# Clinical, Hematological And Biochemical Alterations During The Coarse Of Induced IBDV In Native Saso Chicken Breed

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## ABSTRACT

160 day old chicks hatched from one source were used in the present study, 10 of them were slaughtered to determine the maternal antibodies against IBDV using AGPT. The remaining 150 day old chicks were divided into 3 equal groups each were divided into 2 aliquots one remained as control and the other portion was challenged with $10^3$ VP of IBDV via eye drops at the age of 17,21and 35 day of age.

Obtained result in first age point revealed no clinical symptoms or P.M lesions despite the positive AGP reaction of bursae for IBD viral antigen . But the bursal body weight index revealed immune depression starting from the 7th day of infection. This was also evident from the serology and VVNDV challenge results., the hematological change were anemia, decrease HB% , leuckocytosis and thrombocytophilia besides increase clotting and bleeding time with minor changes in liver and kidney functions

In group challenged with IBDV at 21 days of age results were nearly similar to those challenged at 7days of age but immuno suppression could be detected starting from 3rd day of infection.

In the 3rd age point clinical signs and P.M lesion characteristic for Gumboro were registered, total mortality reached 52% and immuno- suppression was detected in 5th day of challenge, there was a sharp increase in the uric acid and the creatinine levels beside sharp rise in the examined liver enzymes, but they returned to nearly normal levels in the 7th of IBDV challenged .

Results of pathological lesion score could be correlated with the results of BBI, as pathological score of 3.8 and higher values were associated with BBI values indicative of immunosuppression.

It is recorded in the present study that increase bleeding and clotting time was a constant finding in the three age points and there was no clinical disease or P.M lesion in the 1st or 2nd age point . this lead us to conclude that there is an age related resistance properity for IBD infection and this resistance is dependent on bursal ontogeny and maturation . And they should be over looked as initial factors in the pathogenesis of IBD.

## INTRODUCTION

Chickens are the only host known to develop clinical disease and distinct lesion following exposure to IBDV. The economic importance of this disease is manifested by mortality reaching 40%when infection occurs at 3 weeks age and immunosuppression *(1).* Several factors such as breed, genetic lineage , age, immune status, dose and route of inoculation , may influence gumboro disease severity

Managing disease abnormalities in birds requires an understanding of how disease condition changes the biological functions of the body ,because signs of illness frequently subtle *(3)* or may be in apparent as seen with IBD in early or late ages *(4,5)*.

Clinical chemistry is also necessary to evaluate cellular changes and adding clinical pathology data to the physical examination is important in diagnosing organopathies *(6)* .

The aim of the present work is to induce IBD at three age points (7,21, and 35 days of

age) and to study the clinical , hematological , biochemical, virological and histopathological changes after induction of IBD in native saso chicken breed

## MATERIAL AND METHODS

### Material

### Experimental chicks

160 chicks hatched from fertile native saso eggs were used in the present study.

### Lasota virus

NDV Lasota vaccinal strain" intervet", Batch 08809EJ01, was used, the vaccinated birds were receiving $10^3$ ELD50/0.1 ml of NDV Lasota via eye drops

### IBD challenge virus

Virulent IBDV field isolate, previously isolated and identified *(7)*, was used in the present study, the virus titer was $10^{5.5}$ EID50/0.1ml ,challenge dose was prepared to contain $10^3$ vp/dose

### VVNDV challenge virus

VVNDV previously isolated, identified and characterized *(8)* was used in the present study. The virus titer was $10^{7.7}$ EID50 /0.1 ml challenge dose was prepared to contain $10^3$ VP/dose

### IBDV reference precipitating antigen and antisera

IBDV ''serotype 1'' reference antigen and antisera were obtained from the international marketing company, Cairo, Egypt.

### Methods

### Experimental design

160 day old chicks hatched from one source were used in the present study, 10 of them were slaughtered to determine the maternal antibodies against IBDV using AGPT. The remaining 150 day old chicks were divided into 3 equal groups each were divided into 2 aliquots one remained as control and the other portion was challenged with $10^3$ VP of IBDV via eye drops at the age of 17,21and 35 day of age.

In every age point the clinical signs and PM lesions were registered, and at 3rd ,5thand 7th day of inoculation 5 control and 5 infected birds were weighted and their bleeding and clotting time was determined then they were slaughtered, portion of blood was collected on EDTA, while the other portion was used to collect sera . The later was used to study the liver and kidney function besides the serological test. Then the bursa was collected, weighted and divided into two portion one was preserved in 10% formalin to score the bursal lesion while other portion was kept for virological assay. The remaining birds were La sota vaccinated then challenged with VVNDV 10 days post vaccination ,these criteria were used similarly in the remaining two age points *(1)*.

### Clinical and post mortem examination

Experimentally inoculated chicks were examined daily for clinical signs,PM lesion was recorded during autopsy

### Hematological assay

Bleeding time *( 9)* , and clotting time *(10)* was performed, then birds were sacrificed individually to obtain blood. Blood films were prepared for differential leukocytic count. The erythrocytic, total leukocytic and thrombocytic counts were performed using Nutt and Herrick solution *(11)*. Hemoglobin estimation was performed *(12)*. Packed cell volume was estimated using the micro technique *(13)*. The mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) were calculated *(13)*.

### Clinico -chemical assay

Serum gamma glutamyl transferase (GGT) and aspertate aminotransferase (AST) were performed *(14)* as an indicative for liver functions, while serum uric acid and serum creatinine was done *(15)* as an indicative for kidney functions.

Table 1. Shows the experimental design

| Age in (days) | Sampling procedure | mAb for IBDV | Viral inocula | | | EVALUATION CRITERIA | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | A Structual | B Humoral | C Clinical chemistry | | | | D Hematological picture | | | | | | | | | | | | | | | E PATHOLOGY |
| | | | | | | | | Liver function | | Kidney function | | | | | | | | | Differential WBCs | | | | | | | | |
| | | | IBDV. Challenge | NDV Lasota | VVNDV | BB index | | GGT | AST | Uric acid | Creatinine | RBCs | Hb | MCV | MCH | MCHC | WBCS | PLT | Lym | Het | mon | Eos | Bas | BT | CT | |
| 1 | 10 S | + | | | | | | | | | | | | | | | | | | | | | | | | | |
| 7 | 10 S | + | + | | | | | | | | | | | | | | | | | | | | | | | | |
| 10 | 5 inf+5c | | | | | + | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 12 | 5 inf+5c | | | | | + | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 14 | 5 inf+5c | | | | | + | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 14 | 5 S | | | + | | | + | | | | | | | | | | | | | | | | | | | | |
| 23 | | | | | + | | + | | | | | | | | | | | | | | | | | | | | |
| 21 | 10 S | + | + | | | | | | | | | | | | | | | | | | | | | | | | |
| 24 | 5 inf+5c | | | | | + | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 26 | 5 inf+5c | | | | | + | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 28 | 5 inf+5c | | | | | + | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 28 | 5 S | | | + | | | + | | | | | | | | | | | | | | | | | | | | |
| 37 | | | | | + | | + | | | | | | | | | | | | | | | | | | | | |
| 35 | 10 S | + | + | | | | | | | | | | | | | | | | | | | | | | | | |
| 38 | 5 inf+5c | | | | | + | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 40 | 5 inf+5c | | | | | + | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 42 | 5 inf+5c | | | | | + | | + | + | + | + | + | + | + | + | + | + | + | + | + | | + | + | + | + | + | + |
| 42 | 5 S | | | + | | | + | | | | | | | | | | | | | | | | | | | | |
| 51 | | | | | + | | + | | | | | | | | | | | | | | | | | | | | |

BAS=Basophil, Eos=Eosinophil, het=heterophil, Lym=Lymphocyte, Mon=Monocytes, Plt=Plateletes, Tlc=white Corpusles, RBCs=Red Corpusles, Hb=Hemoglobin%, MCV=Mean corpuscular volume, MCH=mean corpuscular hemoglobin, MCHC=mean corpuscular hemoglobin concentration, B.P.score=bursal pathological score, B=Bursa, BBI=Bursal body wt index, NDV=New castle disease virus, IBDV=infectious bursal disease virus, VVNDV=Velogenic NDV, mAb= maternal antibodies Bt=bleeding time Ct= clotting time GGT=gamma glutamyl transferase AST=Aspartate amino transferase S=sera C=control Inf=infected . Control birds were similarly treated but they were not challenged with IBD.

**Evaluation of immunosuppression**

Immunosuppresion following challenge with IBDV was evaluated using HI for NDV *(16)*. Bursal body weight index(BBI) *(17)*. scoring the severity of bursal pathological lesion as described by *(17,18)*. Beside the challenge with VVNDV .

**Agar gel precipitation (AGPT)**

The test was performed as described previously *(19)*.

**Histopathological examination**

Bursal specimens were fixed in 10% formalin , and 5 u paraffin sections were prepared and stained with H&E *(20)* and examined with light microscope.

**Statistical analysis**

Data were statistically analyzed using MSTAT C computer program, F-value was used to determine significance *(21)*.

## RESULTS

Results of the present work are illustrated in Tables (2-6).

## DISCUSSION

In the present study the maternal antibodies (mAb) couldn`t be demonstrated when examined at 1,7,21or 35 days of age using AGPT (Table 1). results of AGPT compares favorably with results obtained using ELISA or VNT *(22)* ,antibody detection using VNT and AGPT gives a reasonable results *(23)*.

The clinical signs of IBDV is dependent upon the age ,breed, mAb level as well as the influence of IBDV strain *(1,24)* ,therefore the clinical picture may vary considerably from the farm ,region ,country or even continent to another.

Clinical IBD is most commonly recognized in susceptible 3-6 week old chicks in both field or experimental infection, day old chicks seldom show any clinical signs or mortality *(5)*. The severity of IBD is directly related to the number of susceptible cells present the bursa *(24)* ,therefore the highest age susceptability is between 3-6 week of age when bursae is at its maximum development

Chickens inoculated with IBDV 42 day post hatching developed severe clinical disease, whereas those inoculated 17 day post hatch remained clinically normal *(25)*, it has been found that IBDV infection in the first week caused severe defect in the humeral immune response *(26-29)*. In the present study the inoculated native saso chicks at 7 and 21 day of age did not express any clinical signs or mortality table (4). Group inoculated with IBDV at 35 days of age showed symptoms and P.M. lesion characteristic of IBD *(1)* , mortality reached 52%. Light breeds expresses high mortality when compared with heavy breeds *(1,24)*.

In the present study the rate of detection of IBDV antigen in the bursa using AGPT was of the same degree in the groups inoculated at 7, 21 or 35 day of age (Table 5) ., these results are in agreement with the previous findings *(5)*.

The evaluation of avian hemogram should involve counting of the various cells per micro liter of blood as well as the cytological evaluation of cells *(27)*. In the present study the hemogram of control birds were similar to the previous findings of for avian species *(30-33)* (Table 2) . In the present study Macrocytic normochromic anemia, decreased hemoglobin, increased bleeding and clotting time ,beside leucocytosis (lymphocytosis and heterophilia) was nearly a constant finding in the challenged groups at the three age points (Table 2),

Several authors (*1,22,34,35*) mentioned that the key to the pathogenesis of IBDV in birds of different ages may lie with the factors involved in the clotting of blood and or an immunologic injury and they mentioned that pathogenesis is not straight forward ,it is recorded in the present study that increased bleeding and clotting time were a constant finding in the three ages of challenge and there was no clinical disease or P.M lesions after the 1st or 2nd challenge despite the presence of IBDV antigens in the bursae of the

Table.2 Shows the mean ± SE of the native saso chicken`s hemogram following the challenge with IBDV at 7, 21,and 35 days of age

| Age (Day) | | RBCS *$10^6$/ul | HB Mg/dl | MCV fl | MCH | Pcv % | MCHC gm/dl | PLT *103/ul | BT sec | CT sec | TLC *103/ul | Differential | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Lym | Hete | Mon | Eos | Bas |
| INFECTED | 10 | 1.686 ±0.2651 | 6.98 ±0.365 | 126.8 ±0.175 | 41.2 ±0.19 | 21.2 ± 0.38 | 32.5 ±0.052 | 30.4 ±0.577 | 79.6 ±0.92 | 110.4 ±0.718 | 35.2 ±0.364 | 16.4 ±0.21 | 7.947 ±0.103 | 1.65 ±0.64 | 2.0 ±1.1 | 0.90 ±1.76 |
| | | ** | ** | * | | * | * | | | * | | ** | * | | | |
| | 12 | 1.706 ±0.3003 | 6.84 ±0.2708 | 122.5 ±0.1433 | 39.8 ±.0796 | 21.2 ±0.284 | 32.29 ±0.0384 | 27.6 ±0.375 | 187. ±0.612 | 207 ±0.5243 | 37.2 ±0.2475 | 20.5 ±0.23 | 8.867 ±0.797 | 2.37 ±0.70 | 3.6 ±0.52 | 0.38 ±3.4641 |
| | | * | *** | * | | | ** | | | *** | * | | | * | *** | |
| | 14 | 1.604 ±0.2082 | 4.92 ±0.2479 | 92.52 ±0.1291 | 30.748 ±0.121 | 15.04 ±0.244 | 31.728 ±0.1294 | 49.6 ±0.155 | 157.8 ±0.845 | 175.2 ±0.715 | 60.4 ±0.6303 | 41.5 ±0.91 | 14.41 ±0.693 | 1.427 ±2.07 | 1.42 ±2.07 | 3.56 ±1.4565 |
| | | *** | *** | | ** | ** | ** | *** | | ** | ** | * | * | | | |
| CONTROL | 10 | 2.7 ±0.4223 | 9.4 ±0.1403 | 104.314 ±0.2064 | 35.642 ±0.276 | 27.68 ±0.214 | 34.296 ±0.0793 | 22.8 ±0.505 | 46.8 ±0.971 | 64.8 ±0.5157 | 29.6 ±0.1762 | 25.5 ±0.23 | 7.84 ±0.041 | 3.44 ±0.68 | 2.77 ±0.91 | 1.4 ±1.7322 |
| | 12 | 2.596 ±0.5042 | 9.58 ±0.1565 | 100.268 ±0.2413 | 38.206 ±0.367 | 25.42 ±0.235 | 37.87 ±0.1437 | 26.8 ±0.226 | 129 ±0.728 | 64.8 ±0.5157 | 29.6 ±0.1762 | 15.7 ±0.37 | 8.467 ±0.591 | 1.12 ±1.87 | 1.14 ±0.21 | 0.3733 ±3.4641 |
| | 14 | 2.682 ±0.313 | 10.3 ±0.2013 | 90.144 ±0.0503 | 38.788 ±0.170 | 24.22 ±0.347 | 37.87 ±0.1437 | 28.8 ± 0.32 | 159 ±0.688 | 64.8 ±0.5157 | 29.6 ±0.2417 | 16.03 ±0.34 | 9.44 ±0.282 | 2.187 ±0.80 | 0.69 ±1.73 | 0.4533 ±3.4641 |
| INFECTED | 24 | 1.652 ±0.2039 | 7.28 ±0.1698 | 139.106 ±0.0580 | 44.128 ±0.053 | 22.94 ±0.163 | 31.73 ±0.0225 | 29.2 ±0.249 | 109 ±0.253 | 130.8 ±0.1929 | 60.8 ±0.2488 | 29.33 ±0.21 | 21.4 ±0.485 | 4.907 ±0.22 | 3.17 ±0.63 | 2.4533 ±0.2290 |
| | | *** | *** | *** | ** | *** | *** | ** | *** | *** | *** | *** | *** | | * | * |
| | 26 | 1.718 ±0.2157 | 7.66 ±0.2673 | 141.36 ±0.1121 | 44.5 ±0.070 | 24.36 ±0.303 | 31.508 ±0.0520 | 58 ±0.109 | 137 ±0.509 | 144.8 ±0.4777 | 61.2 ±0.1355 | 30.47 ±0.08 | 25.67 ±0.257 | 4.96 ±0.17 | 1.6 ±1.74 | 0 #DIV/0! |
| | | *** | ** | ** | ** | * | *** | *** | * | ** | *** | ** | *** | * | | |
| | 28 | 1.746 ±0.2118 | 7.72 ±0.2276 | 140.866 ±0.1167 | 44.206 ±0.063 | 24.54 ±0.256 | 31.656 ±0.0745 | 54.4 ±0.246 | 149 ±0.224 | 198 ±0.1807 | 52.8 ±0.2763 | 29.7 ±0.57 | 17.13 ±0.193 | 2.8 ±0.88 | 2.69 ±2.26 | 2.1066 ±0.3424 |
| | | *** | ** | ** | ** | * | ** | *** | ** | *** | *** | * | ** | | | |

Table 2. continued

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CONTROL | 24 | 2.522 ±0.2141 | 9.84 ±0.11 | 118.082 ±0.1213 | 39.554 ±0.126 | 29.38 ±0.110 | 33.468 ±0.0148 | 20 ±0.316 | 52.4 ±0.377 | 67 ±0.4206 | 23.2 ±0.3575 | 12.67 ±0.633 | 7.84 ±0.041 | 1.547 ±0.521 | 1.013 ±2.24 | 0.90667 ±2.1274 |
| | 26 | 2.522 ±0.2141 | 9.84 ±0.11 | 118.082 ±0.1213 | 39.554 ±0.126 | 29.38 ±0.110 | 33.468 ±0.0148 | 25.2 ±0.181 | 74 ±0.784 | 67 ±0.4206 | 28.4 ±0.2089 | 15.63 ±0.368 | 8.467 ±0.591 | 1.973 ±1.64 | 0.747 ±1.78 | 1.12 ±3.4641 |
| | 28 | 2.522 ±0.2141 | 9.84 ±0.11 | 118.082 ±0.1213 | 39.554 ±0.126 | 29.38 ±0.110 | 33.468 ±0.0148 | 26.8 ±0.309 | 105 ±0.260 | 67 ±0.4206 | 27.6 ±0.2788 | 11.8 ±0.396 | 9.44 ±0.282 | 2.027 ±0.241 | 1.013 ±0.24 | 0.98667 ±2.1164 |
| INFECTED | 38 | 2.254 ±0.2541 | 12.94 ±0.2163 | 175.198 ±0.0843 | 57.532 ±0.073 | 39.38 ±0.205 | 32.84 ±0.0161 | 59.6 ±0.167 | 214 ±0.388 | 315.6 ±0.2587 | 48.4 ±0.1226 | 22.23 ±0.504 | 18.27 ±0.091 | 3.707 ±0.887 | 1.893 ±0.12 | 1.84 ±1.9189 |
| | | *** | *** | *** | *** | ** | | ** | * | *** | *** | * | * | | * | |
| | 40 | 1.882 ±0.1745 | 10.34 ±0.2216 | 167.252 ±0.0675 | 54.762 ±0.069 | 31.74 ±0.267 | 32.414 ±0.0719 | 55.2 ±0.233 | 252 ±1.136 | 358 ±0.4845 | 48 ±0.3281 | 24.8 ±0.365 | 22.63 ±0.532 | 2.693 ±1.18 | 1.947 ±0.45 | 1.92 ±2.2912 |
| | | *** | *** | * | *** | *** | * | *** | | | *** | *** | * | | | | |
| | 42 | 1.936 ±0.1095 | 10.92 ±0.1892 | 171.07 ±0.0616 | 55.746 ±0.072 | 33.8 ±0.202 | 32.294 ±0.0681 | 53.2 ±0.335 | 234 ±0.858 | 336 ±0.5079 | 52 ±0.1961 | 25.6 ±0.176 | 16.97 ±0.278 | 4.267 ±1.126 | 2.773 ±0.87 | 2.08 ±0.1538 |
| | | *** | *** | *** | *** | ** | | *** | * | *** | *** | * | ** | | ** | * |
| CONTROL | 38 | 3.772 ±0.2323 | 16.2 ±0.2029 | 130.588 ±0.0685 | 43.02 ±0.063 | 49.18 ±0.206 | 32.942 ±0.0158 | 42.4 ±0.316 | 142 ±0.625 | 126.6 ±0.4284 | 32.8 ±0.2215 | 15.73 ±0.499 | 7.84 ±0.041 | 1.733 ±0.858 | 0.88 ±1.79 | 1.28 ±2.8284 |
| | 40 | 3.306 ±0.1947 | 17.64 ±0.1279 | 157.728 ±0.0793 | 43.02 ±0.063 | 52.08 ±0.176 | 33.98 ±0.0524 | 36 ±0.222 | 102.6 ±0.491 | 126.6 ±0.4284 | 27.2 ±0.223 | 13.27 ±0.122 | 8.467 ±0.591 | 1.787 ±1.305 | 1.2 ±2.14 | 1.09333 0.30463 |
| | 42 | 3.462 ±0.2639 | 15.14 ±0.1936 | 132.444 ±0.0814 | 43.02 ±0.063 | 45.68 ±0.195 | 33.132 ±0.0160 | 26.8 ±0.309 | 108.6 ±0.277 | 126.6 ±0.4284 | 31.2 ±0.3088 | 10.23 ±1.643 | 9.44 ±0.282 | 2.08 ±0.814 | 0.773 ±1.73 | 1.22667 ±0.1992 |

*p<0.05 **p<0.01 ***p<0.001 Se= standard error

Table.3 Shows the mean ± SE of clinic pathological parameters ,and BBI of the native saso chickens following the challenge with IBDV at 7, 21,and 35 days of age

| | Age(day) | B.WT | BUR.WT | B.BRATIO | B.B.I | URIC ACID | CREATININE | AST | GGT |
|---|---|---|---|---|---|---|---|---|---|
| INFECTED | 10 | 54.692 ±0.2613 | 0.1626 ±0.9633 | 0.003016 ±0.9277491 | 0.9668 ±0.93012 | 7.422 ±0.2548 | 0.62 ±0.254 | 339.3 ±0.1926 | 4.77 ±0.438 |
| | | | | | | | | | * |
| | 12 | 63.5 ±0.1313 | 0.3034 ±1.399 | 0.004822 ±1.4292195 | 1.24862 ±1.42976 | 10.32 ±0.4561 | 0.724 ±0.081947 | 379.58 ±0.3209 | 9.36 ±0.196 |
| | | * | | | | | | | *** |
| | 14 | 71.92 ±0.2913 | 0.1454 ±0.7629 | 0.002044 ±0.7691636 | 0.4092 ±0.7705 | 11.224 ±0.5741 | 0.5604 ±1.076127 | 433.52 ±0.2277 | 10.39 ±0.196 |
| | | * | ** | ** | ** | | | | *** |
| CONTROL | 10 | 62.972 ±0.4353 | 0.1866 ±0.4373 | 0.003114 ±0.7218593 | 1 ±0.72294 | 7.106 ±0.4727 | 0.64 ±0.289801 | 387.2 ±0.2073 | 3.438 ±0.199 |
| | 12 | 76.56 ±0.2512 | 0.2864 ±0.5788 | 0.003864 ±0.7754186 | 0.99924 ±0.77516 | 9.658 ±0.3243 | 0.772 ±0.22391 | 339.1 ±0.3952 | 6.38 ±0.183 |
| | 14 | 92.6 ±0.2419 | 0.4582 ±0.5712 | 0.004996 ±0.6211391 | 0.9988 ±0.62533 | 8.136 ±0.6449 | 0.792 ±0.074484 | 383.32 ±0.2683 | 5.784 ±0.03 |
| INFECTED | 24 | 128.54 ±0.1643 | 0.1462 ±0.4983 | 0.001142 ±0.5597905 | 0.34498 ±0.55869 | 8.308 ±0.2276 | 0.62 ±0.111745 | 244.1 ±0.1496 | 11.96 ±0.103 |
| | | * | *** | *** | *** | | * | | *** |
| | 26 | 163.5 ±0.204 | 0.1556 ±1.1877 | 0.00096 ±1.3511183 | 0.25686 ±1.34125 | 9.8246 ±0.1485 | 0.8 ±0.093541 | 288.54 ±0.2317 | 12.3 ±0.147 |
| | | | *** | *** | ** | *** | *** | | *** |
| | 28 | 170.8 ±0.1447 | 0.112 ±0.8875 | 0.0006464 ±0.7438964 | 0.18282 ±0.58367 | 9.56 ±0.1207 | 0.732 ±0.174308 | 279.2 ±0.2451 | 12.23 ±0.082 |
| | | * | *** | *** | *** | *** | ** | | ** |
| CONTROL | 24 | 172.02 ±0.3793 | 0.5668 ±0.3746 | 0.003314 ±0.2754236 | 1.0092 ±0.25772 | 7.238 ±0.2391 | 0.532 ±0.229292 | 225.76 ±0.2226 | 7.952 ±0.028 |
| | 26 | 181.5 ±0.0976 | 0.6808 ±0.3135 | 0.003754 ±0.3255669 | 0.8992 ±0.68597 | 6.064 ±0.2237 | 0.506 ±0.071802 | 266.16 ±0.3134 | 8.978 ±0.243 |
| | 28 | 194.8 ±0.11 | 0.66 ±0.258 | 0.00333 ±0.2877873 | 0.9236 ±0.40330 | 5.924 ±0.1843 | 0.484 ±0.075066 | 272.18 ±0.0371 | 8.932 ±0.131 |
| INFECTED | 38 | 194.2 ±0.3575 | 0.642 ±0.5681 | 0.003468 ±0.8185237 | 0.980525 ±0.94659 | 47.292 ±1.18 | 1.3225 ±0.450377 | 1072.5 ±0.4998 | 32.27 ±0.898 |
| | | *** | ** | | | * | | *** | * |
| | 40 | 229.8 ±0.14 | 0.576 ±0.6771 | 0.0025 ±0.6183591 | 0.59082 ±0.61772 | 8.408 ±0.3875 | 1.91 ±2.381457 | 471.14 ±0.3639 | 15.35 ±0.617 |
| | | *** | *** | ** | ** | * | | ** | |
| | 42 | 241 ±0.1096 | 0.43 ±0.5601 | 0.001782 ±0.5521383 | 0.41872 ±0.45073 | 8.408 ±0.3875 | 1.91 ±2.381457 | 471.14 ±0.3639 | 15.35 ±0.617 |
| | | *** | *** | *** | *** | * | | ** | |
| CONTROL | 38 | 312.6 ±0.1418 | 1.0924 ±0.2147 | 0.00353 ±0.3583989 | 0.99956 ±0.35867 | 6.084 ±0.5992 | 0.912 ±0.243411 | 347.04 ±0.2462 | 16.71 ±0.295 |
| | 40 | 330.4 ±0.208 | 1.38 ±0.189 | 0.004232 ±0.3560831 | 0.9982 ±0.35399 | 6.08 ±0.4363 | 0.982 ±0.089242 | 296.97 ±0.0893 | 16.06 ±0.408 |
| | 42 | 333 ±0.1319 | 1.32 ±0.29144 | 0.004084 ±0.2869250 | 0.9988 ±0.29025 | 6.08 ±0.4362554 | 0.982 ±0.0892419 | 296.97 ±0.08932 | 16.06 ±0.407 |

Table 4. Shows clinical symptoms, PM lesions after IBDV challenge at 7,21and 35 days of age, beside serological assay.

| Age (day) | Symptoms Post IBDV challenge | P.M. lesion | AGPT-IBD For sera Pre challenge | AGPT-IBD For sera Post challenge | AGPT for bursae Post IBDV challenge |
|---|---|---|---|---|---|
| 10 | No clinical signs could be observed | No characteristic lesion | 0 | 0/5 | 0/5 |
| 12 | | | 0 | 0/5 | 5/5 |
| 14 | | | 0 | 5/5 | 5/5 |
| 24 | No clinical signs could be observed | | 0 | 0/5 | 0/5 |
| 26 | | | 0 | 0/5 | 5/5 |
| 28 | | | 0 | 5/5 | 5/5 |
| 38 | On the 2nd day pi . one birds showed symptoms . Of IBD. , on the 3rd day pi. Two additional birds developed the disease ., on the 4th day pi. Four additional birds developed IBD, and they were badly ill so five of them were picked for experimental work , then from 5th to 8th day pi, 8 birds died (total mortality is thus considered 13 i.e. 52% ). | P.M. lesions characteristic of IBD as previously described (1) was observed | 0 | 0/5 | 2/5 |
| 40 | | | 0 | 0/5 | 5/5 |
| 42 | | | 0 | 5/5 | 5/5 |

Table 5. Shows mortality post IBDV challenge, mortality post VVNDV challenge,beside HI titer

| Age of challenge (day) | Mortality % post IBDV challenge | | Mortality post VVNDV challenge | | HI –GMT titer Pre-challenge with NDV | | HI-GMT titer Post-challenge with NDV | |
|---|---|---|---|---|---|---|---|---|
| | Infected | control | Infected | Control* | Infected | Control* | Infected | Control* |
| 7 | 0 | 0 | 60% | 20% | 2.6 | 6.3 | 5.1 | 10.5 |
| 21 | 0 | 0 | 60% | | 3.5 | | 6.2 | |
| 35 | 52% | 0 | 80% | | 4.4 | | 7.4 | |
| | | 0 | | | | | | |
| | | 0 | | | | | | |

Control birds were Lasota* vaccinated GMT=geometric mean titer

Challenge with VVNDVwas performed at23,37 and 51 day of age .10 days, observation was given

Table 6 Shows the mean pathological score in native saso chickens following infection with IBDV at 7,21and35 days of age

| Age point (days) | INFECTED | | | CONTROL | | |
|---|---|---|---|---|---|---|
| | Individual lesion score | Mean score | BBI | Individual lesion score | Mean score | BBI |
| 10 | (212131213) | 2.4 | 0.9 | (111101010) | 0.4 | 1 |
| 12 | (212141312) | 2.6 | 1.24 | (111101010) | 0.4 | 0.9 |
| 14 | (414141314) | 3.8 | 0.40 | (111101010 | 0.4 | 0.9 |
| 24 | (51512131$) | 3.8 | 0.34 | (111101010) | 0.4 | 1 |
| 26 | (515131314) | 4 | 0.25 | (110101010) | 0.2 | 0.9 |
| 28 | (515131414) | 4.2 | 0.18 | (110101010) | 0.2 | 0.923 |
| 38 | (313131313) | 3.4 | 0.9 | (111111010) | 0.6 | 0.9 |
| 40 | (515151515) | 5 | 0.5 | (111101010) | 0.4 | 0.9 |
| 42 | (513151515) | 4.8 | 0.4 | (111101010) | 0.4 | 0.9 |

three inoculated groups, and that clinical disease was only observed when challenge was performed at 35 days of age , this lead us to conclude that there is an age related resistance for IBD and it is dependent on bursal ontogeny and maturation ,this could be concluded when we consider the reduced pathogenesis of IBD after 70$^{th}$ day of age synchronized with the physiological involution of the bursa in one hand ,and the occurrence of IBD over 70 days of age when an oncogenic virus delays the bursal involution in the other hand, it is also worth to mention that bursectomized chicks don`t develop clinical IBDV*(24)*.

*(24)* interpreted the increased clotting time as follow., after the infection with IBDV there is a dramatic infiltration with T cells around the IBDV replication sites (bursa, spleen , caecal tonsils ) ,the activated T lymphocytes exhibit up regulation of cytokine genes that has an effect on macrophage function with an exacerbated production of premeditators such as (interferon, tumor necrosis factor α, interleukins 6 and interleukins 8 ), this causes a cytokine storm , which induces shock in birds, which in turn became prostrated and reluctant to move, and following this cytokine storm an inflammatory response ensue, this is believed to cause the increased clotting time.

Avian thrombocytes plays a primary role in hemostasis in a manner similar to the mammalian platelets in one hand ,and they perform a phagocytic function in the other hand .Normal thrombocyte count ranges between 20000 to 30000/ul. Thrombocytosis may reflect a rebound response following hemorrhage, or they may compensate for the increase bleeding and clotting time *(27)*, it should be noted that during the determination of clotting time using the glass capillaries ,clot was formed although delayed, this observation may lead us to conclude that hemorrhages seen on skeletal muscles requires brusing ,and increased clotting time well increase the brusing susceptability. .

There is wide variation in the normal leucograms among birds of the same species therefore the values of diagnostic importance must differ greatly from normal reference intervals which are generally much broader than those obtained from domestic mammals *(27)*.

Total leucocyte count greater than 10000/ul is suggestive of leucocytosis, leucocytosis was also a constant findings at all age points in the present study (Table 2). Generally this is due to localized or generalized infection *(6)*. Differential leukocytic count aid in the assessment of leukocytosis because when leukocytosis is caused by inflammation heterophilia is present. Heterophilia and leukocytosis can be associated with infectious agents ,lymphocytosis may be expected with antigenic stimulation associated with infectious agent *(6,27)*

Aspartate Aminotransferase (AST) high activity has been described in association with liver damage *(6)*. AST activity greater than 230 IU/L is considered abnormal and it is indicative of liver damage *(6,36-39)*. Gamma Glutamyl Transferase (GGT), Peptidase constitute a broad group of enzymes of varied specificity and some individual enzymes catalyze the transfer of amino acids from one peptide to another amino acid or peptide. GGT cleaves the gamma glutamyl group from peptides and moves them to an appropriate accepter. GGT is primarily a brush border enzymes with great activity in biliary and renal tubular epithelium, but serum activity of GGT is from biliary origin .Elevation of GGT have been described in association with liver disease *(3)* in the present study AST and GGT levels were constantly higher than in control birds but this increase was sharply higher when infection was induced at 35 days of age and began to subside 7 days post infection (Table3).

Blood creatinine is derived mainly from the catabolism of creatine found in muscle tissue, phosphocreatine is used to store energy in the muscle and its catabolism to creatinine occurs at a steady rate, excretion of creatinine occurs solely via kidney *(6)*, creatine is excreted in urine before it has been converted to creatinine so it is not providing accurate assessment for avian renal function, there is a slim margin between the physiological and pathological

level of creatinine., usually the physiological values are below the detectable range. Normal range of creatinine is 0.1-0.4mg /dl *(6)*. Pathologically severe kidney damage can raise creatinine specially if the filtration rate decreased.Uric acid is the major product of nitrogen catabolism, its synthesis occurs mainly in liver and in renal tubules *(6)*, 90%of blood uric acid is eliminated by tubular secretion, if the blood uric acid concentration exceeds its solubility in the serum, gout may occur, it should be noted that normal chicken uric acid level exceeds 6 times that of human due to the high sodium level in avian plasma and high avian body temperature, both factors increase the uric acid solubility in serum, thus normal uric acid level is 2-11 mgm/dl., Hyperuricemia is a good indicator of renal disease , hyperuricemia can be expected if glomerular filtration decreased below 70-80%.,decreased filtration may occur due dehydration from viral infection but in recent study elevated uric acid was not observed in racing pigeons that were deprived of water for four days *(6)*. In the present study significant alterations in creatinine level compared to control was observed when infection was induced at 35 day of age and it increased sharply 5 days PI and started to decline at the $7^{th}$ day, the uric acid level acted similarly (Table 3). The sharp rise of uric acid and creatinine and the rapid recovery is probably due to the blockage of ureters by the severely swollen and inflamed bursa following IBDV *(24)* , beside the nephrogenic affinity of IBDV *(1)*.

*(17)* used BBI as an indicator for immunosuppresion specially if it was lesser than (0.7), in the present study bursal atrophy was registered 7 days after induced infection at 7 days of age, and started at the $3^{rd}$ day PI when infection was induced at 21 days of age .,and was detected at the $5^{th}$ days PI when infection was induced at 35 day of age .these findings shows the consequences of IBDV infection at different ages (Table 3), these results are in full agreement with the previous findings (*5*). It should be noted that body weight progression in the experimental bird at the three age points reflects the variation in body weight between male and females encountered in native Saso breeds (A mix between native female and red bro-shaver male)

Pathological lesion score is a useful criteria that can be used parallel with BBI to determine the effect of IBDV on the chicken bursae especially when clinical and PM lesions are lacking (Table 6) ,the criteria for scoring the severity of bursal lesion were used *(17)*. In the present study the pathological lesion score in negative control was ranging from 0.2-0.4 but they were negative in AGPT for IBDV antigen (Table 4). Native saso chickens inoculated at $7^{th}$ day of age had bursal lesion score 2.4,2.6 and 3.8 at the $3^{rd}$ ,$5^{th}$ and $7^{th}$ day post challenge respectively ,while It was 3.8,4 and 4.2 at the same intervals when challenge was done at 21 day of age and it was 3.4,5 and 4.8 when challenge was performed at 35 day of age (Table 4). The severity of lesion score in native breeds was comparable to the previous findings of (*37*). In the present study we were able to observe that bursal lesion score 3.8 and over were associated with BBI suggestive of immunosuppresion (Table 6). It should be noted that serology results and challenge with VVNDV result were running parallel to the result of BBI and the pathological lesion score tables *(3-6)*.

## Conclusion

On the contrary to the previous findings, increased clotting time is not a determintal factor in the pathogencity of IBD.

There is an age related resistance properity for IBD and it is dependant on the bursal ontogeny and maturation

Hemorrahges seen on the skeletal muscles of infected birds is not spontaneous but it require brusing. Increased clotting time increases this brusing susceptibility.

The magnitude of the cytokine storm following IBD is responsible for the varied severity of IBD.

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## الملخص العربى

# التغيرات الاكلينيكية والبيوكيميائة والدموية الحادثة بعد العدوى الصناعية بفيروس الجمبورو فى الكتاكيت (الساسو)

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المعمل القومى للرقابة على الانتاج الداجنى بالشرقية * ، وحدة الباثولوجيا الاكلينيكية بمعمل بحوث صحة الحيوان بالزقا.زيق**

اجريت هذه الدراسه لمعرفه التغيرات الأكلينيكيه و البيوكيميائيه والباثولوجيه المصاحبه لعدوي الجامبورو المحدثه في الكتاكيت البلدي الساسو في اليوم (السابع-الواحد وعشرون-والخامس وثلأثون) – استخدم في هذه الدراسة مائه و ستون كتكوت تم تفريخهم من مصدر واحد لضمان تجانس المناعه الأميه قدرالأمكان و قد تم ذبح عشره منهم عند الفقس لدراسه المناعه الأميه المنقوله باستعمال اختبار الترسيب في الأجار الذي لم يدلل علي وجود اجسام مناعيه في الكتاكيت عمر يوم - اما باقي الكتاكيت فقد قسمت الي ثلأث مجموعات متساويه تتكون الواحده من خمسون كتكوت قسمت هي الأخري الي جزئين خمسه و عشرون ظابط و خمسه وعشرون تم تعريضهم لعدوى الجامبورو المحدثة حيث تم تم تقطير ١٠٠٠ وحده فيروسيه في العين من فيروس الجامبورو وذلك في كل مرحله من المراحل السابق الأشاره اليها-تم مراقبه الاعراض المرضيه علي الطيور وفي اليوم الثالث بعد الحقن تم البدء في رصدالتغيرات المزمع دراستها فتم وزن الطيور ثم تحديد زمن النزف و زمن التجلط ثم ذبح الطيور وتجميع جزء من الدم علي مانع تجلط لدراسه التغيرات الدمويه و الاخر استعمل لفصل السيرم الذى استعمل هو الاخر في دراسه وضائف الكبد والكلي والاختبارات السيرولوجيه و تم وزن الحويصله الفبريشيه ثم قسمت هي الاخرى الى جزئين جزء حفظ في الفورمالين لدراسه التغيرات الباثولوجيه والاخر حفظ ليستعمل في اختبار الترسيب في الاجار لتتبع وجود الفيروس وذلك بعد اجراء الصفه التشريحيه –وتم تكرار ذلك في اليوم الخامس والسابع من زمن الحقن وكرر هذا البروتوكول بعد العدوى فى عمر واحد وعشرون وبعد العدوى فى عمر خمسه وثلأثون يوم.

**جاءت النتائج على النحو الأتي**

**ا- في المرحله العمريه الاولي**

لم تظهر علي الطيور أي اعراض مرضيه ظاهريه او أي آفات تشريحيه ظاهريه بالرغم من وجود انتجين الجامبورو في الحويصله الفبريشيه ولكن لوحظ عند عمل معامل وزن الحوصله الفبريشيه الي الجسم حدوث تثنيط مناعي في اليوم السابع من العدوي وقد اكدت علي ذلك نتائج الاختبارات السيرولوجيه و اختبار صد العدوى بفيروس النيوكاسل الحشوى الضاري اما عن التغيرات الدمويه فقد لوحظ حدوث انيميا وانخفاض في نسبه الهيموجلوبين و زياده كرات الدم البيضاء و الصفائح الدمويه و كذلك زياده زمن النزف و التجلط و كانت التغيرات المرضيه طفيفه فى وضائق الكبد و الكلي.

**ب- في المرحله العمريه الثانيه**

جاءت النتائج مشابهه الي حد كبير تلك التي تم الحصول عليها فى نتائج المرحه الاولي فيماعدا ملاحظه حدوث التثبيط المناعي مبكرا حيث بدأ في اليوم الثالث بعد العدوى و استتمر في باقي مراحل الفحص.

**ج- المرحله العمريه الثالثه**

ظهرت في هذه المرحله الاعراض المميزه لمرض الجامبوروفي اليوم الثانى بعد الحقن و ازدادت حده المرض تدريجيا بحيث وصل اجمالي النفوق ٥٢ % و لوحظ حدوث التثبيط المناعي بدأمن اليوم الخامس للعدوى وقد لوحظ فى هذه المرحله حدوث ارتفاع حاد في مستوي حامض اليوريك و الكرياتينين وكذلك انزيمات الكبد في اليوم الخامس بعد العدوى و لكن عاد الي مستوى يقارب قيم ظابط التجربه في اليوم السابع بعد الحقن.

د- من دراسه المراجع السابقه التي تم الربط فيها بين ضراوه عدوى فيروس الجامبورو في مختلف المراحل العمريه و زياده زمن النزف و التجلط فانه في دراستنا هذه قد لوحظ ارتفاع زمن التجلط و النزف في كافه المراحل العمريه محل الدراسه و بدون حدوث نفوق او اعراض مرضيه ظاهريه او آفات تشريحيه فى المرحله العمريه الاولي و الثانيه مما جعلنا نستنتج ان ضراوة عدوي الجامبورو ترتبط بالعمر و قد ترتبط بنضج الحويصله الفبريشيه اذا ما وضعنا في الاعتبار انخفاض شده الاصابه بالجامبورو بعد عمر السبعين يوم من ناحيه .واستمرار امكانيه حدوث عدوى الجامبورو بعد عمر السبعين يوم عند الأصابه باحد الأمراض الفيروسيه التى تؤخر من ضمور الحوصله الفبريشيه .وعدم حدوث اعراض مرضيه فى الطيور التي استؤصلت منها الحوصله الفبريشيه .

ه- امكن عند رصد شده التغيرات الباثولوجيه فى الحوصله الفبريشيه الربط بينها وبين معامل وزن الحوصله الفبريشيه الى الجسم حيث لوحظ ان التثبيط المناعى الذى يحدث اذا ما كان هذا المعامل اقل من (٠,٧) يتطلب ان يكون متوسط شده التغيراتالباثولوجيه (٣,٨) او اكثر.

و- اثناء تحديد زمن النزف فى الطيور المصابه بالجامبورو لوحظ تكون الجلطه بالرغم من زيادة زمن تكونها .و لعدم ملاحظه الانزفه فى اي اماكن اخرى غير عضلات الساق والصدر فانه يمكن الاستنتاج بان تلك الانزفه ناتجه عن كدمه او رضوض وان طول زمن التجلط يساعد على تكونها.