively, and observed for any gross pathological changes. Tissue pieces of liver, lungs, kidneys and bursa of Fabricius of each bird were collected separately in 10% buffered formalin and processed for paraffin embedding, sectioned at 4 to 5 μm thickness, and

TABLE II

The prevalence of gross lesions in broiler chicks in different groups

Organ	Group	Days post challenge			
		5	7	9	11
Liver	I	5/5[1]	4/4	4/4	1/2
	II	1/5	2/4	2/4	2/2
	III	1/5	2/4	1/4	2/2
Kidney	I	5/5	3/4	4/4	2/2
	II	3/5	2/4	4/4	2/2
	III	0/5	0/4	1/4	1/4
Lung	I	5/5	4/4	4/4	2/4
	II	0/5	0/4	0/4	1/2
	III	0/5	0/4	2/4	0/2
Bursa	I	2/5	2/4	2/4	0/2
	II	0/5	0/4	0/4	1/2
	III	0/5	0/4	0/4	0/2

[1]Number of birds with gross lesions/number of birds necropsied.

stained with haematoxylin and eosin. The remaining 5 birds in each group were continued for antibody monitoring up to 4 weeks post challenge (WPC).

RESULTS

Neutralising antibody response

The results of the virus neutralisation test are presented in terms of geometric mean titres in Table I. The NA titre of preinoculation serum from the 3 groups varied from 1:20 to 1:49. The NA titre of serum from birds of the live virus inoculated group remained higher than that of the control and inactivated virus group at 3 WPI when the birds were challenged with homologous virus.

Post challenge response

The NA titres at 1 WPC were 1:375, 1:247 and 1:941 in groups I, II and III, respectively. The challenge virus had the maximum booster effect in the live virus inoculated group where the NA titre continued to increase 3 WPC (Table I).

The prevalence of gross lesions in birds of all the groups is shown in Table II. Of all the organs examined at necropsy, the most frequent lesions in the uninoculated control birds (group I) were in the liver, kidneys, lungs and bursa of Fabricius. At 5 and 7 DPC, livers of all the necropsied birds showed slight enlargement along with petechial haemorrhages on the surface. At 9 DPC the extent of lesions was increased and at 11 DPC, in one bird the liver was found pale, enlarged and mottled whereas the liver was found normal in other birds. The kidneys were swollen, pale and nephrotic, and the lungs were congested in all birds. The bursa of Fabricius was atrophied in some birds at 5, 7 and 9 DPC, however, at 11 DPC no gross change was observed in the bursa. The gross changes in the liver and kidneys of birds of group II were similar to group I, however the severity of lesions and number of birds showing changes were less. Atrophy of the bursa was seen only in one bird at 11 DPC. In group III, the changes in the liver of birds were less severe than in group I and II and no gross change was seen in kidneys at 5 and 7 DPC, although changes were

TABLE III

Severity of histopathological lesions in the liver at different post challenge intervals

Days post challenge	Group	Granular degeneration	Vacuolar degeneration	Necrosis	Intranuclear inclusions
5	I	++(3)	+(1) ++(2)	++(1)	+(3)
	II	+(4)	+(2)	-	+(2)
	III	+(3)	+(2)	-	+(1)
7	I	+(3)	++(2) +++(2)	++(2)	++(3)
	II	+(1) ++(3)	+(3)	-	++(3)
	III	+(2) ++(1)	+(1)	-	+(1)
9	I	+(1) ++(1)	+(2) ++(1)	+(1) ++(2) +++(1)	++(2) +++(2)
	II	+(3) ++(1)	+(1) ++(2)	+(1)	++(2)
	III	+(3)	+(2)	++(1)	++(2)
11	I	-	+++(2)	+(1) ++(1)	+++(2)
	II	-	++(1)	++(1)	+++(2)
	III	+(2)	-	-	+(2)

+ = mild lesions; ++ = moderate lesions; + + + = severe lesions.
Figures in parenthesis indicate number of birds with lesions.
Liver from 5, 4, 4 & 2 birds were examined from each group at 5, 7, 9 & 11 DPC respectively.

comparable to group I and II at 9 and 11 DPC. Only 2 birds were showing mild congestion in the lungs at 9 DPC. No change was seen in the bursa of Fabricius.

The most common histopathological changes observed in the liver were varying degrees of degenerative changes, congestion and haemorrhages and the presence of basophilic intranuclear inclusion bodies (INIBs) in hepatocytes and necrosis. These histopathological changes in the liver are summarised in Table III.

Histopathological changes in the kidneys include varying degrees of congestion and haemorrhages. At 7, 9 and 11 DPC, severe degenerative changes along with coagulative necrosis were observed in the epithelium of the renal tubules of the birds of group I. Changes in the kidneys in group II were similar to those in group I. In group III, there was mild congestion and haemorrhages at 5 and 7 DPC, however, at 9 and 11 DPC the changes were comparable to group I and II but were less severe.

In group I, congestion and oedema was observed in the lungs at 5 DPC. Alveolar septae were thickened and oedematous, and there was infiltration of mononuclear cells. At 7, 9 and 11 DPC, the severity of lesions was greater and accompanied with necrotising bronchitis. In some birds prominent changes were seen in the blood vessels of lungs. The intimal cells were swollen, rounded, vacuolated and projected in the lumen. Adventitia was also thick and oedematous. In group II and III, birds were showing only mild congestion and haemorrhages at 11 DPC.

In group I, mild rarefaction of lymphoid cells was seen in bursa of Fabricius. At 7 and 9 DPC, proliferation of interfollicular connective tissue was also seen. However, at 11 DPC, no change was observed. In group II and III, no change was observed in the bursa throughout the observation period.

DISCUSSION

The neutralising antibodies in group II birds appeared at 1 WPI and rapidly decreased by 3 WPI. The NA response in group III birds appeared at high titre at 1 WPI; however, it marginally decreased at 3 WPI as compared to group II birds. The appearance of NA at 1 WPI was corroborated with the findings of Grimes *et al.* (1977); Maiti (1983); Lal *et al.* (1992). Grimes *et al.* (1977) however, recorded peak titres at 2 WPI following live fowl adenovirus infection as compared to a marginal decrease in the present study. Nevertheless the NA response in the present study was much higher in the live virus inoculated birds (group III) than that recorded in the inactivated virus inoculated group of birds (group II). An attempt was made further to corroborate the *in vitro* and *in vivo* immune response by challenge. The intra-abdominal route for challenge was selected because most of the experimental studies with reproduction of IBH in broilers had been done by this route (Gallina *et al.*, 1973; Wells and Harrigan, 1974; Grimes *et al.*, 1978). The challenge infection, however, had a booster effect on humoral immune response of all the 3 groups as measured by virus neutralisation test. The NA titres in challenged birds of group I and II reached the highest level at 1 WPC and thereafter declined. However, in group III challenged birds the NA level increased to 1 : 941 at 1 WPC and reached a peak of 1 : 971 at 3 WPC. At the fourth WPC the antibodies were still at a high level. These results of a booster effect post challenge were in agreement with those of Yates *et al.*, (1977) for CELO virus and Khalaf (1981) and Holmes *et al.*, (1989) for EDS-76 virus.

The gross and histopathological lesions observed in various organs in group I were similar to those earlier reported in literature (McFerran *et al.*, 1976; Itakura *et al.*, 1974; Grewal *et al.*, 1981; Singh and Oberoi, 1994). On the basis of severity of lesions in various organs in different groups, and appearance and intensity of INIBs it was concluded that the live virus inoculated birds (group III) resisted the challenge to a greater degree than the inactivated virus inoculated birds.

The overall assessment was that the immune response elicited and the resistance to challenge infection was more in the case of live virus inoculated birds than in the birds inoculated with inactivated adjuvanated virus. Though the inactivated adjuvanated vaccine inoculation at one week of age was far from ideal in the commercial situation, it clearly demonstrated the superior immunogenic response of live virus as a candidate for vaccine development against IBH caused by AAV-1.

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REPONSES IMMUNOLOGIQUES CHEZ DES POULETS APRES CHALLENGE ET ANTICORPS NEUTRALISANTS EN UTILISANT DES ADENOVIRUS-1 AVIAIRES INACTIVES OU VIVANTS

Résumé—Les réponses immunologiques utilisant des adenovirus aviaires inactivés ou vivants furent évaluées chez des poulets après challenge et utilisation d'anticorps neutralisants. Le titre d'anticorps neutralisants fut respectivement de 1 : 256, 1 : 64 et 1 : 32 pour les oiseaux inoculés avec des virus vivants, des virus inactivés et sans inoculation et ceci 3 semaines inoculation au moment du challenge: une semaine après le challenge le titre fut de 1 : 941, 1 : 247 et 1 : 375 pour les même groupes. Une stimulation immunologique fut observée, 3 semaines après le challenge, pour le groupe inoculé avec des virus vivants. Une étude histopathologique effectuée après le challenge sur le foie et les reins montrèrent des changements moindres chez le groupe inoculé avec des virus vivants que chez les 2 autres groupes.

ANTICUERPOS NEUTRALIZANTES Y REACCION A LA INOCULACION DEL ADENOVIRUS-1 AVIAR ACTIVO E INACTIVADO EN BROILERS

Resumen—Se evaluaron las respuestas inmunológicas del adenovirus-1 aviar activo e inactivado en broilers con anticuerpos neutralizantes y reacción a la inoculación. El título de anticuerpos neutralizante fue de 1 : 256, 1 : 64 y 1 : 32 en virus activos, virus inactivados y aves control no inoculadas respectivamente a las 3 semanas después de la inoculación, cuando fueron estimulados. A la primera semana después de la estimulación el título de anticuerpos fue de 1 : 941, 1 : 247 y 1 : 375 en virus activos, virus inactivados y broilers control, respectivamente. Hubo un efecto réfuerzo de activación en el grupo de aves inoculadas con el virus activo a partir de la 3 semana después de la inoculación. La evaluación histopatológica después de la inoculación en hígado, riñones, pulmones y bolsa reveló cambios menos graves en el grupo inoculado con virus activos que en los otros grupos comparados.