

## THE EFFECT OF SALMONELLA ENTERITIDIS INFECTION ON IMMUNE RESPONSE AGAINST NEWCASTLE DISEASE (ND) IN CHICKENS

By

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### SUMMARY

The relationship between chronic infection with *Salmonella enteritidis* in chickens and the immune response against ND vaccination using Hitchner B1 vaccine was studied. *Sal. ent.* infection proved to have an immunosuppressive effect on the immune response against ND considering post mortem lesions, seroconversion, histopathological changes and mortalities after VVNDV challenge as criteria.

Calenk et al., 1991). *Salmonella enteritidis* may be present in contaminated eggs and poultry meat (Anon; 1989).

Prevention of the highly contagious septicaemic ND is accomplished by sanitation and vaccination programmes. The efficiency of ND vaccine may be affected by the presence of other bacterial pathogens infecting the bird which consequently affect the immune status of the bird.

This investigation is planned to study the effect of chronic infection of *Salmonella enteritidis* as carrier pathogen on the efficiency of immune response against ND.

### INTRODUCTION

Avian Salmonellosis is one of the problems of economic concern to all phases of the poultry industry from production to marketing. Recently; *Salmonella enteritidis* was proved to be a major disease problem for chickens in different localities, as some strains produce as high as 20% mortality in chickens. On the other hand chronic salmonellosis is considered one of the major problems which cannot be ignored (Nagaraja et al., 1991). *Salmonella enteritidis* is principally egg-borne infection and is of public health significance (Sockett and Roberts; 1991 and

### MATERIAL AND METHODS

#### I- Experimental chicks:

One hundred, one-day old male LSL chicks were used in this study. Ten chicks were randomly taken and subjected to clinical, post mortem and bacteriological examinations which proved to be healthy and free from bacterial infections. The remaining 90 chicks were divided into 3 equal groups consisting of 30 each.



## 2- Bacterial inoculum:

Salmonella enteritidis strain isolated from the intestine (cecum) of broiler chicks was used. This strain was identified after Kauffmann (1972), Krteg and Holt (1984) and Edward and Ewing (1972).

## 3- Vaccine:

A commercial lentogenic ND virus vaccine (Hitchner B<sub>1</sub> strain), 1000 dose vial, Intervet International B.V.Co., batch No. 50286 B, 1995, was reconstituted in phosphate buffer saline (PBS). Each bird received intraocularly  $10^6$  ELD<sub>50</sub>.

## 4- Challenge virus:

A velogenic viscerotropic ND virus (VVNDV) strain obtained from Vet. Serum and Vaccines Research Institute, Abassia Cairo, Egypt (Sheble and Reda 1976) was used. The virus was twice passaged via the allantoic cavity of 10-day-old embryonating chicken eggs. Allanto-amniotic fluid was harvested from dead embryos and stored at -20°C. till used. ELD<sub>50</sub> was  $10^{7.5}$ .

## 5- Haemagglutination (HA) test:

The test was performed to determine four haemagglutinating units (4 HAU) used in HI test according to (Beard, 1989).

## 6- Haemagglutination inhibition (HI) test for NDV:

The test was applied against 4 HAU according to

(Beard, 1989). Calculation of geometric mean (GM) was determined according to (Brugh, 1978).

## 7- Blood samples:

Blood samples were taken weekly before vaccination, post challenge and tested for HI antibody titers against ND. In calculating the geometric mean (GM) of HI titers, those more than  $\log_3$  were considered to be positive.

## 8- experimental design:

Five days old experimental chicks were divided into 3 equal groups (1-3) consisting of 30 each. The first group (group 1) was inoculated orally with 18-hours broth culture of Salmonella enteritidis in a dose of  $1.5 \times 10^4$  CFU for 2 successive days. Ocular vaccination against ND using HB1 vaccine was carried out for the groups 1 and 2. Blood samples were collected for 4 weeks before challenge for HI titer determination. The third group (group 3) was reared in a separate room away from the other groups and kept as negative control.

Chicks of all groups were challenged ocularly with 0.1 ml of VVNDV per chick. All chicks were kept for 3 weeks observation period post challenge during which mortality; clinical signs and blood samples for HI testing as well as lesions were recorded (Beard and Easterday; 1967).

Specimens of bursae of Fabricius, spleen and thymus glands from different experimental groups were fixed in neutral formaline (10%) and collected fro histopathological examinations



according to Culling (1963).

## RESULTS

The obtained results are showed in tables 1-2. The challenge virus killed all unvaccinated control birds (group 3) between 5-6 days post challenge and exhibited haemorrhagic lesions in the digestive tract whereas the groups vaccinated afforded 68% and 88% protection for vaccinated infected *Salmonella enteritidis* (group 1) and vaccinated non-infected (group 2) groups respectively. All birds tested for antibody to NDV by the HI test were approximately zero before vaccination. Post challenge HI titers indicated that they were higher in positive control group (group 2) than in the group inoculated with *Salmonella enteritidis* (group 1).

The symptoms appeared after challenge during 1<sup>st</sup> and 2<sup>nd</sup> weeks as ruffled feathers, dullness, off food, drooping of wings and diarrhoea which was more prominent in group 1.

The post mortem lesions of vaccinated dead birds were congestion of heart and liver, enteritis which was more prominent in group 1 and petechial haemorrhage on cecal tonsils. Moreover; there was ulcer with petechial haemorrhage on glandular stomach which was recorded only in group 1 after 2<sup>nd</sup> week post ND challenge.

The histopathological findings were described as follows:

**A-Group 1:** (*Salmonella* infected, vaccinated, and challenged):

**1- Bursa of Fabricius:** Showed atrophy of lymphocytic follicles which showed severe depletion of lymphocytes. Sometimes complete absence of lymphoid follicles was observed which was replaced by debris and cysts. Severe plical edema accompanied with great thickening of hyperplastic epithelial cells were detected. (Fig. 1).

**2- Spleen:** It was affected with capsular and subcapsular edema with congested blood vessels, sometimes destruction of blood vessels wall was observed. Also, there was slight depletion of lymphocytes. (Fig. 2).

**3- Thymus glands:** showed slight congested blood vessels and mild degeneration of thymocytes.

**B- Group 2:** (Vaccinated, non-infected, and challenged);

**1- Bursa of Fabricius:** It was affected with slight plical edema and mild degree of cystic formation. The epithelium of some plica suffered from hyperplasia, also there was mild depletion of lymphocytes.

**2- Spleen:** showed dispersion of capsular fibres due to edema, sometimes there was capsular haemorrhage. Lymphocytic depletion was detected accompanied with haemorrhage in splenic parenchyma. (Fig. 3).

**3- Thymus glands:** revealed haemorrhage and severe congestion of cortical blood vessels, while necrosis was observed in medullary part accompanied with pronounced depletion of thymocytes. (Fig. 4).



Table (1): Results of HI for chicken groups vaccinated with NDV

Time of sampling	Chick groups	Haemagglutination Inhibition Geometric mean (HIGm)		
		Group 1	Group 2	Group 3
Pre-vaccination		0.00	0.25	0.78
1st W. Post Vaccination		3.96	3.68	0.38
2nd W. Post Vaccination		4.50	4.14	0.25
3rd W. Post Vaccination		4.00	4.70	0.12
4th W. Post Vaccination		3.70	5.10	0.05
1st W. Post Challenge		8.50	9.20	-
2nd W. Post Challenge		8.70	9.80	-
3rd W. Post Challenge		6.80	7.80	-

Group (1) = Vaccinated, Salmonella enteritidis infected chicks.

Group (2) = Vaccinated non-infected chicks.

Group (3) = Non-vaccinated, non-infected chicks.

Table (2): Results of challenging of chickens with NDV

Group No.	No. of chicks	Mortalities/Week							
		1st		2nd		3rd		Total	
		No.	%	No.	%	No.	%	No.	%
1	30	2	6.6	4	14.3	2	8.3	8	26.7
2	30	2	6.6	1	3.6	-	-	3	10.0
3	30	30	100.0	-	-	-	-	30	100.0

Group (1) = Vaccinated, Salmonella enteritidis infected chicks.

Group (2) = Vaccinated, non-infected chicks.

Group (3) = Non-vaccinated, non-infected chicks.





Fig. (1): Bursa of Fabricius showing severe plical edema with hyperplastic epithelium. group 1 (H & E X 100).

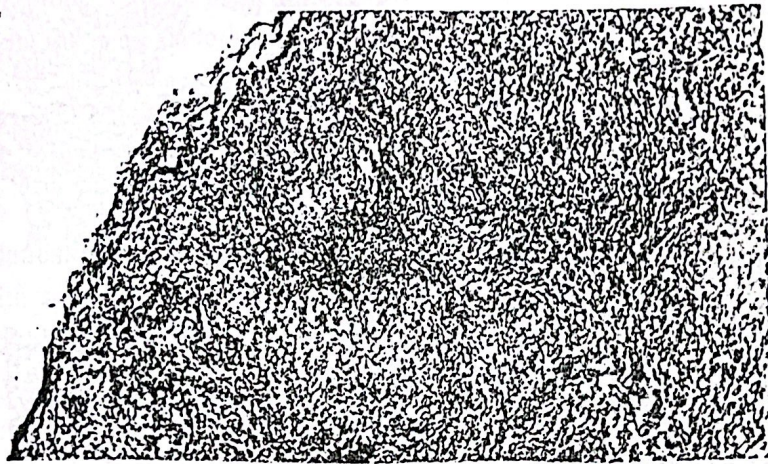


Fig. (2): Spleen showing coagulative necrosis. group 1 (H & E X 100)

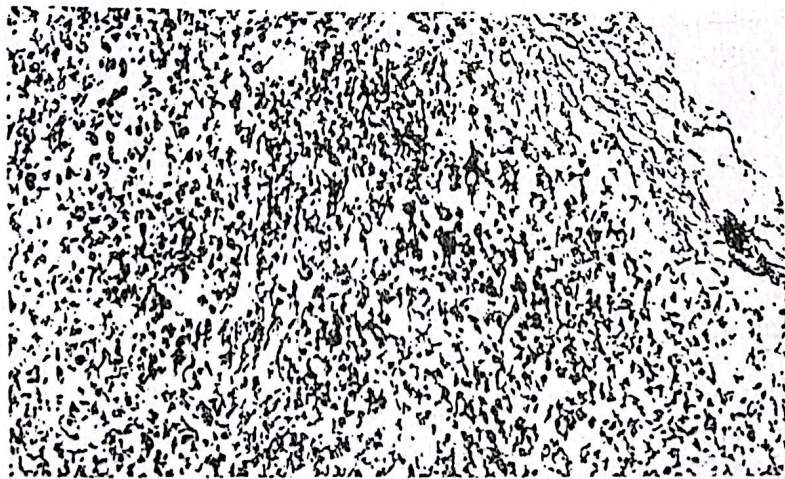


Fig. (3): Spleen showing capsular haemorrhage and depletion of lymphocytes. group 2 (H & E X 250).





Fig. (4): Thymus showing severe haemorrhage and congestion group 2 (H & E X 100).

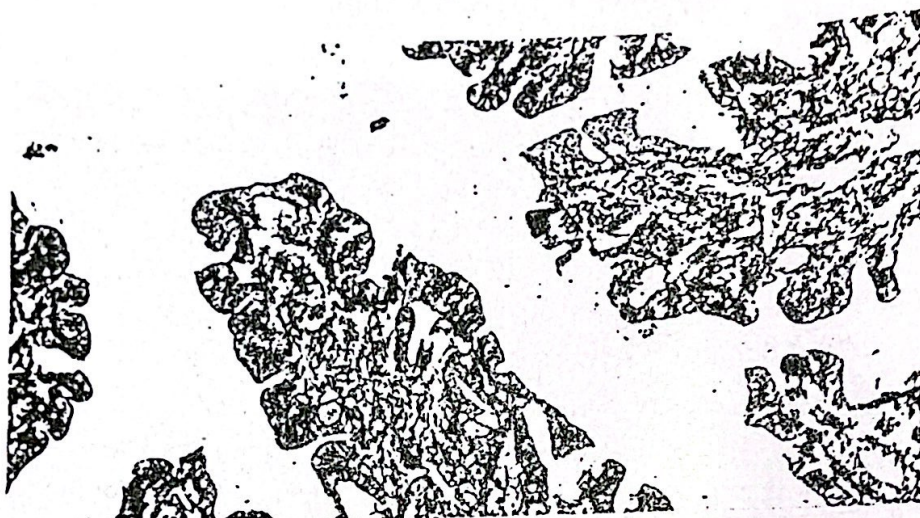


Fig. (5): Bursa of Fabricius showing pronounced depletion of lymphoid follicles and cystic formation. group 3 (H & E X 100).

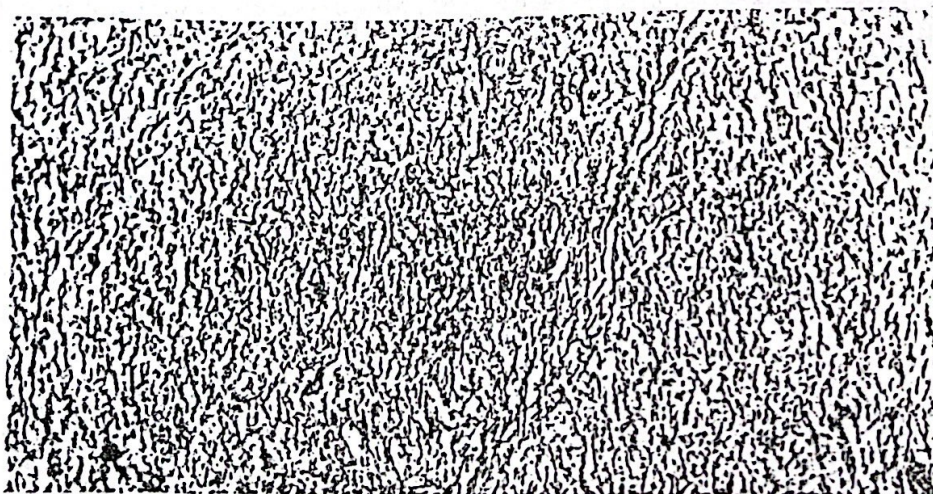


Fig. (6): Thymus showing severe degenerated thymocytes. group 3 (H & E X 100).



**C- Group 3: (Non-vaccinated, non-infected, and challenged):**

- 1- **Bursa of Fabricius:** exhibited subepithelial edema and congested blood vessels. Pronounced lymphocytic depletion of lymphocytic follicles. Hyperplastic changes of plial epithelium in addition to cystic formation. (Fig. 5).
- 2- **Spleen:** showed focal coagulative necrosis with severe depletion of lymphocytes. Thickening in the wall of blood vessels, sometimes focal proliferation of the endothelium which protruded to the lumen were observed. Haemorrhage, congestion and perivascular edema were prominent.
- 3- **Thymus glands:** indicated congestion of blood vessels with severe depletion of thymocytes and haemorrhage and also severe degeneration of thymocytes. (Fig. 6).

## DISCUSSION

Immunopotentiators and immunosuppressives are factors that influence positively or negatively chickens immune response (Beck; 1974; Faragher et al., 1974, Brunner and Muscoplat; 1980, Armanious et al., 1991, Tizard, 1992, Soliman et al., 1992 and Saad et al., (1993). However; reviewing the available literature and to the best of our knowledge, there is no previous investigation that has been carried out to clarify the possible effect of chronic salmonellosis in chickens on their immune response against ND. In the present investigation three criteria were adopted for studying this relationship, including

severity of pathological changes, seroconversion as well as mortality rate after challenging with VVNDV.

The recorded post mortem lesions were prominent in vaccinated infected group (group 1) as compared with vaccinated non-infected group (group 2) after VVNDV challenge.

Results of seroconversion revealed higher geometric mean (GM) of HI titers in vaccinated non-infected group as compared with the vaccinated Salmonella enteritidis infected group at different intervals post ND vaccination or challenge. However; these titers gradually decreased in the non-vaccinated non-infected negative control group (group 3) until approximately vanished at 4 weeks post vaccination (Table 1).

Results of challenging revealed mortality rate of 26.7%, 10.0% and 100% in vaccinated Salmonella enteritidis infected group, vaccinated non-infected group and non-vaccinated non-infected group respectively (Table 2).

Regarding the histopathological findings of bursa of Fabricius of group 1 (Salmonella enteritidis infected group) revealed atrophy of lymphocytic follicles with severe depletion of lymphocytes and sometimes complete absence of lymphoid follicles that were replaced by debris and cyst. These findings might explain the immunosuppressive effect of Salmonella enteritidis infection.

Regarding the aforementioned results it could be concluded that chronic infection with Salmonella enteritidis in chickens has an immunosuppressive



effect on ND vaccination. This conclusion may add another significance to the importance of salmonella infection as a potent pathogen threatening poultry industry.

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