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PREPARATION OF GUMBORO DISEASE VACCINE FROM INTERMEDIATE STRAIN AND ITS FIELD APPLICATION PRIVATE

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SUMMARY

A new live infectious bursal disease (IBDV) vaccine (Intermediate strain) was prepared, titrated and tested experimentally and in the field. Different vaccinal programs were tried and the immune response was evaluated. The results obtained from both experiment and field application proved that the prepared new vaccine has a good protection against virulent IBDV either in case of high maternally immune chicks or low maternally immune chicks.

INTRODUCTION

Viral immunosuppressive infections considered one of the important problems facing the economics of poultry industries. The most serious cause of this immunosuppressive infections is the IBDV where it causes suppression of the antibody response to other viral and bacterial disease as Newcastle, Marek's (Cho, 1970), laryngotracheitis (Rosenberger and Gelb, 1978). The effect of IBD on cell-mediated immunity responses is transient and less obvious than that on the humoral responses. Sivanandan and Maheswaran (1989) observed suppression of cell mediated immunity responsiveness using the lymphocyte blasttransformation assay.

Immunization of chickens still the principal method used for control of IBD in chicken especially immunization of the breeder flocks to confer parental immunity to their progeny and protect the chicks from early immunosuppressive infections.

Different choices of live vaccines are available according to its degree of virulence as mild, mild intermediate and intermediate. The present study try to develop a new live vaccine from an intermediate IBD strain and its evaluation experimen-

tally and in the field.

MATERIAL AND METHODS

1. Virus strains:

i. Vaccinal strains:

a. Intermediate strain of IBDV:

A standard vaccinal IBD strain identified as (intermediate) was supplied by Dr. Salwa El-Assely from Arkansas University, USA.

b. Bursavac M strain of IBDV and D78 strain virus as an attenuated strains:

It was supplied by the College of Agricultural Science, Delwar University, USA.

c. IBDV inactivated vaccine:

A locally prepared combined multiple pathotype inactivated IBDV vaccine, Veterinary Serum and Vaccine Research Insitute, Abbasia, Cairo, Batch No. 112004.

ii. Challenge virus of IBDV:

IBDV bursal homogenate isolated locally from El-Fayoum Governorate, Egypt (1997) with a titre of 107 EID50/ml (Nadia, 2001).

2. Embryonated chicken eggs:

9-11 days old embryonated chickens (Specific Pathogen Free, Egg Production Farm, Nile SPF eggs, Koum Oshiem, Fayoum, Egypt) were used for propagation, preparation and titration of the prepared batches of IBDV strains.

3. Experimental chicks:

One hundred and fifty, 1-day-old, Hubbard chicks (Ministry of Agriculture) were reared under complete hygienic measures in isolated and disinfected wire floored cages and fed on commercial broiler ration. These chicks were divided into 5 equal groups for the experimental part of the study.

4. Cell cultures:

African green monkey kidney cells (Vero) established by Yasumura and Kawatika (1963) were used in SNT to estimate IBDV neutralizing antibodies in the sera of vaccinated chickens.

5. Virus propagation:

IBDV was propagated after the method of Hitchner (1970). The obtained virus was propagated for 2 passages and titrated in embryonated chicken eggs via allantoic sac inoculation and the titre was expressed and calculated according to method of Reed and Muench (1938) and reported to be 10^7 EID_{50}/ml for intermediate prepared vaccine, $10^{7.9}$/ml for Bursavac and 107.5/ml for D78.

6. Quality control:

The experimentally prepared vaccine was subjected to quality control measures as described by Stone et al. (1979).

7. Serological tests:

a. Serum neutralization test (SNT):

It was carried out estimating the neutralizing anti-

bodies against IBDV according to the method of Villegas (1990).

8. Bursal body weight ratios:

Random five chicken were chosen from each group at intervals of 6, 10 and 14 days post primary vaccination, slaughtered for determination of bursal/body weight ratio according to Tsai and Saif (1992).

9. Bursal histopathology:

Five birds from each group were scarified, bursae were taken and examined histopathologically to evaluate the degree of bursal infection. The relative degree of severity of bursal changes was noted microscopically for each individual bursae. Diffuse follicular necrosis and atrophy, lymphoid depletion, cellular infiltration, infolding of the overlaying epithelium, and interstitial edema and hemorrhage all were scored using a scale of 0-5 to indicate the relative degree of severity. A score of 0 indicated unmarkable lesions, and scores of 1-5 corresponded to minimal, slight/mild, moderate, moderately severe and severe/high lesions, respectively (Sharma et al., 1989).

10. Challenge:

Five chickens from each groups were inoculated intraoccularly by 10^3 EID_{50}/dose of virulent IBDV 30 days post vaccination. The chickens were observed for 15 days for any clinical symptoms or deaths.

Experimental Design:

Chicks were divided into 5 groups 30 for each as follows:

First group:

It was vaccinated with the experimentally prepared IBD vaccine (intermediate strain) at 16 days old and followed by inactivated IBDV vaccine at 22 day old.

Second group:

It was vaccinated with mild strain of IBDV (Bursavac strain) at 16 day old followed by the intermediate prepared vaccine at 22 day old.

Third group:

It was vaccinated with prepared intermediate strain of IBDV only at 16 day old.

Forth group:

It was vaccinated with mild strain of IBDV (Bursavac strain) at 16 day old followed by intermediate strain of IBDV D78 at 22 day old.

Fifth group:

It was left as non-vaccinated control group.

Each chicken in the vaccinated groups received intraocularly 10^3 EID_{50}/dose of live vaccine according to its group and 1/2 ml I/M for inactivat-

ed IBDV vaccine group. Ten random blood samples were collected weekly from each group for 12 weeks post vaccination. Sera were separated, collected and stored at -20°C for detection of corresponding antibodies against IBDV.

Field application of the prepared intermediate strain of IBDV:

A total number of 26,000 one day old chicks were reared in 3 different farms in Wadi El-Natroon were vaccinated with the prepared vaccine as recorded in table (4). All flocks were evaluated for immunological response of IBDV with SNT, clinical signs, death and post mortem for bursal changes were recorded.

RESULTS AND DISCUSSION

As most viral diseases, vaccination is the sole option to prevent infectious bursal disease in chickens. Several factors influence the design of vaccination programmes to protect against IBDV. The level of maternal antibody against IBDV in newly hatched chicks determines suitable time for vaccination with live IBDV vaccine. Chicks which hatch with high IBDV maternal antibody titres will be refractory to all live IBDV vaccines (Skeeles et al., 1979, Lucio and Hitchner, 1979). Therefore, the level of breeder IBDV antibody titres can be the ultimate factor which determines the clinical manifestation of IBDV infections in the progeny.

Field application of the newly prepared vaccine was carried out on both chickens with high and low maternal antibody level. Meanwhile, the experimental part of this study was carried out on low maternally immune chicks.

Group (1) was vaccinated with the prepared vaccine and followed by inactivated IBDV, group (2) was vaccinated with Bursavac vaccine followed by prepared vaccine, group (3) was vaccinated with prepared vaccine only and all these groups were compared with one of the vaccinal program used in field (Bursavac followed by D78).

Table (1) illustrated the result of bursa/body weight indices for vaccinated and challenged birds which revealed that all groups showing approximately the same body weight ratio at 6, 10 and 14th day post vaccination and slightly increased than the control group. Regarding for bursal/body weight indices of all vaccinated group with different vaccinal programs and after challenge were shown in the table (1). All bursae collected from all vaccinated and challenged birds had B/B weight index higher than 0.7 indicating that they were normal bursae and did not affect by vaccination with the prepared vaccine compared with the non-vaccinated group after challenge. The results agreed with Lucio and Hitchner (1979).

The results of immunological response of vaccinated and challenged chicks tested by serum neutralization test (SNT) at different weeks post vaccination and one week post challenge were shown in table (2). It was noticed that all vaccinated groups gave good immune responses which appeared in high titre of SNT till the end of experiment. These results agree with Lukert and Saif (1991) who reported that intermediate viruses overcome VN titres of 1:250. Regarding to the SNT of challenge birds after one week post challenge and 4 weeks post vaccination in the same table show that the titre decreased after challenge test for all groups due to some neutralization of challenge virus antibodies.

Challenge test for five birds from each vaccinated and non-vaccinated groups revealed that all control birds showing sever symptoms typical for IBDV infection, but the vaccinated groups showed no symptoms and no mortalities.

Histopathological examination:

The histopathological examination during the experiment revealed that all vaccinated groups before and after challenge were with normal size and histological appearance, while the first and third groups were apparently normal although there was some inter-follicular edema degeneration in the center of some follicles and centrofollicular replacement of some lymphoid follicles (Table 3 and Fig. 1 and 3).

Groups (2 and 4) bursa of Fabricius of chick showed follicles formed from cortex and medullary zones, mild hyperplastic (activated) lymphoid follicles with reduction in the inter-follicular space (Table 3 and Fig. 2 and 4). The results agreed with Mazariegos et al. (1990) who proved that mild IBDV vaccines cause little or no detectable pathology, whereas intermediate vaccines cause measurable bursal atrophy clinical reaction.

Group (5) control (Fig. 5) chicks of control group of experiment which experimentally infected with IBDV showed that the medullary area in some bursal follicles revealed depletion or degeneration and necrosis represented by homogenous eosinophilic masses with pyknotic nuclei. Other follicles revealed cystic cavities in the medullary zones and fibroblastic proliferation in the inter-follicular tissue. The covering epithelia showed hyperplasia and vesicle formation.

According to the field application of the intermediate strain of IBD in farm (I) it was found sever reaction of chicken after one week from vaccination in form of morbidity and mortality which reach about 30% with typical lesion of bursa of Fabricius this may be due to absence of maternal antibodies. On the other hand, farm II gave good protection with a mild reaction reach (0.6%) the general health condition of the flock is very good allover the period of rearing. These results refer to primary vaccination with Bursavac strain of

IBDV which initiate the immune response and protect the birds.

Referring to the farm III which gave a moderate reaction after vaccination reach 2% inspite of presence of maternal antibody. This condition may be due to the reaction of hotness of the strain and irregular individual variation of maternal antibodies. This flock show a healthy condition allover the period of breeding and weight ranges 1600-2200 kg with mean 1900 kg.

Field evaluation of intermediate strain of IBDV vaccine could show improved performance in terms of better production data, less problems with IBDV-related to secondary infections and good response to live vaccines applied in the progeny.

Conclusion

Intermediate strain of IBDV can be used for vaccination of broilers and layers as booster dose after vaccination with mild strain of IBDV for chicks with moderate and high levels of maternal antibody which interfered considerably with the immunogenicity of these vaccine.

Table (1): Bursal/Body (B/B) weight indices in vaccinated and challenged birds

Group	Type of vaccination	Days Post Vaccination						7 days post challenge	
		6		10		14			
		B/B ratio*	B/B index**	B/B ratio*	B/B index**	B/B ratio*	B/B index**	B/B ratio*	B/B index**
1	Intermediate IBDV	0.0015	1.783	0.0022	1.582	0.0022	3.1445	0.000609	0.614
	then inactivated IBDV	0.00108	1.273	0.00224	1.611	0.00224	0.9153	0.00104	1.052
2	Bursavac than	0.001	1.169	0.0044	3.165	0.0044	2.198	0.00169	1.704
	intrmediate IBDV	0.001	1.169	0.0048	3.509	0.0048	2.170	0.000346	0.348
3	Intermediate IBDV only	0.0015	1.783	0.00297	2.142	0.00297	2.052	0.00283	2.853
		0.00158	1.855	0.0043	3.117	0.0043	1.872	0.00224	2.25
4	Bursavac then	0.0013	1.520	0.0032	2.308	0.0032	2.629	0.00178	1.801
	D78 IBDV	0.0012	1.403	0.0033	2.379	0.0033	1.8100	0.00477	4.818
	Control	0.0011		0.0010		0.0010		0.00134	
5		0.00061		0.00179		0.00179		0.000645	
	Mean	0.000855		0.00139		0.00166		0.000992	

* Bursal / body weight ratio = Bursal weigh / Body weight

** Bursa / body weight index = Bursal/body weight ration of vaccinated birds / Bursal/body weight ration of control birds

Table (2): The mean titre of neutralizing antibodies against IBDV of different vaccinated chicken groups

Group	Weeks Post Vaccination								One week post challenge
	1	2	3	4	6	8	10	12	
1	8	24	48	256	256	380	512	256	96
2	4	16	48	128	128	128	512	768	48
3	16	24	48	128	128	320	512	256	32
4	6	32	96	128	256	256	256	128	16
Control	10	8	8	8	4	2	0	0	32

* Antibody titre = The reciprocal of serum dilution which neutralized and inhibited the CPE of 100-200 $TCID_{50}$ of the virus.

Table (3): Histopathological incidence and bursal lesion scores of different groups

Group	Days post vaccination	Bursal lesion						Bursal lesion scores
		Diffuse follicle		Lymphoid depletion		Infiltration		
		Atrophy	Necrosis	Cortical	Medullary	Macrophag	Heterophils	
1	6	-	+	+	+	+	+	4
	10	+	-	-	-	-	-	4
	14	+	-	+	+	+	+	5
2	6	-	-	-	-	-	-	2
	10	+	-	+	+	-	-	4
	14	+	+	+	+	+	+	5
3	6	+	+	+	-	+	+	5
	10	+	-	+	+	+	+	4
	14	+	-	+	+	+	+	4
4	6	-	-	+	-	-	-	2
	10	+	+	+	+	+	+	5
	14	-	-	-	-	+	-	2
5	6	-	-	-	-	-	-	0
	10	+	-	-	-	-	-	0
	14	+	-	-	-	-	-	0

Table (4): Results of field application in three differnet farms

No. of farm	Type of bird	Total number	Prevace. Antibody	Days Post Vaccination						Mortality		PM lesion	SNT
				1	6	12	14	15	18	28	Age/day	%	
I	Balady layer	5000	0	IB spray	HBI drinking	-	-	IBDV intermediate strain	LaSota		21-30	30%	Typical lesion of IBDV in bursa (enlarged-congested)	ND*
II	Balady layer	5000	0	IB	HBI	IBDV Bursavac strain		IBDV intermediate strain	LaSota		16-25	0.6%		42.66**
III	High line broiler	16000	32**	IB	HBI		IBDV intermediate strain		LaSota-IB	LaSota	41-21	2%	Enlarged bursa	48

* ND: Not Done

** Mean of serum neutralizing antibody titre= mean of the reciprocal of serum dilution which neutralize and inhibit the CPE of 100 $TCID_{50}$ of IBDV.

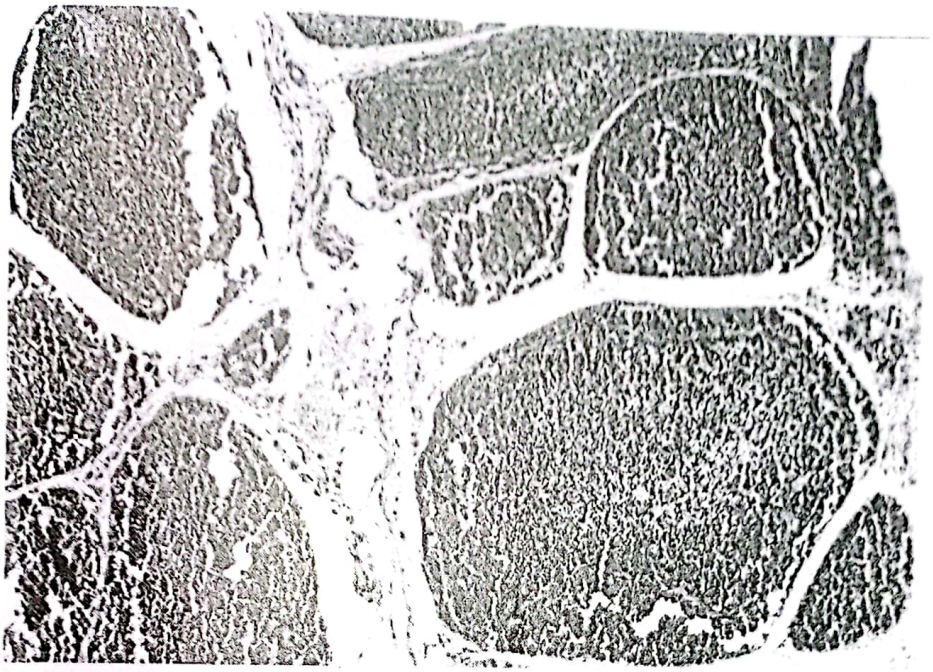

Fig. (1): Group (1) vaccinated with the prepared intermediate IBDV vaccine and followed by inactivated IBDV vaccine
(10 day old after vaccination) (H & E x100)

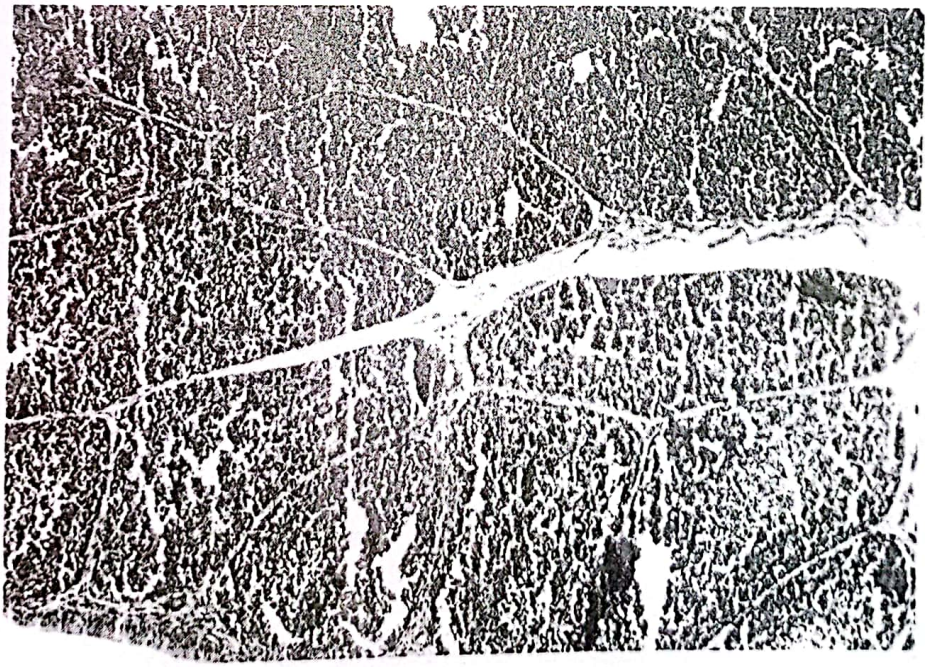

Fig. (2): Group (2) vaccinated with mild strain of IBDV (Bursavac strain) followed by the intermediate prepared IBDV vaccine
(10 day old after vaccination) (H & E x100).

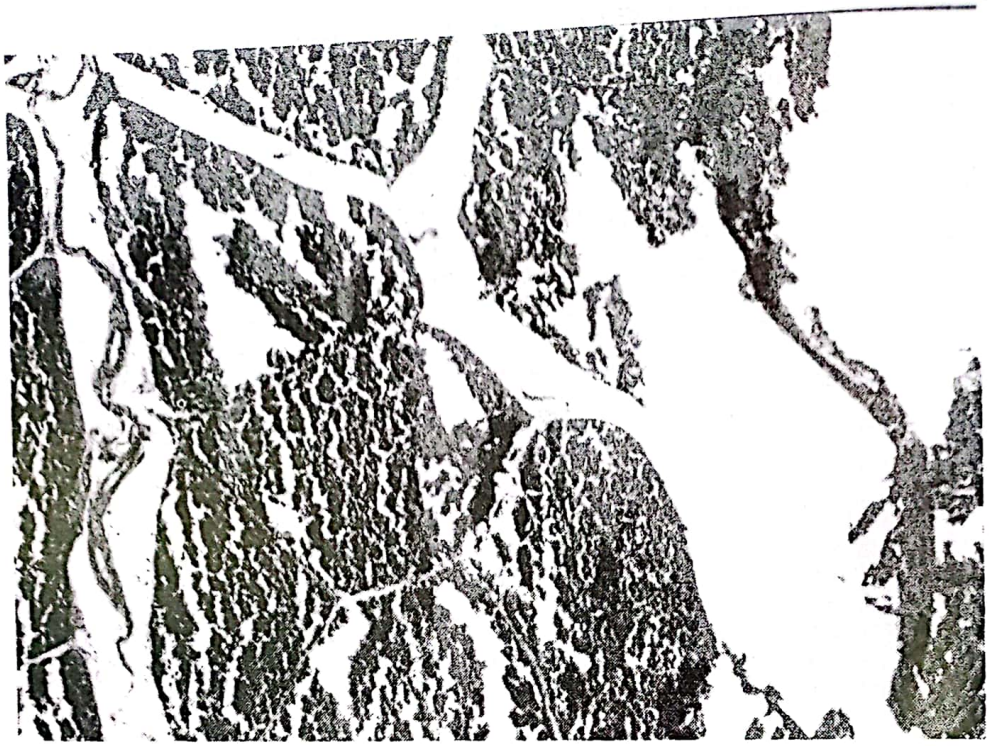

Fig. (3): Group (3) vaccinated with prepared intermediate strain of IBDV only

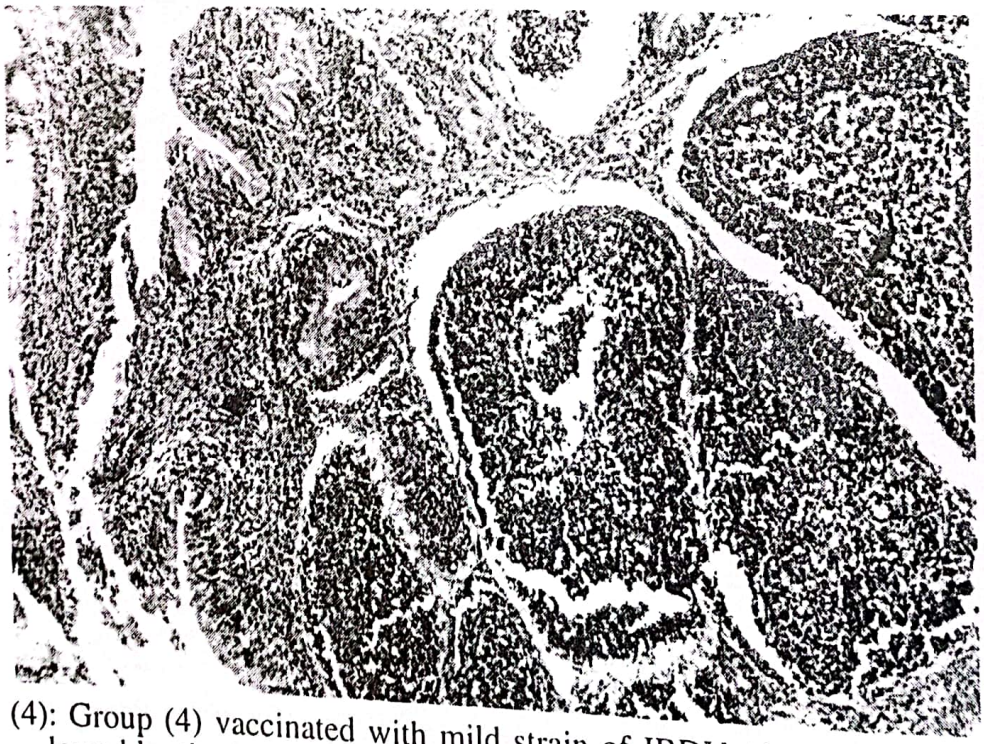

Fig. (4): Group (4) vaccinated with mild strain of IBDV (Bursavac strain) followed by the intermediate strain of IBDVD78 (10 day old after vaccination) (H & E x100).

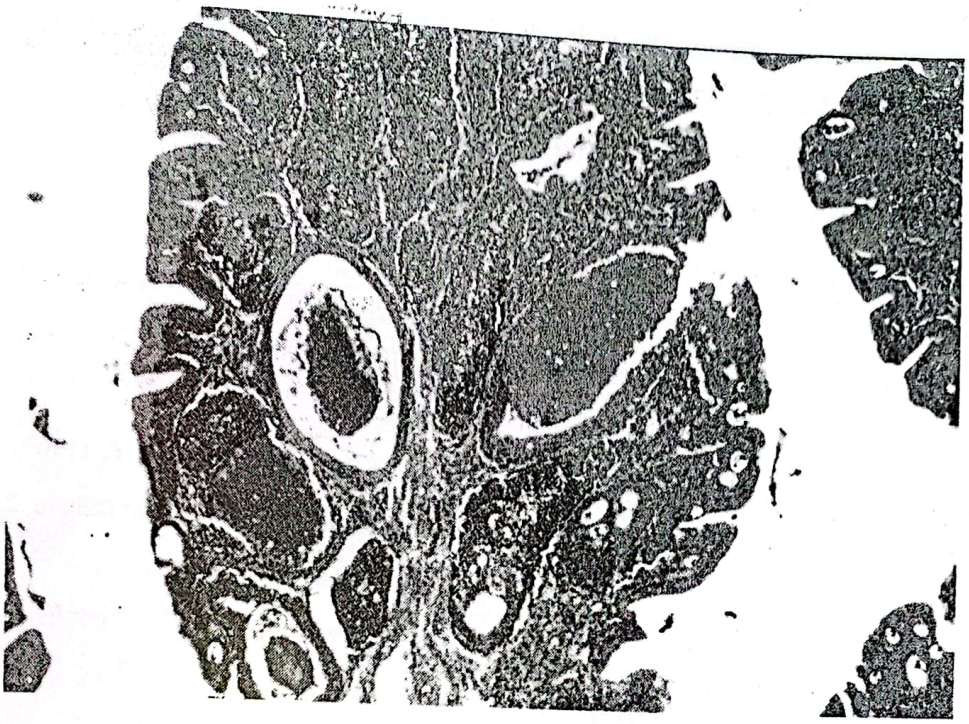

Fig. (5): Group (5) control non-vaccinated after challenge (H & E x 100).

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