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# VALUATION OF EMERGENCY VACCINATION AGAINST INFECTIOUS URSAL DISEASE (IBD) WITH OR WITHOUT SOME NON-SPECIFIC MMUNOSTIMULANTS

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## SUMMARY

A trial for emergency vaccination against IBDV 48-hour post experimental infection of 29-day old chikcs was evaluated. The emergency vaccine was given with or without some immunostimualnts, namely; Pind-Avi (Fowl pox virus inactivated by gamma rays), Ultracorn® (Corynebacterium cutis lysate), Levamisole hydrochloride® and transferrin. The results revealed that emergency vaccination of infected chicks was useless in controlling mortality or bursal damage (which was evidenced by bursal lesion score & bursal lymphoid tissue lesions). The use of these immunostimulants alone improved the protection % but had not restored the bursal damage with varying degree of stimualtion of non-specific immune response in the following order: Transferrin, ultracorn®, Pind-Avi finally levamisole. The combination of emergency vaccine with immunostimulant didn't show a significant control point.

## INTRODUCTION

Infctious bursal disease (IBD) is a common disease of chickens. The virus of IBD is widespread and tends to infect most commercial flocks of chickens early in life.

Although vaccination against the disease is the main and unique tool for its prevention, some flocks undergo servere losses in spite of vaccination either due to antigenic diversity or the variant challenging viruses from classical vaccinal strains (Jackwood and Saif 1987) or the possible interference with the maternal antibodies (Van Den Den Berg and Meulemans, 1991).

The emergence of acute or very virulant IBDV outbreaks appeared in Egypt and are still occurring since 1989 and have caused serious economic losses to the developing poultry industry despite vacciantion (El-Batrawi, 1990 & Ahmed, 1991).

Currently, a great potential has been reported for the use of immunostimulants to improve the immune response, increase the non-specific resistance to infections and to minimize their deleterious effects (Brunecher, et al., 1986; Afifi, 1990 & Kutkat, 1992).

The present investigation was designed to evaluate the use of some immunostimulants with or without simultaneous emergency vaccination in reducing losses from IBD in experimentally infected chicks.

## MATERIAL AND METHODS

**1) Chickens:-**

Day-old commercial Hubbard broiler chicks obtained from a private company were used in this experiment. The chicks were floor- reared and fed on a balanced commercial ration.

**2) Immunostimulants:-**

**a- Pind-Avi**

Gamma ray-inactivated fowl pox virus strain HP 1-428 with an initial titer of $10^{7.5}$ $KID_{50}$/ml was kindly obtained from Prof. Dr. I. Reda, Fac. Vet. Med., Cairo Univ. in the form of lyophilized powder. This powder contained 32 protecting units per 0.1ml when diluted in 5 ml phosphate-buffered saline (PBS). The preparation was used by subcutaneous injection at a dose of 0.3 ml/50 gm body weight (b.w.) (Mayr et al., 1986).

**b- Utracorn®**

A complete lysate of Corynebacterium cutis at a concentration of 20 mg/ml produced by Virbac Company, was subcutaneously injected at 40ul/500 g body weight (b.w.).

**c- Levamisole®**

Levamisole HCl sterile solution (7.5%) Lot No. 973007 obtained from Pharmachism, Bulgaria was used by subcutaneous injection at a dose of 7.5 mg/kg body weight (b.w.) (Goranov and Bonovskal, 1987).

**d- Transferrin**

It was prepared by precipitation of chicken serum by ammonium sulphate and chromatography on diethyl amino ethyl (DEAE) cellulose. The technique adopted was that of Bezkorovainy et al. (1963), modified by Awaad (1975) and Kutkat (1988).

The chromatography on DEAE-cellulose ion exchange column was done by the procedure of Sober et al.(1956) and the protein content of the preparation was determined according to Peters (1968).

**Characterization of the prepared transferrin was achieved by:**

1- Spectral analysis after Sober et al. (1956) and Roop and Putman (1967).
2- Electrophoresis, using the technique of polyacrylamide gel after Graber and Williams (1953).
3- Determiantion of total iron-binding capacity after (Kutkat 1992).

preparation was used at a concentration of g/ml BGM (semi-defined buffered liquid ium after Rainard (1986) and inoculated utaneosly at 10 mg/40 gm body weight (b.w.) kat 1992).

**BD vaccine:-**

8 IBDV vaccine (batch No. 7060 B) produced Intervet International B.V. Boxmeer, Holland, h virus titre of $10^6$ $EID_{50}$ was used raocularly for vaccination of experimental cks.

**IBD infected virus:-**

very virulent IBDV (vvIBDV) isolated and ntified from a natural outbreak in 36-day old oiler flock (El-Batrawy, 1990) was used as a rsal homogenate in infecting the experimental icks by the intraocular route.

**. Agar gel precipitation test (AGPT):**

he test was used to demonstrate the presence of aternal antibodies to IBDV in examined chicken era as described by Wood et al. (1979).

**- Histopathology:-**

Specimens from bursae of experimental chicks were fixed in formaline-saline 10% and embedded in paraffin. Sections were cut at 6 micron thickness and stained with haematoxyline and eosin (Harris, 1898).

The severity of microscopic lesions of bursal lymphoid tissue lesions was scored 0-4 on the basis of lymphoid necrosis and/or depletion according to Sharma et al. (1989) as follows;

0= less than 5% of the lymphoid follicles (per field) affected.

1= 5-25% of the lymphois follicles (per field) affected.

2= 25-50% of the lymphoid follicles (per field) affected.

3= 50-75% of the lymphoid follicles (per field) affected.

4= More than 75% of the lymphoid follicles (per field) affected.

**Experimental design:**

Experimental chicks as day-old were floor reared and fed on commercial raton. Maternal antibody waning in those chicks was followed up at different intervals starting from day 1 up to day 27 of age, using groups of 20 serum samples/ time interval. They were examined individually by the AGPT.

Three hundred chicks at 27 day old were divided into 15 identical groups, 20- chicks per each, and used to carry out four simultaneous experiments (Table 1):

**Experiment (A):** Twenty chicks were infected intraocularly with $10^{4.4}$ $EID_{50}$/bird of vvIBDV (El-Batrawi and El-Kady, 1990) at 27 day old (group I). The chicks were vaccinated at 48 hrs post-infection (immediately after the onset of clinical signs) using one vaccine dose of D-78 IBD vaccine by intraocular instillation as emergency vaccination.

**Experiment (B):** Eighty chicks were infected

intraocularly with $10^{4.4}$ $EID_{50}$. bird of vvIBDV at 27 day old. The chicks were vaccinated at 48 hrs post-infection (PI), using one vaccine dose of D-78 IBD vaccine by intraocular instillation as emergency vaccination. The chicks simultraneously treated with different immunostimulants as follows: the chicks was divided into four groups (2, 3, 4 and 5); 20 chicks each. Each chick of the 2nd group were subcutaneously (s/c) inoculated with 0.3 ml/50 g.w. of Pind-Avi. The chicks of the 3rd group were individually s/c inoculated with 40 ul/500 g of Ultracorn®. The birds of the 4th group were individually s/c inoculated with 7.5 mg/kg b.w. of levamisole. the chicks of 5th group were individually s/c inoculated with 10 mg/500 g b.w. of transferrin.

**Experiment (C):** Eighty chicks were infected intraocularly with $10^{4.4}$ $EID_{50}$/bird of vvIBDV at 27 day old. At 48 hrs PI, the chicks were divided into four groups (6, 7, 8 and 9); 20 chicks each. Each chick of the 6th group was s/c inoculated with 0.3 ml/50 g b.w. of Pind-Avi. The chicks of the 7th group were individually s/c inoculated with 40 ul/500 g b.w. of Ultracorn®. The birds of the 8th group were individually s/c inoculated with 7.5 mg/kg b.w. of Levamisole. The chicks of 9th group were individually s/c inoculated with 10 mg/50 g b.w. of Transferrin.

**Experiment (D):** Eighty chicks were divided into four gorups (10, 11, 12 and 13); 20 chicks each (at 29 day old). Each chick of the 10th group was s/c inoculated with 0.3 ml/50 g b.w. of Pind-Avi. The chicks of the 11th group were individually s/c inoculated with 40 ul/500 g of Ultracorn®. The birds of the 12th group were individually s/c inoculated with 7.5 mg/kg b.w. of Levamisole. The chicks of 13th group were individually s/c inoculated with 10 mg/500 b.w. of Transferrin.

Another three groups were kept as control gorups, one of them served as a control challenged gorup (group 14), one of them served as a control vaccinated non-challenged group (group 15), and the other gorup (group 16) was kept as non vaccinated non challenged blank control group.

All birds were kept under observation for 10 days post-vaccination and/or immunostimulation. Moralities and lesion score of dead birds were recorded. Percentage of survival and actual protection due to protection levels were calculated according to the following formula: Survival% of vaccianted infected birds minus survival% of non-vaccinated infected birds. In addition, histological sections were prepared from bursae of three birds in the experimental groups which died and/or were scarificed daily during the period of observation.

**Table (1): Experimental design :**

| Exp. | Gp. | No. of | I/O infection | I/O emergency vaccination | S/C treatment with immunostimulants β | | | |
|---|---|---|---|---|---|---|---|---|
| No. | No. | birds | with vvIBDV | with D-78 IBD vaccine @ | Pind-Avi | Ultracorn | Levami-sole | Transferrin |
| A | 1 | 20 | + | + | – | -- | -- | – |
| B | 2 | 20 | + | + | + | – | – | – |
| | 3 | 20 | + | + | – | + | – | – |
| | 4 | 20 | + | + | – | – | + | – |
| | 5 | 20 | + | + | – | – | – | + |
| C | 6 | 20 | + | – | + | – | – | – |
| | 7 | 20 | + | – | – | + | – | – |
| | 8 | 20 | + | – | – | – | + | – |
| | 9 | 20 | + | – | – | – | – | + |
| D | 10 | 20 | – | – | + | – | – | – |
| | 11 | 20 | – | – | – | + | – | – |
| | 12 | 20 | – | – | – | – | + | – |
| | 13 | 20 | – | – | – | – | – | + |
| control infected non-vacc | 14 | 20 | + | – | – | – | – | – |
| control vacc. non-ifect. | 15 | 20 | – | + | – | – | – | – |
| Control blank | 16 | 20 | – | – | – | – | – | – |

Exp.No. = Experiment Number. Gr.No. = Group Number.

I/O = Intraocular Instillation. S/C = Subcutaneously.

@ = Emergency vaccination was performed immediately after the onset of clinical signs.

β = Treatment with different immunostimulants was carried out simultaneously with the emergency vaccination.

## RESULTS

The protection rates in Table (2) showed no much difference between infected non-emergency vaccinated and infected emergency vaccianted groups, while protection ranged from 45% to 65% in groups which received the emergency vaccine simultaneously with immunostimulants. On the other hand, the gorups which received the immunostimulants alone 48 hours post infection, the protection rates were ranging from 45% to 70%.

The mean of lesion score in dead or sacrificed birds was tabulated in Table (3) in the form of figures for examined organs, generally, it was the highest in infected vaccinated and infected non vaccinated groups, while it was lesser in groups received immuostimualnts alone than those received immuostimulants together with the vaccine specially at the second and third days post treatment.

In Table (4) the severity index of bursal lymphoid tissue lesions in dead and or sacrificed birds after treatment with the immunostimulants and or vaccination with live IBDV vaccine in different experimental groups showed maximum lymphocyte necrosis and lymphocyte depletion in groups 1 and 14 (infected vaccinated and infected non vaccinated), followed by groups which received the immunostimulants simuataneously with the vaccine (2, 3, 4 and 5) while in groups 6, 7, 8 and 9 were undergone slightly less severity indices however, in some groups the lymphocyte necrosis was equal or somewhat higher than those infected non treated.

**Table (2): Pattern of mortalities and the protection rates among different experimental groups of chickens**

| Exp No. | No. | Bird Treatment | No. of birds | Patterns of mortality per day post-treatment with immunostimulants and/or vaccination | | | | | | | Mortality | Survival % | Protection %[2] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | | |
| A | 1 | Infec. & vacc. | 20 | 1 | 2 | 10 | 0 | 1 | 1 | 0 | 15/20 | 25 % | 15 % |
| B | 2 | Infec.&vacc.& PA | 20 | 1 | 2 | 3 | 1 | 0 | 1 | 0 | 8/20 | 60 % | 50 % |
| | 3 | Infec.&vacc.& UC | 20 | 1 | 1 | 4 | 1 | 0 | 1 | 0 | 8/20 | 60 % | 50 % |
| | 4 | Infec&vacc.& Lev. | 20 | 1 | 1 | 5 | 1 | 2 | 0 | 0 | 9/20 | 55 % | 45 % |
| | 5 | Infec.&vacc.& Tf. | 20 | 2 | 0 | 1 | 1 | 1 | 0 | 0 | 5/20 | 75 % | 65 % |
| C | 6 | Infec.& PA | 20 | 1 | 2 | 4 | 0 | 1 | 0 | 0 | 8/20 | 60 % | 50 % |
| | 7 | Infec.& UC | 20 | 1 | 1 | 3 | 2 | 0 | 0 | 0 | 7/20 | 65 % | 55 % |
| | 8 | Infec.& Lev. | 20 | 2 | 2 | 3 | 0 | 1 | 1 | 0 | 9/20 | 55 % | 45 % |
| | 9 | Infec.& Tf. | 20 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 4/20 | 80 % | 70 % |
| D | 10 | PA | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/20 | 100% | - |
| | 11 | UC | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/20 | 100% | - |
| | 12 | Lev. | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/20 | 100% | - |
| | 13 | Tf. | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/20 | 100% | - |
| Con infec | 14 | Infec.& non-vacc. | 20 | 1 | 6 | 10 | 1 | 0 | 0 | 0 | 18/20 | 10% | - |
| vac. | 15 | Vacc.&non-infec. | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100% | - |
| blan | 16 | Non-vacc.& non-infec. | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 % | - |

Exp. No. = Experiment number. Gr. No. = Group number. Infec. = Infected group with vvIBDV.
Vacc.= Vaccinated group with live IBDV vaccine(D-78).
Con. Infec. = Control challenged only not vaccinated nor treated group.
Con. Vac. = Control vaccinated only not challenged nor treated group.
Control blank = Control non-infected non-vaccinated & non-treated group.
PA = Pind- avi UC = Ultracom® Lev. = Levamisole Tf. = Transferrin.
1 - No. Of dead birds/ No.of infected birds.
2 - Protection % = % survival of infected- vaccinated minus % survival of infected non-vaccinated or % survival of infected- vaccinated &treated with different immunostimulants minus % survival of infected non-vaccinated & non-treated.

**Table (3): Gross lesion mean score in dead or sacrificed birds after treatment with immunostimulants and/or vaccination.**

| Exp No. | | Bird Treatment | Gross lesions: Days post treatment with immuno-stimulants | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1st | | | | 2nd | | | | 3rd | | | | 4th | | | | 5th | | | | 6th | | | |
| | | | B | M | K | P | B | M | K | P | B | M | K | P | B | M | K | P | B | M | K | P | B | M | K | P |
| A | 1 | Infec & vacc. | 2 | 2 | 1 | 0 | 3 | 3 | 3 | 2 | 2 | 3 | 3 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 2 | 0 | 1 | 1 | 1 | 0 |
| B | 2 | Infec.& vacc.& PA | 1 | 0 | 1 | 1 | 2 | 1 | 2 | 0 | 2 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 |
| | 3 | Infec.& vacc.& UC | 1 | 0 | 1 | 1 | 2 | 1 | 2 | 0 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 |
| | 4 | Infec.& vacc.& Tf | 2 | 0 | 1 | 0 | 3 | 2 | 2 | 1 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | - | - | - | - |
| | 5 | Infec.& vacc.& Lev. | 2 | 1 | 0 | 0 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | - | - | - | - | - |
| C | 6 | Infec.& PA | 2 | 1 | 0 | 0 | 2 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | - | - | - | - | - |
| | 7 | Infec.& UC | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | - | - | - | - | - | - | - | - | - |
| | 8 | Infec.& Lev. | 2 | 1 | 2 | 0 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | - | - | - | - | - |
| | 9 | Infec.& Tf | 2 | 1 | 3 | 0 | 1 | 0 | 2 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | - | - | - | - | - |
| D | 10 | PA | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 11 | UC | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 12 | Lev. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 13 | Tf | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Con. infec. | 14 | Infec.& non-vacc | 2 | 1 | 3 | 0 | 3 | 3 | 3 | 2 | 3 | 2 | 3 | 1 | 2 | 2 | 2 | 1 | 1 | - | - | - | - | - | - | - | - |
| | | vacc | | | | | | | | | | | | | | | | | | | | | | | | | |
| Con. vac | 15 | Vacc.& non-infec. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | - | - | 0 | - | - | - | - |
| Con. blank | 16 | Non-vacc& non-infec. | - | - | - | 0 | - | - | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | - | - | - | - | - | - | - | - |

B = Bursal gross lesions :
0 : no gross lesions.
1 : congesuon of bursa or showing atrophy.
2 : gelatinous yellowish transudate covering the serosal surface wit oedma and hyperemia.
3 : enlargement with petechial or echymotic hemorrhage on the mucosal surface.

M = Muscle gross lesions :
0 : no gross lesion
1 : petcheal hemorrhage on thigh muscle or pectoral muscle.
2 : slight hemorrhage on thigh muscle and pectoral muscle.
3 : patches of hemorrhage on thigh muscle and pectoral muscle.

K = Kidney gross lesions :
0 : no gross lesions.
1 : slight nephritus.
2 : moderate nephritis and nephrosis in some lobes of kidneys.
3 : nephritis and nephrosis of the two kidneys.

P = Proventriculus gross lesions :
0 : no gross lesions.
1 : petecheal hemorrhage on junction between proventriculus and gizzared.
2 : petcheal & echymotic hemorrhage on junction between proventriculus and gizzare

Table (4): Severity index of bursal lymphoid tissue lesions of dead and scarified chickens after treatment with some immuno stimulants and /or vaccination with live IBDV vaccine (D-78).

| Exp. No. | Gr. No. | Bird Treatment | Bursal lymphoid tissue lesion at X days post treatment with some immunostimulants and/or vaccination represented by severity index | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1st day | | 2nd day | | 3rd day | | 4th day | | 5th day | | 6th day | |
| | | | L.N. | L.D. | L.N. | L.D. | L.N. | L.D. | L.N. | L.D. | L.N. | L.D. | L.N. | L.D |
| A | 1 | Infec & vacc. | 3 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | – | – | – | – |
| B | 2 | Infec.&vacc.& PA | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 2 | 2 | 2 | 2 |
| | 3 | Infec.&vacc.& UC | 3 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 |
| | 4 | Infec.&vacc.&Lev | 4 | 3 | 4 | 3 | 3 | 3 | 3 | 2 | 2 | 3 | – | - |
| | 5 | Infec.&vacc.& Tf. | 2 | 1 | 3 | 2 | 3 | 2 | 2 | 1 | 1 | 2 | - | - |
| C | 6 | Infec.& PA | 2 | 2 | 4 | 3 | 4 | 2 | 1 | 2 | 1 | 2 | – | - |
| | 7 | Infec.& UC | 1 | 1 | 3 | 2 | 3 | 2 | 2 | 2 | – | - | – | – |
| | 8 | Infec.& Lev. | 3 | 3 | 4 | 3 | 2 | 2 | 2 | 1 | 2 | 2 | – | – |
| | 9 | Infec.& Tf. | 1 | 2 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 0 | – | - |
| D | 10 | PA | - | - | - | - | - | - | - | - | - | - | - | - |
| | 11 | UC | - | - | - | - | - | - | - | - | - | - | - | - |
| | 12 | Lev. | - | - | - | - | - | - | - | - | - | - | - | - |
| | 13 | Tf. | - | - | - | - | - | - | - | - | - | - | - | - |
| Con. infec | 14 | Infec.& non-vacc. | 3 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | - | - | - | - |
| Con vac | 15 | Vacc.& non-infec. | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| Con blan | 16 | Non-vacc.& non-infec. | - | - | - | - | - | - | - | - | - | - | - | - |

Infec. = Infected group with vvIBDV. Vacc.= Vaccinated group with live IBDV vaccine(D-78).
Con. Infec. = Control infected only not vaccinated nor treated group.
Con. Vac. = Control vaccinated only not challenged nor treated group.
Control blank = Control non-infected non-vaccinated & non-treated group.
PA = Pind- avi UC = Ultracom® Lev. = Levamisole Tf. = Transferrin.
L. N. = Lymphocyte necrosis. L.D. = Lymphocyte depletion.

## DISCUSSION

Commercial flocks in IBD endemic areas may be protected against IBD by vaccination. Several types of commercial vaccines are available, but the proper age at which live virus vaccines should be administered is often difficult to determine. If the level of maternal antibody is high at the time of vaccination, the antibody may interfere with the vaccine virus and prevent the virus from establishing infection and initiating an immune response, or may prevent active immune response by negative feed-back mechanisms (Sharma and Rosenburger, 1987). On the other hand, if vaccination is delayed until after the maternal antibody has waned, there may be a crucial period during which passive and active immunity may be at subprotective levels (Sharma and Rosenburger, 1987); consequently, outbreak of the disease may occur inspite of vaccination.

In facing the ugly storm of IBD infection, it would be legal to use different tools of defense. This has initiated the current work as an attempt to overcome the destructive action of IBDV on humoral arm of immunity by two methods.

Firstly, it was necessary to investigate the efficiency of the intermediate IBD vaccine D-78 in emergency vaccination, experiment A. When ocular vaccination was adopted at 48 hour after infection with vvIBDV isolate in group 1 carrying severe 15% protection comparing to infected non-vaccinated group 14 (Table 2), severe gross lesions in bursa, muscles and kidneys (Table 3), bursalas well as 4 severity index in bursal histologic lesions (lymphoid necrosis and lymphoid depletion) (Table 4) (Fig. 1). T protection% against mortality might be attribu to interferon production, whereas infection pathogenic or non-pathogenic IBDV indu production of interferon that can be reached p levels within 2 to 3 days of virus infection (G et al., 1979 & Lukert and Saif, 1991). T challenge virus of IBDV induced interferon fo days then continued the production of interfe by emergency vaccinal strain resulting this low protection% but did not prevent the gross lesio in some internal organs or bursal tissues. T result accords with (Sultan, 1994) who conclud that emergency vaccination can thus be helpf when an outbreak occurs in one flock multi-house farms where protection of adjace houses appears necessary. Vaccination aft infection has entered a flock proved to have r value.

Secondly, it was important to evaluate th stimulation of the cellular branch of immuni (non-specific immune response) by differe immunostimulants as Pind-Avi, Ultracorn® Levamisole and Transferrin. When s/c injection o these immunostimulants was adopted 48 h post-infection revealed 50%, 55%, 45% and 70% protection%; respectively (Table 2). Also, the resulted in moderate (in Pind Avi & Ultracor treated groups), severe (in Levamisole treate group) and mild (in Transferrin treated group gross lesions in bursa, muscles, kidneys an proventriculus (Table 3). The results of histologi bursal lesions revealed that the severity index ha ranged 3, 2, 4 and 1.75 in infected treated group with Pind-Avi, Ultracorn®, Levamisole an Transferrin; respectively comparing to 4 i

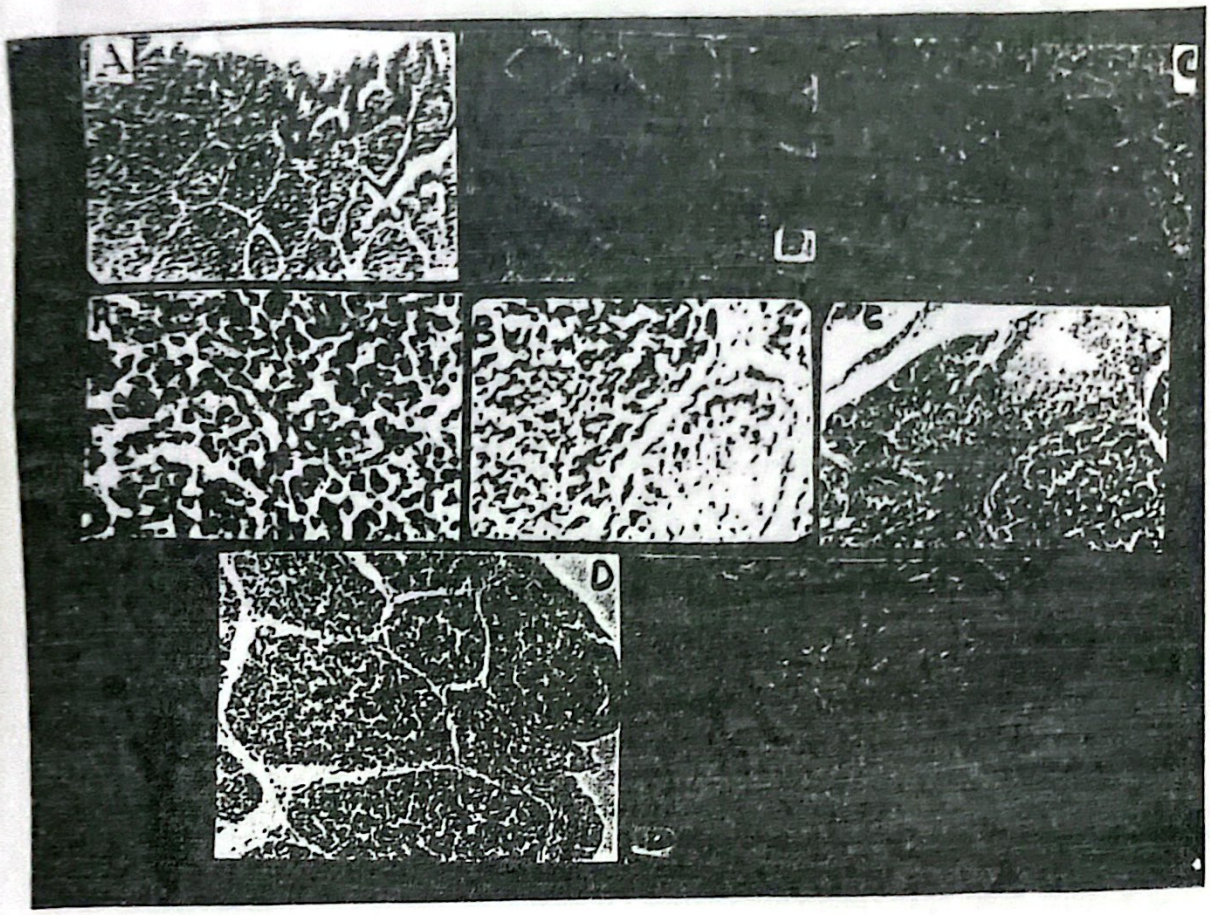



**Fig. (1)** : Sections of the bursa of Fabricus from birds of group (16), (15), (14) and group (1) at 5 days post- vaccination (H &E.X 4 & 10).

A- Bursa of control blank group (16), shows normal follicles and normal intrafollicullar separation.

B- Bursa of control vaccinated group (15) shows little intrafolicullar oedem (X4), infiltration of lymphocytes and heterophiles (X10).

C- Bursa of challenged birds with vvIBDV group (14) shows injured follic inflammatory oedema separates the follicles (X4). Central necrosis with depletion of lymphocytes and fibroplsia of the intrafollicular connective tissue(X10).

D- Bursa of birds 48hr vaccinated with intermediate D-78 vaccine post-infection with vvIBDV group (1) shows depletion of lymphoid center and hyperplasia of the bursal epithelium oedema within and around follicles and hemorrhage.

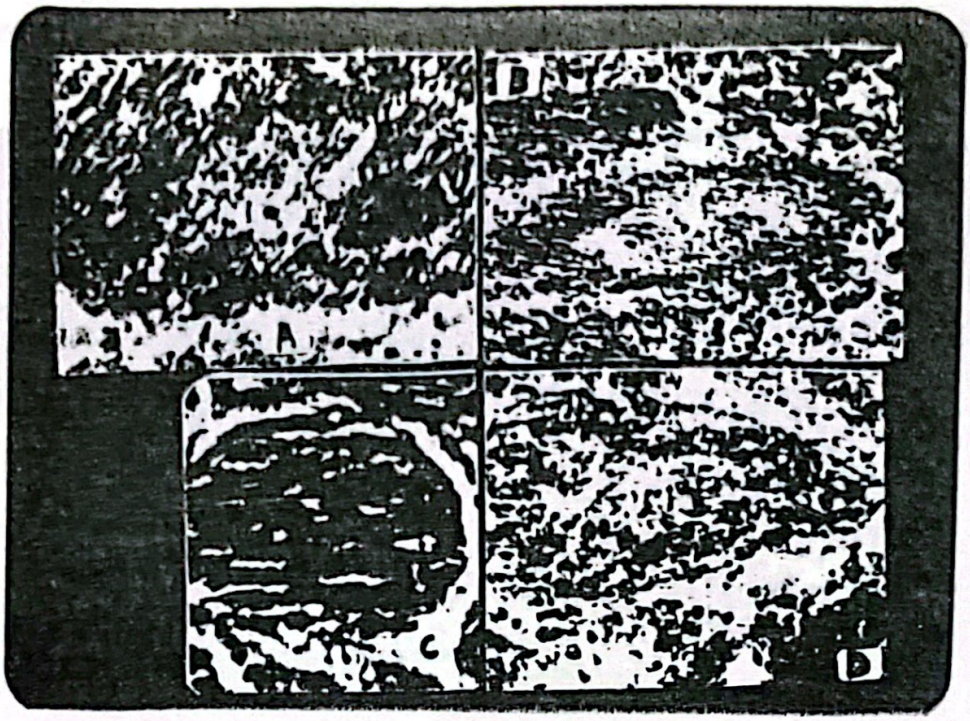



**Fig. (2) :** Sections of the bursa of Fabricus from birds of groups ( 2), (3), (4) and (5). At 5 days post-vaccination and treatment. (H & E X10).

A- Bursa of birds was emergenced vaccinated simultaneously with Pind-Avi 48hr post-infection with vvIBDV showing severe eosinophilic necrotic material together with pyknosis and karyolysis.

B- Bursa of birds was emergenced vaccinated simultaneously with Ultracorn® 48hr post-infection with vvIBDV showing depletion of lymphoid center which contains necrotic lymphocytic and eosinophilic debris.

C- Bursa of birds was emergenced vaccinated simultaneously with levamisole 48hr post-infection with vvIBDV showing marked necrosis and necrobiotic changes (few macrophages) with bursal hemorrhages .

D- Bursa of birds was emergenced vaccinated simultaneously with Transfermune 48hr post-infection with vvIBDV showing moderate follicular hyperplasia and marked activation of cortico-medullary reticular cells.

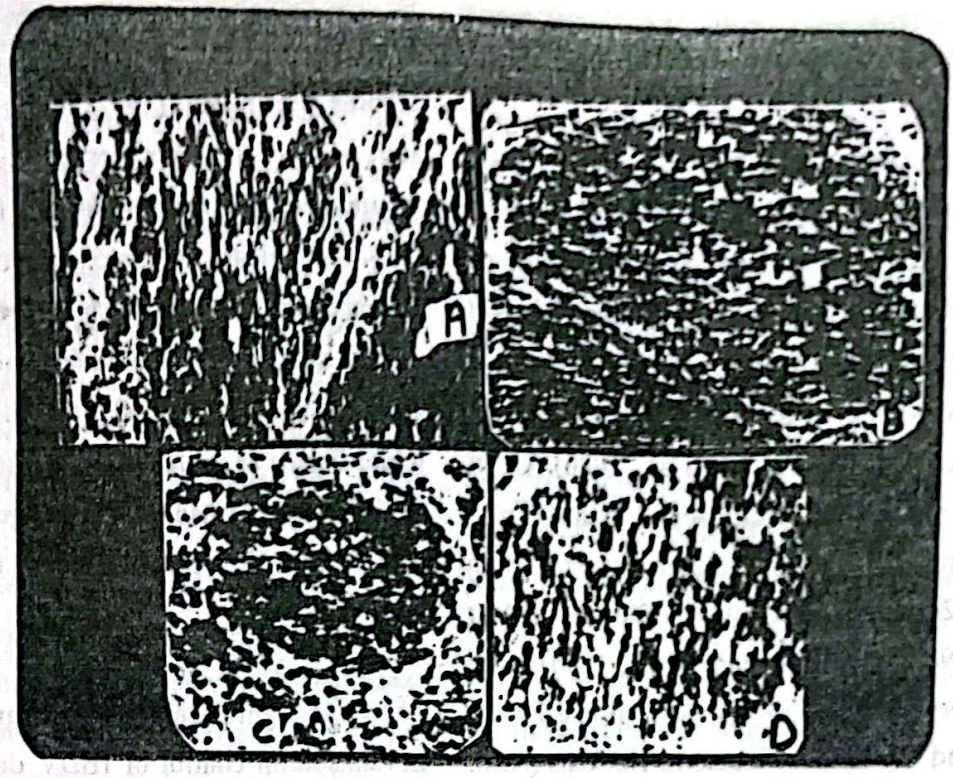


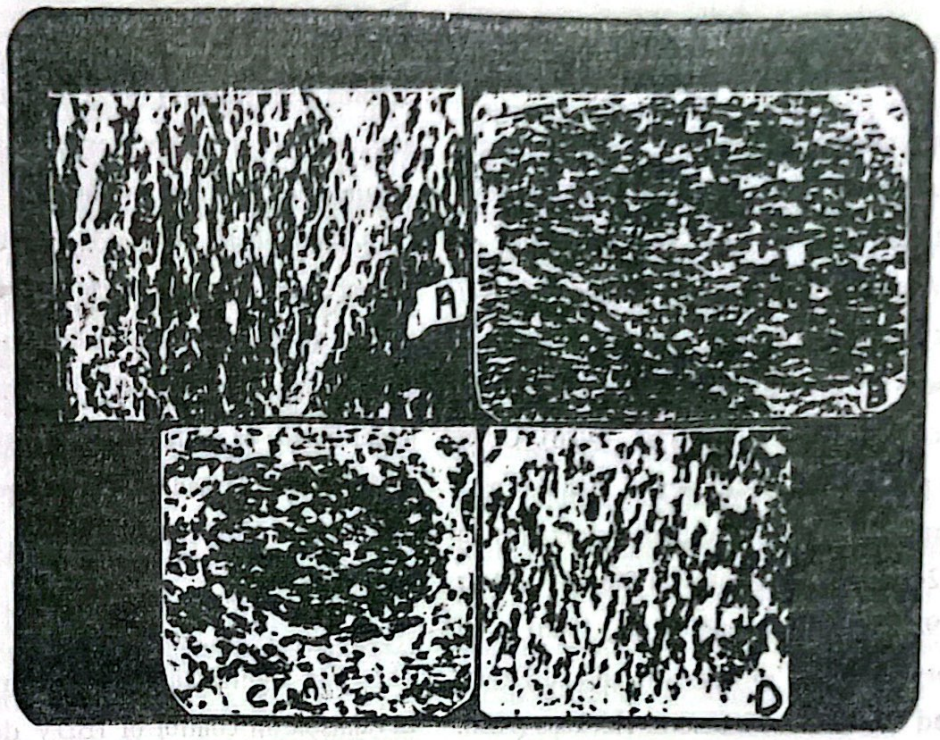

**Fig. (3)** : Sections of the bursa of Fabricus from birds of groups (6), (7), (8) and(9). At 5 days post- treatment. (H &E X10).

A- Bursa of birds treated with Pind-Avi 48hr post-infection with vvIBDV group (6) showing darkly staining corticomedullary areas within injured follicles.The space is full of purulent exudate with lymphocytic depletion and moderate necrosis.

B- Bursa of birds treated with Ultracorn® 48hr post-infection with vvIBDV group (7) showing moderate necrosis, lymphoctosis and heterophilic infiltration.

C- Bursa of birds treated with levamisole® 48hr post-infection with vvIBDV group (8) lymphocytic loss with dark staining cortical rims due to residual lymphocytic nuclear debris.

D- Bursa of birds treated with Transferrin 48hr post-infection with vvIBDV group (9) shows slight transfer central necrosis and lymphocytic depletion.

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infected non-treated group (Table 4) (Fig. 3).

The paraimmunization effect of Pind-Avi might be attributed to stimulation of spontaneous cell-mediated cytotoxicity and activation of macrophages & lymphocytes including T-helper lymphocytes which consequently activated B-lymphocytes plus its stimulation of cytolytic serum activity (Brunecher et al., 1986). Other previous studies on the efficacy of Pind-Avi as a paraimmunizer against vesicular stomatitis in mice in 24 hr pretreatment with Pind-Avi (Mayr et al., 1986 & Buttner and Mayr, 1986), viral respiratory infection in horses (Hell and Fisher, 1984) and Newcastle disease in chickens (Afifi, 1990) augment our findings.

Enhancement of protection against mortality and gross lesion development in Ultracorn® treated birds may be explained by the postulate of Ttizard (1984) that non-specific activation of macrophages occurs by Corynebacterium, which promotes antibody formation that is it promotes B-lymphocyte activity. Also, activated macrophages release large quantities of proteinases which activate the complement component and release interferon (non-specific antiviral) which in turn activates the natural killer cells. As well as activated macrophages release interleukin1 which stimulates T-helper cells, followed by activation of B-lymphocytes which synthesis immunogloblins. All these sequences end by complete enhancement of immune response. Our results agreed with Kutkat (1992) in improvement of protection of chickens against IBDV and Eid et al. (1995) in protection of chickens against fowl pox infection .

The highest poteniating effect of Transferrin (Tf.) accords with Kutkat (1992) might be due to the activation of lymphocytes by supplying iron requirement for transformation (Tormey et al., 192) that release mediator for enhancing the natural cytotoxic cells, which in turn eliminate the foreign antigens. On the other hand, Tf. inhibits virus attachment to susceptible host cells to a minor degree as postulated by Martin and Jandle (1959). Also Awaad (1975) suggested that Tf. constitutes a first line of defense in the face of foreign antigens.

The non-significant modulation effect of Levamisole in control of IBDV disagreed with Kodama et al. (1980) & Narang et al. (1994) who recorded an increase in the survival rate of chickens treated with Levamisole against Marek's disease virus infection, moreover, oral treatment of levamisole raised the protection% against Emeria tenella, (Onaga et al. 1984 and Afifi, 1990) who resulted improvement of protection rate against Newcastle disease in chickens. As well as results of Singth and Dhawedkar (1993) who concluded that Levamisole treatment may be useful to poultry farmer for prevention of diseases arising in birds immunosuppressed by subclinical IBD; Panda (1993 & 1994) who used Levamisole and vitamin E-selenium for controlling of IBD, Rao et al. (1994) & Amer et al. (1994) who proved that the best time of administration of Levamisole is 3 days after IBD vaccination.

The use of an emergency vaccination simultaneously with the immunostimulators improved the protection% if compared to vaccinated, nontreated group which showed a

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Transferrin simultaneously given with IBD vaccination recorded the best results of severity index (SI) of bursal lymphoid tissue lesion (SI=2) compared to infected vaccinated group (SI =4 ) & challenged group (SI = 4). Followed by Ultracorn with vaccine then Pind-Avi with vaccine, finally Levamisole with the vaccine (Table 4) (Fig. 2).

These results correlated to the results of gross lesion score of bursa, muscle, kidneys and proventriculus (Table 4). Previous literature indicated that these immunostimulants when simultaneously given with the vaccine improved the protection rate as a preventive tool but not as a control one (Afifi, 1990; Kutkat, 1992 and Eid, 1995).

The use of these immunostimulants had no damage effect on bursal tissue while they induced infiltration of monocytes in the lymphoid tissue (Fig.4) which are considered the first cells of immune response to prepare the immune system to defend against any foreign antigen.

Eventually, it is already established that the fate of the battle between the host and pathogen

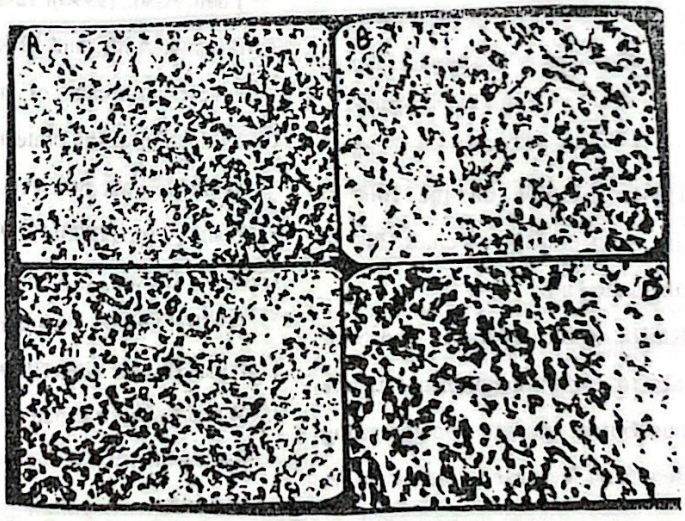


Fig. (4) : Sections of the bursa of Fabricus from birds of groups (10), (11), (12) and (13). At 5 days post-treatment. (H &E X10).

A- Bursa of birds treated with Pind-Avi group (10) showing macrophages and lymphocytic infiltration with mitotic figures.

B- Bursa of birds treated with Ultracorn® group (11) showing macrophages and lymphocytic infiltration with mitotic figures.

C- Bursa of birds treated with levamisole® group (12) showing macrophages and lymphocytic infiltration.

D- Bursa of birds treated with transferrin group (13) showing macrophages and lymphocytic infiltration with mitotic figures with high degree.

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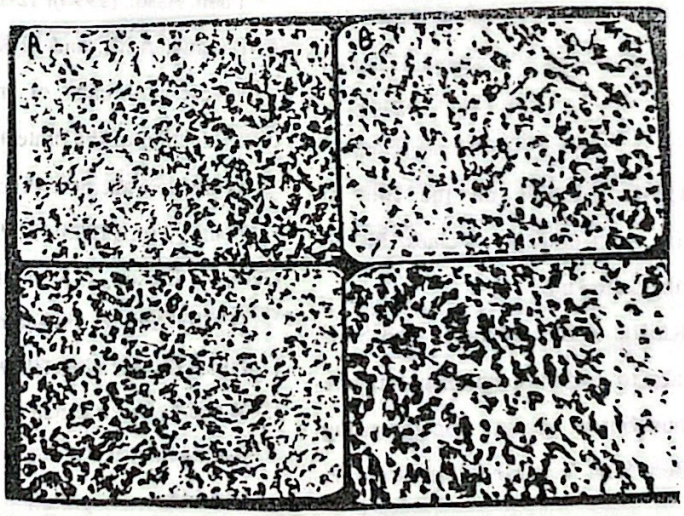

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depends mainly on two factors: the defense of the host and the virulence of the attacking agent, the former is expressed by the natural resistance (non-specific immunity) and immune status (specific immunity). Accordingly, the non-specific immunity could help in lowering the losses, as mentioned before, produced by IBD under experimental conditions. This is because the infection with IBDV compromises the humoral (specific immunity) and local immune system. The cellular immune system is also affected, but that effect is transient and of lower magnitude (Lukert and saif, 1991), So we can be able to stimmulate this part of immunity which consequently stimulates indirectly the humoral one, but does not repair the destroyed bursal lymphoid tissue lesions.

## REFERENCES


Afifi, Manal, A. (1990): Studies on the role of immunostimulants in using poultry vaccines. Ph. D. Thesis. Fac. Vet. Med., Cairo Univ.

Amer, M.M.; D.E.G. Khilfa; A.S.E. Metwalli and S.M. Gergis (1994): Trials to improve chicken immune response to infectious bursal disease vaccine using immunostimulants. Beni-Suef Vet. Med. Red., Vol. IV, No. 2/1, July.

Awaad, M.H. (1975): Studies on E.Coli infection in chickens. Ph. D. Thesis. Fac. Vet. Med., Cairo Unviersity.

Bezkorovainy, A.; Max, E.; Rafelson, J.R., and Vinay Likhite (1963): Isolation and partial characterization of transferrin from normal human plasma. Archives of Biochemistry and Biophysics, 103: 371-378.

Brunecher, M.G., Erfle, V., and Mayr, A. (1986): Comparison of the effect of viral paraimmunity inducers Pind-Avi and Pind-Orf with that of BCG, Corynebacterium parvium and levamisole on the growth of radiation induced murine oesteosarcoma. J. Vet. Med. B, 33: 188-195.

Buttner, M., and Mayr, A. (1986): Tests on protection against viral diseases. Can. Immun. Microbiol. Infect. Dis., 9 (213): 205-215.

Eid, G.; Waffa A. Zaghloul, A.H.; H. Awaad, M.A. Bastamy and A. Michael (1995): Role of Corynebacterium cutis as an immune stimulant on the immune response of chickens against fowl pox virus. Vet. Med. J., Giza, Vol. 43, No. 2: 219-229.

El-Batrawi, A.M. (1990): Studies on severe outbreaks of infectious bursal disease. I- The natural and experimental disease. Proc. 2nd Sci. Conf. Egypt. Vet. Poult. Asso., (1990): 12-14 March; p. 239-252.

El-Batrawi, A.M., and El-Kady, M.F. (1990): Studies on severe outbreaks of infectious bursal disease. II- Evaluation of the protectiveness of some commercially available vaccines. Proc. 2nd Sci. Conf. Egypt Vet. Poult. Asso., (1990): 12-14 March; P. 253-262.

Gelb, J.; Eidson, C.S.; Fletcher, O.J., and Kleven, S.H. (1979): Studies on interferon induction by infectious bursal disease virus (IBDV). II. Interferon production in White Leghorn chickens infected with an attenuated or pathogenic isolate of IBDV. Avian Dis., 23 (3): 634-645.

Goranov, K.H., and Bonovska, M. (1987): Enhancement by levamisole of the functional and cytochemical status of leukocytes and lysozyme in blood of sheep. Veterinaromeditsinski, Nauki, 24 (2): 72-76.

Garbar, P., and Williams, C.A. (1953): A method permitting the combined study of the electrophoretic and immunochemical properties of a mixture of proteins; application to blood serum. Biochem. Biophys. Acto., 10:193.

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Garbar, P., and Williams, C.A. (1953): A method permitting the combined study of the electrophoretic and immunochemical properties of a mixture of proteins; application to blood serum. Biochem. Biophys. Acto., 10:193.

ris, F. (1898): Carlton Histological Technique. 4th Ed. (1967), Oxford University Press, New York, Toronto.

H. And Fisher, J. (1984): Efficacy of active paraimmunization in horses by tracheal adniinistration of (Duphamunavi virus). Prakitische Tierarzt; 65 (11): 976-979.

kwood, D.H., and Saif, Y.M. (1987): Antigenic diversity of IBDV. Avian Dis., 31 (4): 766-770.

dama, M.; Mikami, T., and Izawa, M. (1980): Effect of Nat. levamisole on pathogenesis of Marek's disease. J. Cancer Inst. Vet. 65, No. 1: 155-159.

uby, J. (1994): In Immunology. Immune regulation and tolerance Chapter 16, pp. 370.

Kutkat, M.A. (1988): Studies on the control of Enterobacteriaceae in poultry. M.V.Sc. Thesis. Fac. Vet. Med., Cairo University.

Kutkat, M.A. AH. (1992): In vitro and in vivo studies of the effect of some biological preparations on certain poultry pathogens. Ph. D. Thesis (Poultry Disease), Fac. of Vet. Med., Cairo Unviersity.

Lukert, P.D., and Saif, Y.M. (1991): Infectious bursal disease. In: Diseases of Poultry, ninth edition. A.A.A.P., Iowa State University Press Ames, Iowa, USA, pp.648-663.

Martin, C.M., and Jandle, J.H. (1959): Inhibition of virus multiplication by transferrin. J. Invest., 38: 1024.

Mayr, A.; Buttner, M.; Pawlaw, S.; Erfle, V.; Mayr, B.; Brunner, R. and Osterkarm K. (1986): Comparative studies on the immunostimulating (paraimmunizing) effectiveness of BCG, Levamisole, Corynebacterium parvuum and preparations of pock viruses in various in vitro and in vivo tests. J. vet. Med. B., 33, 321-339.

Narang, G.; Mu, Kharol and Jagjit-Singh (1994): Effects of Levamisole hydrochloride along with herpes virus of turkey vaccine on shedding of Mark's disease virus from feather follicles. Indian Journal of Virology, 10 (1): 55-59.

Onaga, H.; Tajina, M., and Ishii, T. (1984): Effect of levamisole on the immune response of chickens to infection with Eimeria tenella. 261 Bakteriol. Mikrobiol. Orig., 256 (3): 322-327.

Panda, S.K. (1993): Effects of Levamisole and vitamin E-selenium on experimental infectious bursal disease virus infection. Indian J. of Vet. Pathology, 17 (2): 162-163.

Panda, S.K. and Rao, A.T. (1994): Effect of Levamisole on chicken infected with infectious bursal disease (IBD) virus. Indian Vet. J., 71; 5, 427-431.

Peters, J. (1968): In: Clinical Chemistry. Principals and Technics. Hoper-Row, New York, 14: 1147.

Rainard, F. (1986): Bacteriostasis of Escherichia coli by bovine lactoferrin, transferrin and immunoglobulins ($IgG_1$, $IgG_2$ & IgM) acting alone or in combination. Vet. Microbiol. Vet. Microbiol. 11: 103-115.

Rao-TVS; S.C. Mishra and TN. Taiswal (1994): Effect of Levamisole on complement levels in chicks vaccinated and challenge infected with IB virus. Indian Veterinary Journal, 71 (1): 8-11.

Roop, W.E., and Putram, F.W. (1967): Purification and properties of human transferrin C and a slow moving genetic variant. J. Biol. Chem., 242:2507-2513.

Sharma, J.M.; and Rosenburger, J.K. (1987): In: Avian Immunology: Basis and Practice. Infectious bursal disease and Reo virus infection of chickens. Immune reponse and vaccine control, Vol. II, chapter (10): 143-157.

Sharma, J.M.; Dohms, J.E., and Metz, A.L. (1989): Comparative pathogenesis of serotype 1 and variant serotype 1 isolates of infectious bursal disease virus and their effect on humoral and cellular immune competence of specific-pathogen free chickens. Avian dis., 33: 112-124.

Singh, K.C.P. and Dhawedkar, R.G. (1993): Immunomodulating effects of levamisole in chicks immunocompromised by infectious bursal disease virus. Tropical Animal Health and Production, 25 (1): 11-14.

ris, F. (1898): Carlton Histological Technique. 4th Ed. (1967), Oxford University Press, New York, Toronto.

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Sober, H.A.; Gutter, F.J.; Wyckaff, M.M., and Peterson, E.A. (1956): Chromatography of proteins. II. Fractionation of serum protein on anion-exchange cellulose. J. Am. Chem. Soc., 78: 756-763.

Soliman,R.; Reda, I.; Yossef, S.A.H., and Refai, M. (1991): Studies on the effect of ultracorn® on chicken immune response to Newcastle disease virus vaccine. J. Egypt. Vet. Med. ass., 51-387-400.

Sultan, H.A.E.R., (1994): Studies on infectious bursal disease in chickens Ph. D. Thesis, Fac. Vet. Med. Alexandria University.

Tizard, I. (1984): In: Immunology, An Introduction. Philadelphia, New York, Chicago, P. 289.

Tormy, D.C.; Imrie, R.C., and Muller, G.C. (1972): Identification of transferrin as lymphocyte growth promotor in human serum. Expl. Cell. Res., 74: 163.

Van Der Berg, T.P., and Meulemans, G. (1991): Acute infectious bursal disease in poultry: protection afforded by maternally derived antibodies and interference with live vaccination. Avian Pathology, 20: 409-421.

Wood, G.W.; Muskett, J.C.; Hebert, C.N., and Thornton, D.H. (1979): Standardization of the quantitative agar gel precipitation test for antibodies to infectious bursal disease. J. Biol. Stand., 7: 89-96.

Sober, H.A.; Gutter, F.J.; Wyckaff, M.M., and Peterson, E.A. (1956): Chromatography of proteins. II. Fractionation of serum protein on anion-exchange cellulose. J. Am. Chem. Soc., 78: 756-763.

Soliman,R.; Reda, I.; Yossef, S.A.H., and Refai, M. (1991): Studies on the effect of ultracorn® on chicken immune response to Newcastle disease virus vaccine. J. Egypt. Vet. Med. ass., 51-387-400.

Sultan, H.A.E.R., (1994): Studies on infectious bursal disease in chickens Ph. D. Thesis, Fac. Vet. Med. Alexandria University.

Tizard, I. (1984): In: Immunology, An Introduction. Philadelphia, New York, Chicago, P. 289.

Tormy, D.C.; Imrie, R.C., and Muller, G.C. (1972): Identification of transferrin as lymphocyte growth promotor in human serum. Expl. Cell. Res., 74: 163.

Van Der Berg, T.P., and Meulemans, G. (1991): Acute infectious bursal disease in poultry: protection afforded by maternally derived antibodies and interference with live vaccination. Avian Pathology, 20: 409-421.

Wood, G.W.; Muskett, J.C.; Hebert, C.N., and Thornton, D.H. (1979): Standardization of the quantitative agar gel precipitation test for antibodies to infectious bursal disease. J. Biol. Stand., 7: 89-96.