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COMPARATIVE STUDY ON TWO COMMERCIAL HOT VACCINAL STRAINS OF IBDV

By

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ABSTRACT

The comparative effect of two hot vaccinal strains of IBDV was conducted by vaccination of chicks with various minimal dose requirements of two commercially available hot IBD vaccine (IBD-Blen and 228E vaccine). The three different titres were $10^{2.0}$ EID_{50}/dose, $10^{2.5}$ EID_{50}/dose and $10^{3.5}$ EID_{50}/dose. The immune response was determined by challenging birds with a known virulent IBD virus and by measuring of ELISA antibody titre against IBD virus following vaccination. Other parameters were also considered such as protection rate, bursal index and the immune suppressive effect of IBD strains was measured using NDV vaccine. The high titre dose of 3.5 log_{10} Nobilis 228E and IBD-Blen vaccines could protect 100% of chickens, the lower titre dose 2 log_{10} is still protective against virulent challenge IBD virus causing minimal bursal lesion.

INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious viral infection of chickens that causes lymphoid degeneration of the bursa of Fabricius (BF) and suppression of humoral immune response (**Ivanyi and Morris, 1976 and Allan *et al.*, 1984**). Infectious bursal disease infection at early stage (3-6 weeks old) is known to interfere with the bird's immunity later in life (**Hirai *et al.*, 1974 and Giambrone *et al.*, 1977**). During last years, it is generally accepted by many investigators allover the world, that the bursa of Fabricius plays a significant role in the immunity of chickens. Since IBD is now considered a wide spread infection of commercial chickens with high morbidity rate up to 100% and mortality rate of 25% or more in some instances, moreover birds that survive the infection suffer a lot from reduced immune response to subsequent vaccination making the birds vulnerable for the attack by other diseases (**Faragher *et al.*, 1974**). Control of the disease through management and

sanitation are not adequate to control IBD (**Van den Berg and Meulemans, 1991**). So, the vaccination is considered the principle method used for the control of IBD in chickens. Ideally, an IBD vaccine should elicit a prompt and long lasting protective antibody response against virulent field strains, with lake of injury to immune system. So, the present investigation is planned to evaluate two different commercial hot vaccinal strains for control of IBD by giving satisfactory protection as well as the immune response against Newcastle disease vaccination.

MATERIAL AND METHODS

Material:

1. Chickens:

540 commercial Hubbard broiler chicks were obtained from United Company for Poultry Production (UCPP) as one day old. All the birds were reared in isolated room and provided with a commercial starter ration until used in the experiment.

2. Embryonated chicken eggs:

SPF eggs were obtained from SPAFAS, USA, as zero day old and kept in the incubator at 37°C till 9-11 days of age. They were used in titration of viral strains of NDV and IBD strains and antisera.

3. Viruses:

Virulent strain:

a. Virulent strain of NDV:

A velogenic viscerotropic NDV local strain was isolated and identified by **Sheble and Reda (1976)**. its titre was $10^{8.1}$ EID_{50}/ml.

b. Virulent Gumboro disease virus strain:

It was kindly supplied by **Dr. Afaf H.A. (1990)**. Its titre was 10^{8} EID_{50}/ml.

2. Vaccinal strain:

a. BioLaSota:

Lentogenic strain against Newcastle disease (ISBI) Batch No. (989305). Its titre was $10^{9.7}$ EID_{50}/ml. It was used in vaccination of experimental chickens at 21 days old through eye drop route with a dose of 10^{6} EID_{50}/ml.

b. Gumboro disease vaccines:

Two commercial hot vaccines against Gumboro disease were used:

i. Nobilis strain 228E:

Live vaccine against Gumboro disease (Intervet International B.V., Boxmeer, Holland), Batch No. (90766A). Its titre was $10^{7.2}$ EID_{50}/ml. It was used in vaccination of experimental chicks at 14 days old through eye drop with a dose of $10^{2.0}$, $10^{2.5}$ and $10^{3.5}$ EID_{50}/dose.

ii. IBD-Blen:

Live vaccine against Gumboro disease (Sanofi Animal Health), Batch No. (2305H1U). Its titre was $10^{6.8}$ EID_{50}/ml. It was used in vaccination of experimental chicks at 14 days old through eye drop with a dose of $10^{2.0}$, $10^{2.5}$ and $10^{3.5}$ EID_{50}/dose.

4. Serum samples:

Blood samples were collected from all chickens pre-vaccination as well as at the time of 7 and 14 days post vaccination. Each serum sample was separated, inactivated at 56°C for 30 minutes and examined for measuring antibodies against IBD using ELISA test and against ND using HI test.

Methods:

Haemagglutination Inhibition (HI) test:

It was done according to **Majujabe and Hitchner, (1977).**

Enzyme linked immunosorbent assay (ELISA):

It was performed according to **Snyder *et al.*, (1986).**

Bursal Index:

It was carried out according to **Lucio and Hitchner, (1979).**

The Bursa:Body Weight (B.B.) index was calculated by the following formula:

$$\text{B.B. Index: } \frac{\text{B.B. ratio}}{\text{Mean of B.B. ratio of uninfected control groups}}$$

Chicken with an index lower than 0.7 was considered to have bursal atrophy.

Histopathological examination:

It was carried out according to **Nakamura *et al.*, (1990).**

Experimental Design:

540, one day old, commercial broiler chicks were used. They were subjected weekly for measurement of maternal antibodies against Gumboro disease using ELISA till the time of vaccination. The chickens were divided into four groups:

Trials were made to vaccinate the chickens using two hot commercial IBD vaccines (IBD-Blen and 228E) using three different titres per dose of vaccination ($10^{2.0}$, $10^{2.5}$ and $10^{3.5}$ EID_{50}/dose) and compare between:

1. The effect of IBD vaccinal titre and immunity gained by chicken as determined by ELISA titre and protection percentage against challenge with virulent IBD virus.
2. The effect of Gumboro vaccination with three different titres on the immune response to NDV.
3. The effect of vaccination using three different titres on bursa of Fabricius.

540 chicks used in these trials were used. They were divided into 4 groups:

Group (1):

Contained 210 birds and were divided into 6 subgroups each group contained 35 birds.

The birds in each subgroup received either $10^{2.0}$ EID_{50}/dose, $10^{2.5}$ EID_{50}/dose or $10^{3.5}$ EID_{50}/dose of IBD Blen and 228E IBD virus strain. The birds were vaccinated at 14 days old via eye dropings.

Five days post vaccination, 5 birds from each subgroup were sacrificed and the bursae were weighed for calculation of Bursa:Body Weight ratio and subjected to histopathological examination. On the 7 and 14 days post vaccination, blood samples were collected for serum separation and examination for Gumboro disease antibodies using ELISA. On the 21st day post vaccination with IBD vaccine, all vaccinated chickens and the control were challenged using virulent IBD virus via conjunctival route. Each bird received $10^{2.0}$ EID_{50}/bird.

Group (II):

Contained 120 chickens and was divided into 6 subgroups, each contained 20 birds. The birds in each subgroup were vaccinated with 10 field dose of either $10^{2.0}$ EID_{50}/dose, $10^{2.5}$ EID_{50}/dose or $10^{3.5}$ EID_{50}/dose of both IBD Blen and 228E virus strain vaccine. After 5 days post vaccination, birds sacrificed and bursae were subjected to histopathological examination..

Group (III):

Contained 120 chicks, 14 days old, were divided into 4 subgroups each contained 20 birds using for immunosuppression tests with IBD strain. The four groups were vaccinated with one field dose via the recommended route of vaccination with different virus titre of two hot IBD vaccines ($10^{2.0}$ EID_{50}/dose, $10^{2.5}$ EID_{50}/ml and $10^{3.5}$ EID_{50}/dose, individually). Seven days post vaccination with IBD vaccine, all the birds were vaccinated with LaSota strain NDV via drinking water route. Each bird received 10^{6} EID_{50}/dose. At 7 and 14 days post vaccination with NDV vaccine, serum samples were collected from all chickens for measuring of antibody titre against NDV vaccine using HI test and all groups were challenged with virulent (VVNDV) 14 days post vaccination and the protection rates were determined in each subgroup.

Group (IV):

Contained 90 birds and used as positive and negative controls.

RESULTS AND DISCUSSION

Despite advance in vaccine development and antibody determination, infectious bursal disease virus (IBDV) incidence and associated disease problems still occur. The virus infected chickens become immunodepressed and they fail to respond to routinely used poultry vaccines (**Sharma, 1986**). Since the recording of the disease a lot attempts have being tried to produce safe, effective vaccines, which are now available in the market in the form of mild,

intermediate or hot live vaccines. The efficiency of these vaccines for the control of IBD and the obtained immunity following vaccination of chickens were also studied (**Giambrone and Closser, 1990**). Signs of immunosuppression caused by IBDV infection include the inability to respond to vaccine with adequate antibodies (**Allan *et al.*, 1972**). Newcastle disease vaccines are most suitable as an indicator in experimental studies to measure immunosuppression, as has been shown by **Faragher *et al.*, (1974)**. The present study was designed to vaccinate chicks with various minimum requirement per dose of two commercial hot IBD virus strain (IBD Blen and 228E virus strain). The three different titres were $10^{2.0}$ EID_{50}/dose, $10^{2.5}$ EID_{50}/dose and $10^{3.5}$ EID_{50}/ dose.

The immune response was determined by challenging birds with a known virulent IBD virus and by measuring ELISA antibody titres against IBD virus following vaccination. Besides, determining the protection rate and bursa body weight (B.B) index and the immuno-suppressive effect of IBD strains was measured using NDV vaccine as an indicator in this experimental studies.

As shown in Table (1), the geometric mean ELISA titres at 7 days post vaccination were 412, 490 and 590 by Nobilis 228E by dose of 2 log_{10}, 2.5 log_{10} and 3.5 log_{10}, respectively. While, the IBD-Blen vaccine induced 518, 611 and 687 ELISA antibody titre at doses of $10^{2.0}$, $10^{2.5}$ and $10^{3.5}$, respectively. The immune responses were increased after 14 days post vaccination to 6160, 7020 and 9942 ELISA antibody titre by Nobilis 228E at doses of $10^{2.0}$, $10^{2.5}$ and $10^{3.5}$, respectively. Similar results were recorded by **Naqi *et al.*, (1983) and Solano *et al.*. (1986)**. While the IBD-Blen elicited higher titres than 228E where it gave 7880, 8622 and 11400 at doses of $10^{2.0}$, $10^{2.5}$ and $10^{3.5}$, respectively. The protection rates as shown in Table (2) were 100% in different dose titres except 2.0 log_{10} of Nobilis 228E, while the unvaccinated challenged control showed 90% mortality. Our result was in agreement with that of **Giambrone and Clay, (1986)**.

The histopathogical study of the two vaccines as indicated in Table (3) revealed that the higher dose titre of IBD-Blen ($10^{3.5}$ EID_{50}) induced in moderate lymphoid depletion and follicular atrophy, while Nobilis 228E vaccine at $10^{3.5}$ EID_{50} dose induced marked bursal lesion in comparison with the titre dose $10^{2.0}$ EID_{50} which was mild (around 2). The unvaccinated control revealed extensive lymphocyte depletion and atrophied follicle. It was clarified that low titre dose had lower pathogenicity than that of higher ones ($10^{3.5}$). These results agreed with that obtained by **Muller (1986); Mazariegos *et al.*, (1990) and Nieper and Muller, (1994)**.

As shown in Photos (1-6), the histopathological study revealed that the chickens vaccinated with either IBD-Blen vaccine or 228E vaccine ($10^{2.0}$ EID_{50}) showed slight lymphocytic depletion and interlobular oedema, while in case of ($10^{2.5}$ EID_{50}), they showed lymphocytic depletion, stromal fibroplasia between follicles and presence of macrophages. But, in case of ($10^{3.5}$ EID_{50}), the

picture was more severe representing in lymphoid follicles necrosis, appearance of reticular mass and focal areas of hyperplastic proliferation. The aformentioned results are in agreement with those obtained by **Winterfield *et al.*, (1972) and Ayoub *et al.*, (1982)** who found that the histopathological examination of bursa revealed a varying degree of changes according to the attenuation degree of the different vaccine strains of IBDV. Also, **Amal, (1999)** found that the hot strain of IBD vaccine induced severe depletion of lymphoid follicles, degeneration and necrosis of lymphocytes, interfollicular oedema with inflmmatory cells, aggregation and there results agreed with those of **Sharma *et al.*, (1989)**.

The immunosuppression study in the vaccinated chicken (Tables 5 and 6) revealed that non of all vaccinated groups showed immunodepression as indicated by good HI immune response and good protection rates over 90% against velogenic ND challenge virus in spite it was less than that of IBD unvaccinated control. The above mentioned results are in agreement with **Hiari *et al.*, 1980; Sivandan and Mahaswaran, 1980; Thangavelu *et al.*, 1998 and Lam, 1998).**

In conclusion, in spite of the high titre dose of Nobilis 228E and IBD vaccines could protect 100% of chicken, the lower titre dose either 2 $\log_{10}$ or 2.5 $\log_{10}$ is still protective against virulent challenge IBD virus causing minimal bursal lesion.

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Table (1): Comparison of ELISA antibody titre of vaccinated chicken with different titre dose of IBD-Blen and Nobilis 228E vaccines.

Days	Nobilis 228E vaccine			IBD-Blen vaccine			Control unvaccinated challenged	Unvaccinated unchallenged control
	$10^{2.0}$	$10^{2.5}$	$10^{3.5}$	$10^{2.0}$	$10^{2.5}$	$10^{3.5}$		
1 day old (Prevacc.)	12200			12200			12200	12200
7 days old (Prevacc.)	3320			3320			3320	3320
14 days old (Prevacc.)	680			680			680	680
7 days post vaccination	412	490	590	518	611	687	220	220
14 days post vaccination	6160	7020	9942	7880	8622	11400	60	60
7 days post challenge	8890	9862	12260	10110	12010	13210	450	22

Table (2): Challenge test of the vaccinated and unvaccinated groups against IBDV.

Subgroups	No.	Mortality					No. of Dead / Total	Protection %
		Days Post Challenge						
		1	2	3	4	5		
$10^{2.0}$ Blen	20	0	0	0	0	0	0/20	100 %
$10^{2.5}$ Blen	20	0	0	0	0	0	0/20	100 %
$10^{3.5}$ Blen	20	0	0	0	0	0	0/20	100 %
$10^{2.0}$ 228E	20	0	0	1	1	0	2/20	90 %
$10^{2.5}$ 228E	20	0	0	0	0	0	0/20	100 %
$10^{3.5}$ 228E	20	0	0	0	0	0	0/20	100 %
Control unvaccinated challenged	20	0	2	3	9	4	18/20	10 %

Table (3): The pathogenicity of two vaccines at different titre doses.

Subgroups		5 Days post vaccination with one field dose		5 Days post vaccination with 10 field dose	
		Bursal Index	Bursal lesion score	Bursal Index	Bursal lesion score
Nobilis 228E vaccine	$10^{2.0}$	0.88	2.0	0.89	2.0
	$10^{2.5}$	0.87	2.1	0.87	2.1
	$10^{3.5}$	0.77	2.6	0.70	2.8
IBD-Blen vaccine	$10^{2.0}$	0.83	2.2	0.80	2.3
	$10^{2.5}$	0.83	2.2	0.82	2.35
	$10^{3.5}$	0.72	2.9	0.75	3.0
Non vaccinated non challenged		0.0	0.0	0.0	0.0

Table (4): The bursal lesion score and indices of chicken vaccinated with Nibilis 228E and IBD-Blen and challenged with virulent IBD strain.

Subgroups		Bursal Index	Bursal lesion score
Nobilis 228E vaccine	$10^{2.0}$	0.86	2.2
	$10^{2.5}$	0.86	2.2
	$10^{3.5}$	0.72	2.6
IBD-Blen vaccine	$10^{2.0}$	0.80	2.3
	$10^{2.5}$	0.80	2.3
	$10^{3.5}$	0.70	2.7
Non vaccinated non challenged		0.56	4.0

Table (5): The immune response of chicken vaccinated with LaSota after 7 days of IBD-Blen or Nobilis 228E vaccination as measured by ELISA.

Time (Days)	Nobilis 228E vaccine			IBD-Blen vaccine			Control unvaccinated IBD and ND vaccineated	Control unvaccinated IBD unvaccinated ND
	$10^{2.0}$	$10^{2.5}$	$10^{3.5}$	$10^{2.0}$	$10^{2.5}$	$10^{3.5}$		
Pre-vaccination	2.8	3.0	2.8	2.8	2.9	2.8	2.9	2.9
7 days post vaccination	7.8	7.8	7.3	7.8	7.7	7.3	8.1	2.6
14 days post vaccination	8.7	8.7	8.2	8.7	8.5	8.1	9.3	2.0

Table (6): Protection rates against ND after vaccination with IBD-Blen or Nobilis 228E vaccines.

Subgroups		Mortality	Protection rate
Nobilis 228E vaccine	$10^{2.0}$	0/10	100 %
	$10^{2.5}$	0/10	100 %
	$10^{3.5}$	0/10	100 %
IBD-Blen vaccine	$10^{2.0}$	0/10	100 %
	$10^{2.5}$	0/10	100 %
	$10^{3.5}$	1/10	90 %
Control unvaccinated ND & challenged		9/10	10 %
Control unvaccinated IBD and vaccinated ND and challenged		0/10	100%

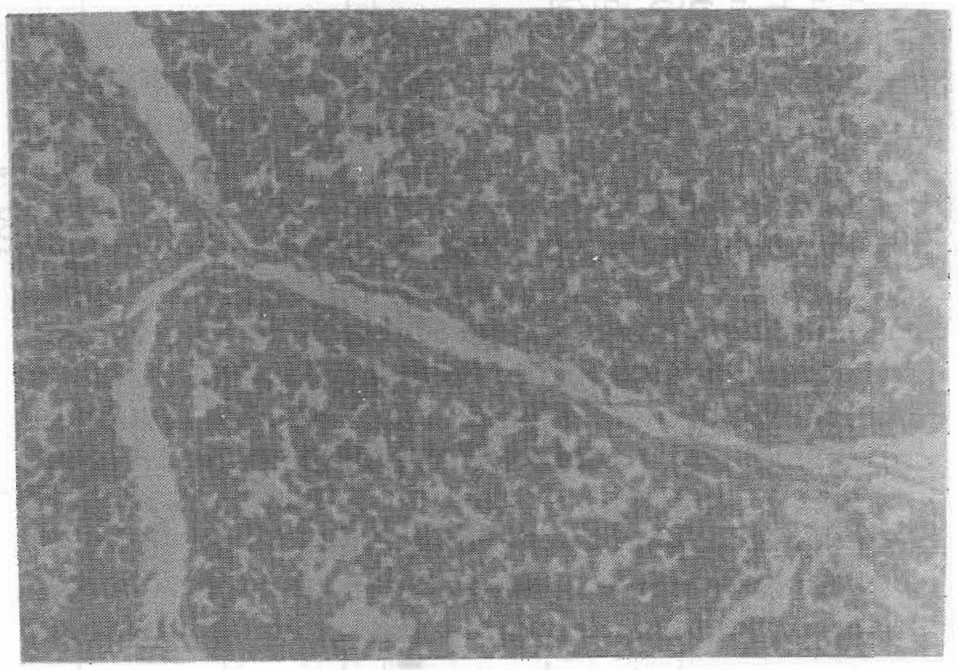

Photo (1): Bursa of Fabricius of chicken vaccinated with IBD-Bien vaccine ($10^{2.0}$ EID_{50}/dose) showing slight lymphocytic depletion and macrophages are seen in some areas.

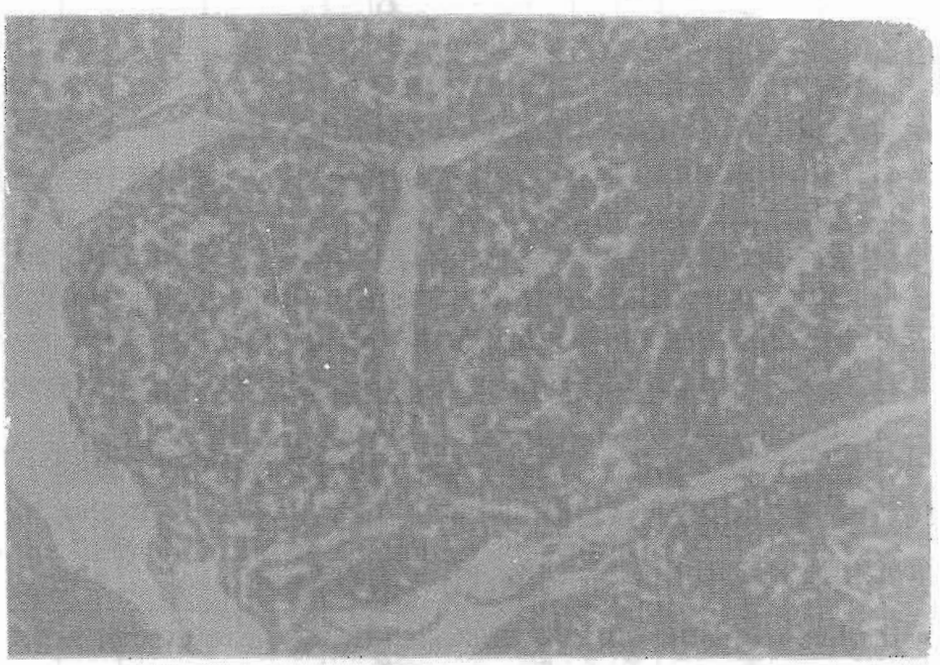

Photo (2): Bursa of Fabricius of chicken vaccinated with 228E vaccine ($10^{2.0}$ EID_{50}/dose) showing slight interlobular oedema.

Photo (3): Bursa of Fabricius of chicken vaccinated with IBD-Blen vaccine ($10^{2.5}$ EID_{50}/dose) showing lymphocytic depletion, others begin to activate. Lymphocytes are lost from some follicles in cortex and medulla.

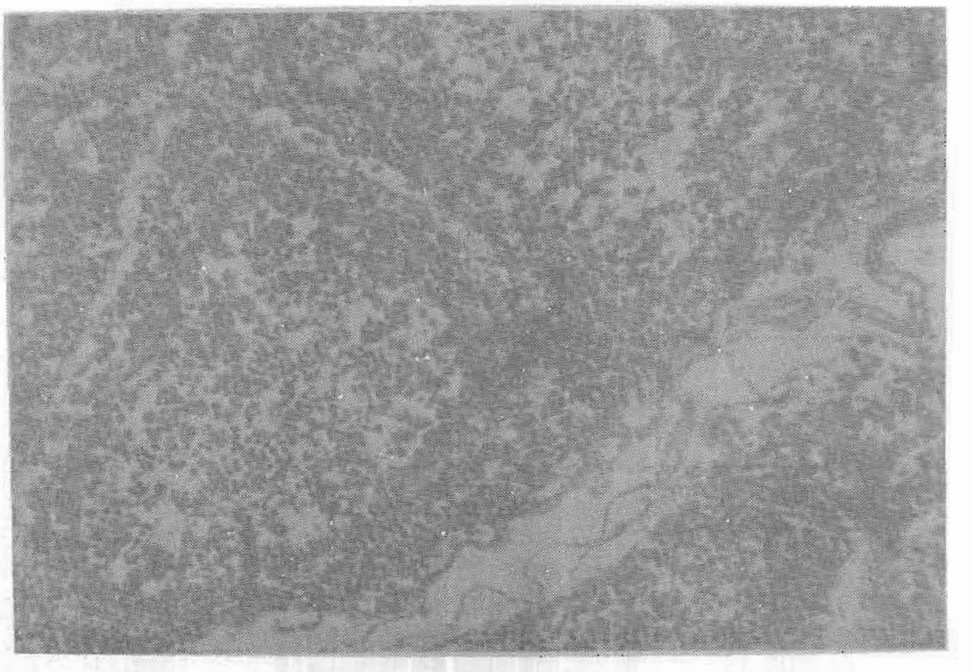

Photo (4): Bursa of Fabricius of chicken vaccinated with 228E vaccine ($10^{2.5}$ EID_{50}/dose) showing lymphocytic depletion. Lymphocytes are lost from some follicles in cortex and medulla. Some areas between follicles showed stromal fibroplasia. Macrophages are seen.

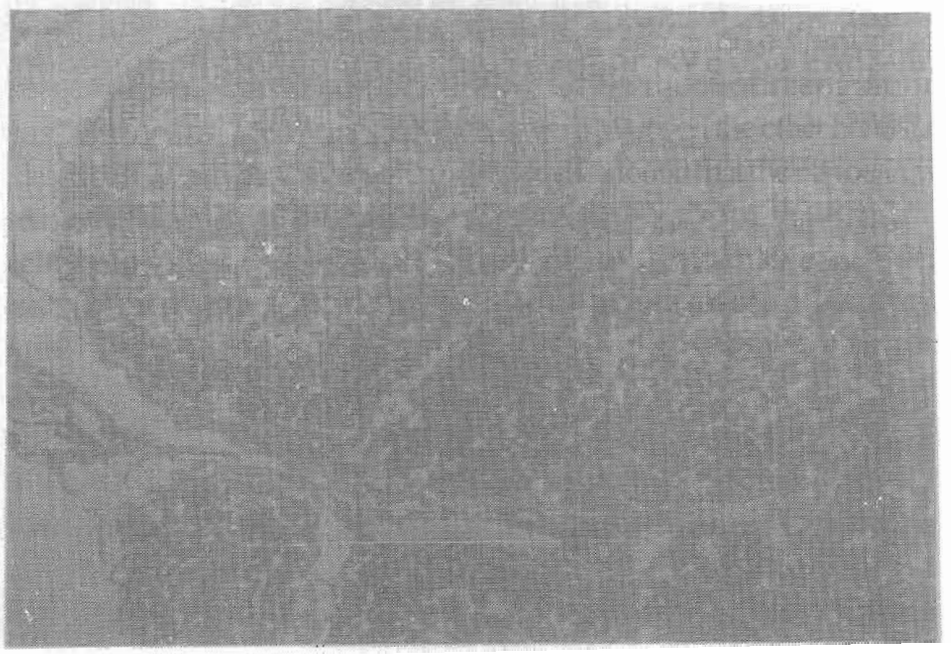

Photo (5): Bursa of Fabricius of chicken vaccinated with IBD-Blen vaccine ($10^{3.5}$ EID_{50}/dose) showing that the central lymphocytic depletion of the bursal lymphoid follicles with necrosis, lymphocytosis and appearance of reticular mas. Small, multiple cystic spaces within the bursal follicles are seen.

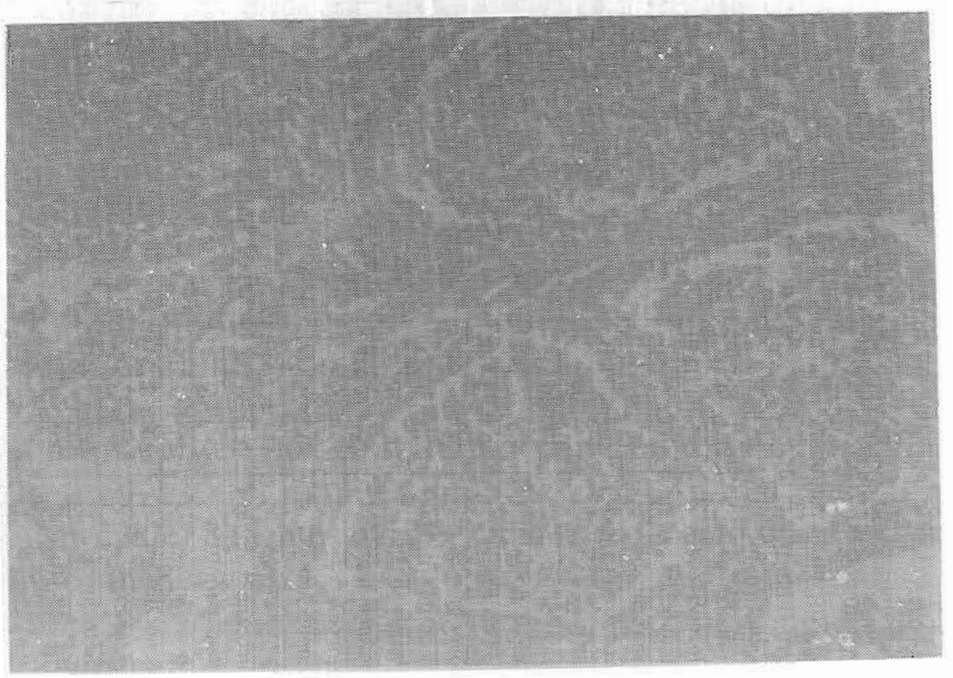

Photo (6): Bursa of Fabricius of chicken vaccinated with 228E vaccine ($10^{3.5}$ EID_{50}/dose) showing that the bursal epithelial lining showed focal denuded areas and few focal areas of hyperplastic proliferation.

Photo (7): Bursa of Fabricius of chicken challenged with IBD virulent virus revealed that some follicles showing extensive lymphocytic depletion and contained macrophages. The lumen follicles showing cellular debris. The reticular fibers undergo metaplasia and give rise to acinar structure.

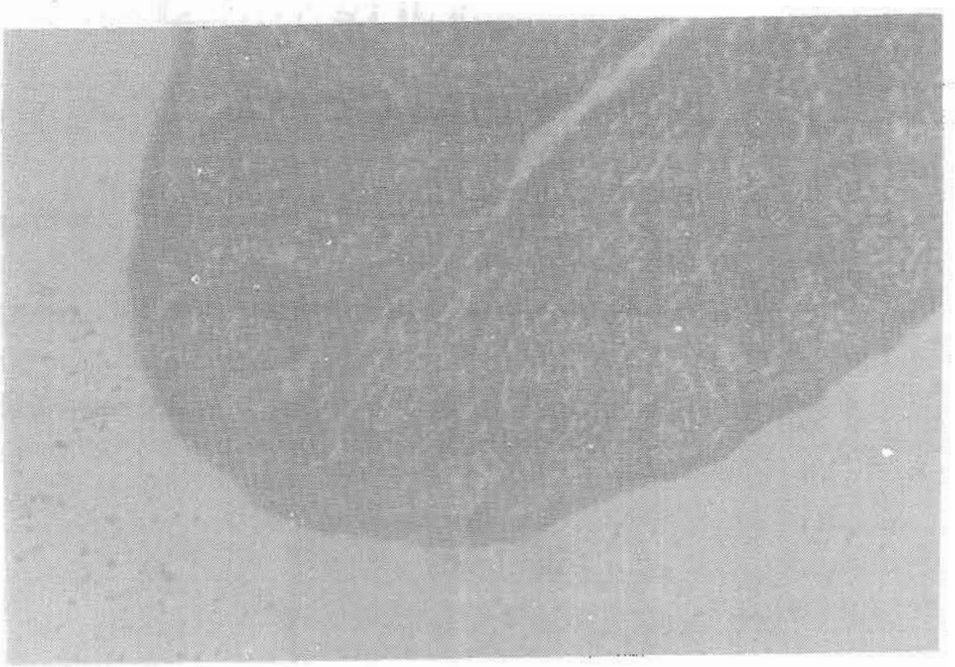

Photo (8): Bursa of Fabricius of negative control chicken showing normal struscture

الملخص العربي

دراسة مقارنة بين عترتين شديدة الضراوة من اللقاحات الحية لمرض الجمبورو

الهام عطا الأبياري* عبد الحكم محمود محمد* ناصر عباس يوسف*

سوزان سيد المهدي* نادية محمد حسن**

* المعمل المركزي للرقابة علي المستحضرات الحيوية البيطرية - العباسية - القاهرة

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أجريت هذه الدراسة كمقارنة بين ثلاث قوي مختلفة هي ($2.0\ \log_{10}\ EID_{50}$/dose, $3.5\ \log_{10}$/EID_{50}/ dose, $2.5\ \log_{10}\ EID_{50}$/dose) للقاحين شديدة الضراوة ضد مرض الجمبورو.

وتم قياس المستوي المناعي للطيور المحصنة باستخدام أختبار الأليزا ، كذلك مستوي الحماية باجراء التحدي وكانت نسبتها ١٠٠ % في الثلاث قوي العيارية. كذلك تم قياس معامل وزن الجسم ووزن غدة فابريشي.

كما تم دراسة التأثير المثبط لكل قوة عيارية لكل لقاح علي حدة بقياس الأستجابة المناعية في الطيور المحصنة بلقاح اللاسوتا. وقد وجد أي من القوي العيارية ليس له تأثير مثبط ، ولوحظ أن أقل اصابة لغدة فابرشي كانت باستعمال قوي عيارية $2.0\ \log_{10}\ EID_{50}$/dose.