

The therapeutic effect of non-specific immunoglobulins in controlling colisepticemia and Newcastle disease infections in chickens

Samy, M. M.*; Saad, A. E. M.; Hassanin, Z. A.W.*; Selim, A. A.*; Awaad, M. H.** and Bakry, H. H.

Faculty of veterinary Medicine Benha University

*Animal health Research Institute, Dokki

**Faculty of veterinary Medicine Cairo University

Abstract

The focus of this study was to assess the therapeutic effect of non-specific immunoglobulins (Igs) derived from bovine species in the control of colisepticemia and Newcastle disease infections in chickens. Bovine Igs were evaluated for the therapeutic effect against chicken colibacillosis and Newcastle disease infections. The obtained results revealed that bovine Igs had a potential therapeutic effect on chicken colibacillosis and Newcastle disease infections.

Key words: *Immunoglobulins, Non-specific immunoglobulins, colisepticemia, Newcastle, chicken therapy.*

Introduction

Poultry industry constitutes a significant sector of the world economy. The intensive poultry production provides a good media for viral and bacterial diseases to be established. *Newcastle disease (ND)* and *colibacillosis* amongst most viral and bacterial diseases that threaten poultry industry worldwide.

Velogenic ND (vND) outbreaks are extremely costly. Their control measures, including vaccination, represent a continuing loss to the industry (Leslie, 2000). In developing countries with endemic vND represents an important limiting factor in the development of commercial poultry production and the establishment of trade links. The constant losses from vND severely affect the quantity and quality of human food on marginal diets. Therefore, the economic impact of vND should not only be measured in direct losses in commerce but also the effect on human health and loss of potential socioeconomic growth (Spradbrow, 1992 and Sen *et al.*, 1998).

Escherichia coli (*E. coli*) infection is the most common enteropathogen that invades birds following vaccine application (Nakamura *et al.*, 1992 and Hassanein *et al.*, 2001). *E.coli* infection causes marked gross and microscopic bur-

sal and thymic lesions (Hassan and Hassanein 1999) that demonstrated the temporary nature of bursal atrophy which resulted in transient humoral immunosuppression.

The direction towards the use of environmentally friendly preparations in the therapy of poultry pathogens has been recommended by World Health Organization (WHO). Consequently; the usage of natural control methods was emerged. Already; several investigators reported on the use of bovine Igs as prophylaxis of colibacillosis in calves to control morbidity and mortality. They have been used in prevention of experimentally induced *colisepticemia* in calves (Cadman *et al.*, 1994).

Bovine Igs have been also used in prevention and control of chicken colibacillosis in chickens (Awaad, 1975 and Youseif *et al.*, 2005).

The present investigation is dedicated to study the possible therapeutic effectiveness of concomitant usage of bovine Igs-rich fraction on both chicken *colisepticemia* and vND infections.

Material and Method

Preparation of bovine rich fraction from calf serum:

It was carried out according to Awaad (1975) and Shepherd and Dean (2000) as follows;

bovine serum was concentrated using 40% ammonium sulphate to precipitate IgG. The volume of the serum to be fractionated was placed in a beaker to which slowly added equal volume of 80% ammonium sulphate solution while gently stirred. The mixture was left for four hours at room temperature. The precipitated globulin was collected by centrifugation at 6000 rpm for 30 minutes in cooling centrifuge. The supernatant fluid was decanted. The precipitate was resuspended in distilled water to the original volume of serum. The precipitation was repeated three times as outline before. The third precipitated globulin was resuspended in half the original volume of serum. The globulin solution was placed in a dialysis bag of visking tubing (27/32 size) and ammonium sulphate was removed by dialyzing at 4°C against frequent changes of 0.85% NaCl PH 8.0, until barium chloride test gave negative result. The globulin solution was removed from the dialysis bag and sterilized by filtration (0.45 swirex filter). After preparation of the IgG-rich fractions, it was dispensed aseptically in a sterile plastic 50 ml bottles and stored at -20°C.

Sterilization of bovine immunoglobulins: According to (Cruickshank *et al.*, 1970 and Awaad, 1975).

Experimental Design

Experiment 1:

Effect of bovine immunoglobulins on the treatment of experimentally infected broiler chicks with *E.coli serogroup O1*.

Fifty-five day old broiler chicks obtained from a commercial hatchery and produced from *E.coli serogroup O1* unvaccinated parents were used in this experiment. Five chicks were randomly selected, sacrificed and subjected to bacteriological examination to prove that they were free from any bacterial infections specially *E.coli serogroup O1*. The birds were floor reared and given food and water ad libitum. The chicks were not vaccinated against any viral infection. Thirty chicks were divided into two groups and kept in separate cages and each was inoculated with *E.coli serogroup O1* in a dose of 1.5×10^8 CFU/bird orally at 5 days of age. The remaining chicks were divided into 2 groups consisting of 10 chicks each. One group was injected with Igs only while the other one was kept as control. Three days post injection the chicks of the 1st and 3rd groups started to show clinical signs (off-food, ruffling of feathers, huddling together, loss of body weight followed by increase mortalities) The 1st group was injected with bovine Igs when mortalities began (Table 1).

Table (1). Study the effect of bovine immunoglobulins on the treatment of experimentally infected broiler chicks with *E. coli serogroup O1*

Groups	No. of chicks	<i>E.coli serogroup O1</i> ** injection	Ig Injection*
1 (Infected treated)	15	+	+
2 (Non infected treated)	10	-	+
3 (Infected untreated)	15	+	-
4 (Blank control)	10	-	-

* Ig injected S/C with 0.15 mg/dl concentration after appearance of 1st clinical signs.

** *E.coli serogroup O1* orally injected with 1.5×10^8 CFU/bird at 5 days old.

+ = Injected

- = Not injected

Experiment 2:

Studying the therapeutic effect of bovine immunoglobulins in *NDV* infected broiler chickens.

Fifty-five, day old broiler chickens obtained from a commercial hatchery were used. Five chicks were sacrificed and examined bacte-

riologically to detect any bacterial infection while the remaining chicks were kept under observation for detection of any clinical signs. Thirty chicks were divided into two groups and kept in separate cages and each was inoculated with 0.5 ml of 10^3 *NDV* I/M at 30 days old. The remaining chicks were divided into two

groups of 10 each. One group was injected with bovine Igs only while the other kept as

negative control (Table 2).

Table (2). Study the effect of bovine immunoglobulins on the treatment of *NDV* infection in broiler chickens.

Groups	No. of chicks	<i>NDV</i> inoculation*	Ig inoculation**
1 (Infected treated)	15	+	+
2 (Non infected treated)	10	-	+
3 (Infected untreated)	15	+	-
4 (Blank control)	10	-	-

**NDV* inoculated I/M 0.5 ml at 30 days old.

**Ig injected S/C with 0.15 mg/dl concentration after appearance of symptoms.

+ = Injected

- = Not injected

Results

Experiment 1:

Effect of bovine immunoglobulins on the treatment of experimentally infected broiler chicks with *E.coli serogroup O1*.

The results of the therapeutic effect of bovine immunoglobulins on experimentally infected broiler chicks with *E.coli serogroup O1* are shown in Tables (3) and figure (1). It was clear that 3 days post inoculation with *E.coli serogroup O1* the chicks of the 1st and 3rd groups began to show clinical signs including off-food, ruffled feathers, huddling together, loss of body weight followed by increased mortalities. After the appearance of the 1st mortality

the 1st group was injected with a dose of 0.15mg/dl/bird bovine immunoglobulins. After Igs inoculation; the clinical signs decreased and the vitality of the birds increased till reached normal and the mortalities decreased in comparison with the untreated infected group. After Igs treatment; 2 birds / group were randomly taken weekly, sacrificed and subjected to postmortem examination as well as subjected to bacteriological examination. Birds of group 3 (infected untreated) showed septicemia manifested as congestion of parenchymatous organs with petechial or ecchymotic hemorrhages in heart, liver, spleen and kidneys with lung congestion and airsacculitis.

Table (3). The therapeutic effect of bovine immunoglobulins on the treatment of experimentally infected broiler chicks with *E.coli serogroup O1*.

Groups	No. of chicks	<i>E.coli serogroup O1</i> injection*	Igs Injection**	Morbidity %	Mortality	Mortality %
1 (Infected treated)	15	+	+	100%	2	13%
2 (Non infected treated)	10	-	+	0%	0	0%
3 (Infected untreated)	15	+	-	100%	6	40%
4 (Blank control)	10	-	-	0%	0	0%

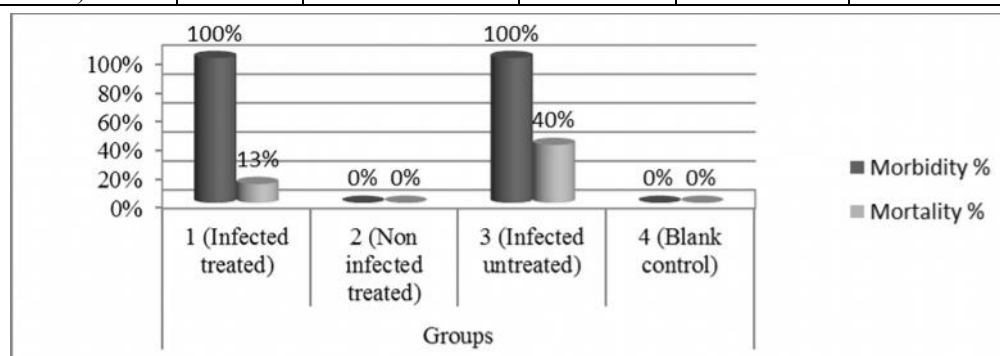


Figure (1). Histogram of the morbidity and mortality percentages of studying the therapeutic effect of Igs in experimentally infected chickens with *E.coli serogroup O1*

Histopathological findings; the liver of the infected untreated group showed severe degenerative changes (Fig. 2 a and b), with severe congestion, mononuclear infiltration with compensatory air capillaries in the lung (Fig. 2 c and d). The heart showed edema, mononuclear infiltration, myocarditis and pericarditis (Fig. 2 e). The bursa showed depletion with cystic formation and heterophylic infiltration (Fig. 2 f). The Igs treated group showed only degenerative changes in liver in the 2nd week post treatment (Fig. 2 g and h) which became mild on examination at the 3rd week (Fig. 2 i). The non infected treated group showed normal histomorphological finding at all examined weeks (Fig. 2 j and k).

Experiment 2:

Studying the therapeutic effect of bovine immunoglobulins in *NDV* infected broiler chickens.

The results of the effect of bovine immunoglobulins on the treatment of *NDV* infection in broiler chickens are illustrated in table (4) and figure (3). Nine days post *NDV* injection

the chicks of the 1st and the 2nd groups began to show clinical signs manifested as off-food, greenish diarrhea followed by nervous manifestations in the form of torticollis, trempling, ataxia, with daily mortality, as shown in table (4). The 2nd group was injected with bovine immunoglobulins 2 days after appearance of 1st clinical signs. After bovine Ig inoculation in the second group at the day 41 of age there was marked decrease in mortalities within 2 days post inoculation and the clinical signs began to decrease by time and the birds returned to their normal feed and water consumption and their vitality became normal even those birds that were showing nervous manifestations (like torticollis and tremors) improved clinically within 2 weeks post inoculation.

Histopathological findings; the *NDV* infected untreated group showed severe pneumonia in lung (Fig. 4a and b) with degenerative changes in liver (Fig. 4c and d) and severe bursitis with depletion in bursa (Fig. 4e). The *NDV* infected and Igs treated

Table (4). Shows the effect of bovine immunoglobulins on the treatment of *NDV* infection in broiler chickens

Groups	No. of chicks	<i>NDV</i> inoculation*	Ig inoculation**	Morbidity%	Mortality no.	Mortality%
1	15	+	+	100%	2	13%
2	10	-	+	0%	0	0%
3	15	+	-	100%	15	100%
4	10	-	-	0%	0	0%

*Each chick inoculated with 0.5 ml *NDV* intranasally at 30 days old.

** Ig inoculated with 0.15 mg/dl S/C at 41 days old.

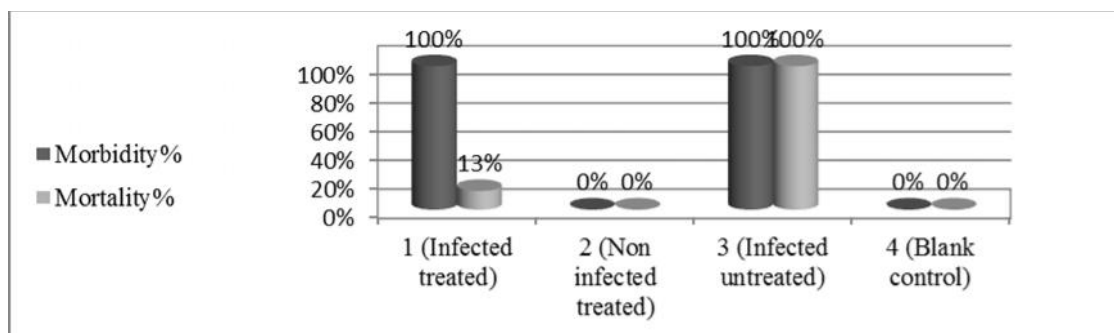


Figure (3). The morbidity and the mortality rates of different groups in experiment 2.

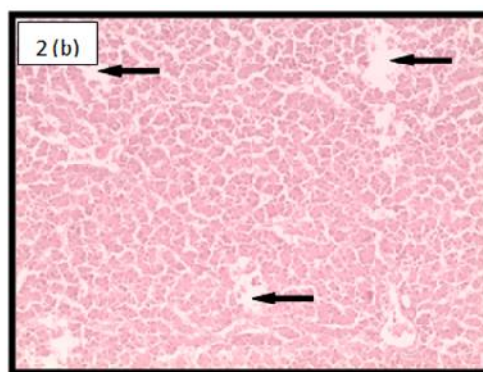
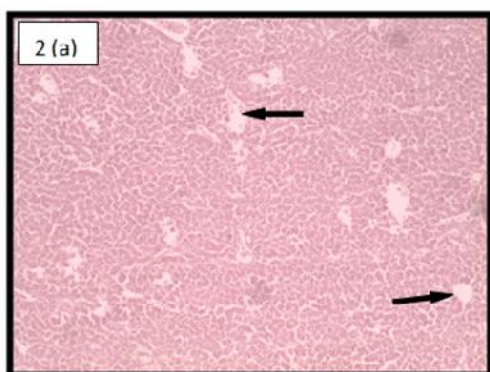


Fig. (2a and b): Degenerative changes in liver of the *E. coli* infected Igs untreated group (H&E X10 and X20 respectively).

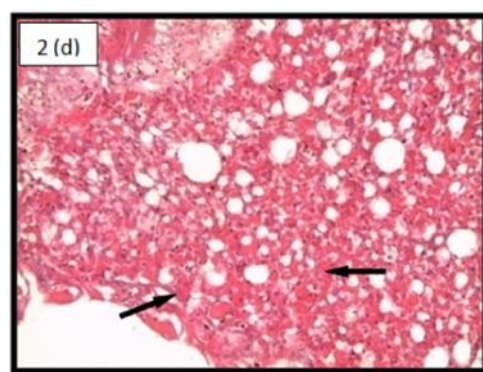
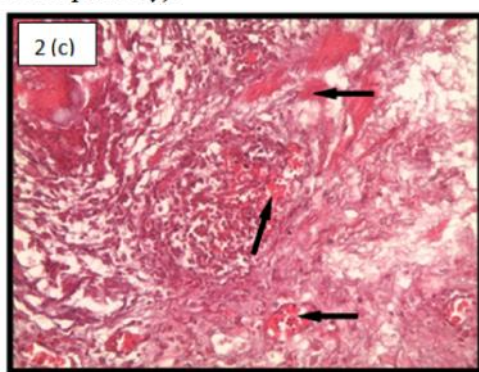


Fig. (2c and d): Severe congestion, mononuclear infiltration with compensatory air capillaries in the lung of the *E. coli* infected Igs untreated group (H&E X10 and X20 respectively).

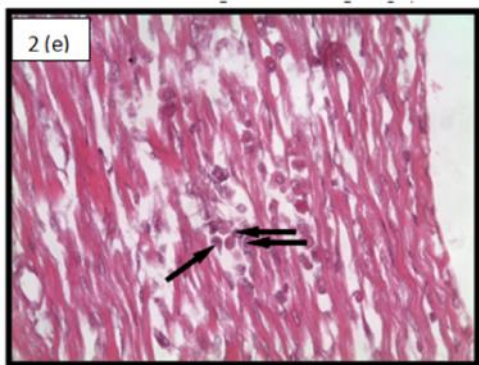


Fig. (2e): The *E. coli* infected Igs untreated group shows edema, mononuclear infiltration (arrows), myocarditis and pericarditis (H&E X20).

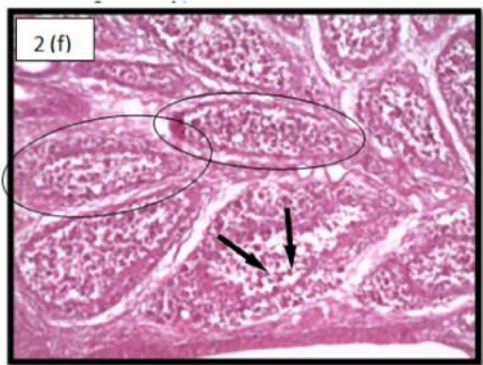


Fig. (2f): The *E. coli* infected Igs untreated group shows depletion of bursa with cystic (black circles) and heterophylic infiltration (arrows) (H&E X10).

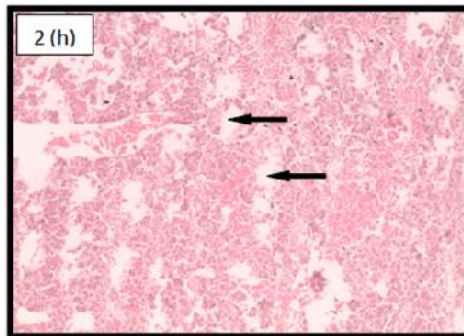
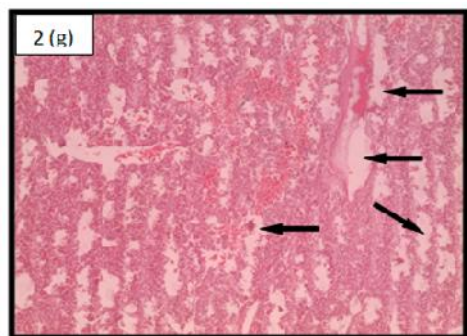


Fig. (2g and h): The *E. coli* infected and Igs treated group shows degenerative changes in the liver the 2nd week post treatment (H&E X10 and X20 respectively).

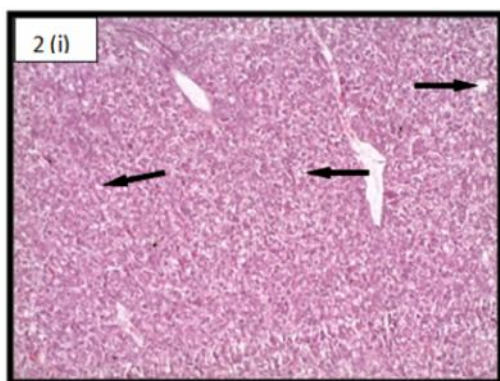


Fig (2i): The *E.coli* infected and Igs treated group shows mild degenerative changes in the liver at the 3rd week post treatment (H&E X10).

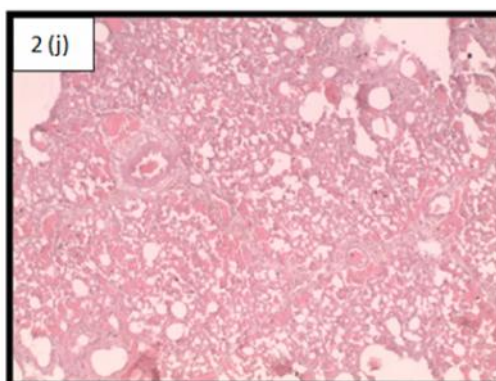


Fig. (2j): The *E.coli* infected and Igs treated group shows normal lung at the 3rd week post treatment (H&E X10).

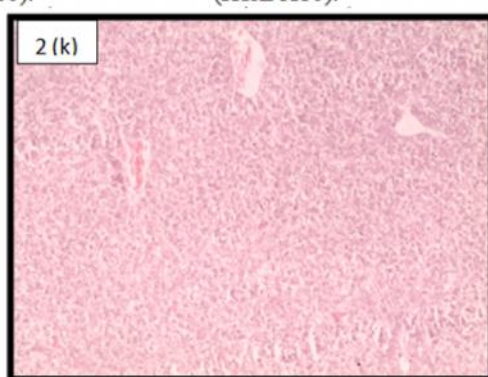


Fig. (2k): The *E.coli* non infected and Igs treated group shows normal liver (H&E X10).

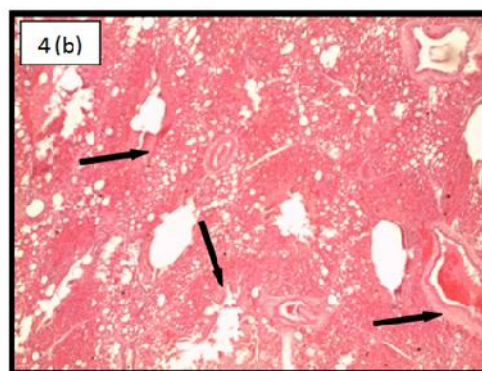
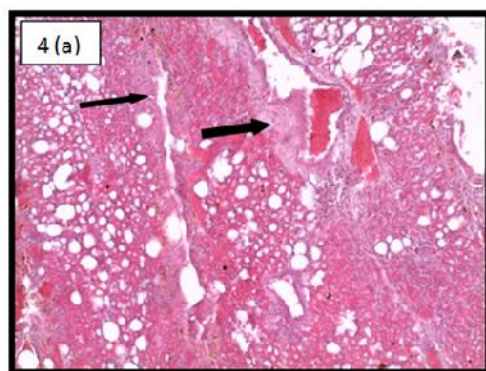


Fig. (4a and b): The *NDV* infected untreated group shows severe pneumonia in the lung (H&E X10)

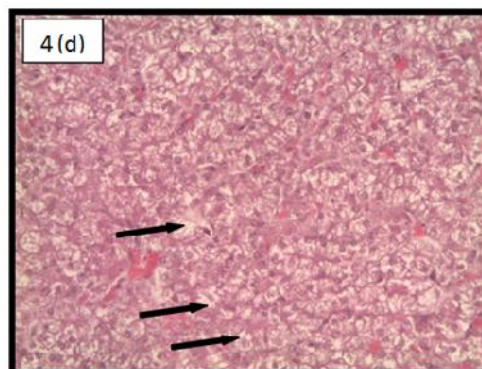
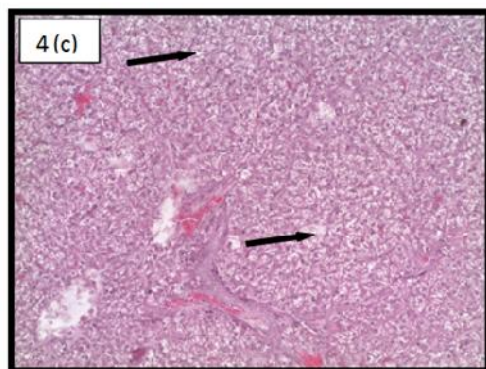


Fig. (4c and d): The *NDV* infected untreated group shows degenerative changes in the liver (H&E X10 and X20 respectively).

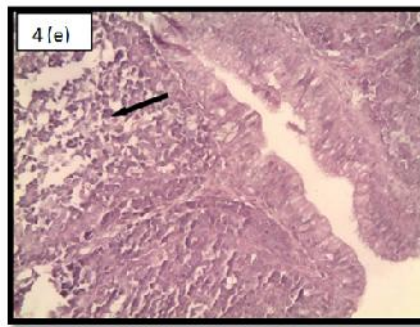


Fig. (4e): The *NDV* infected untreated group shows severe bursitis with depletion in the bursa (H&E X10 and X20 respectively).

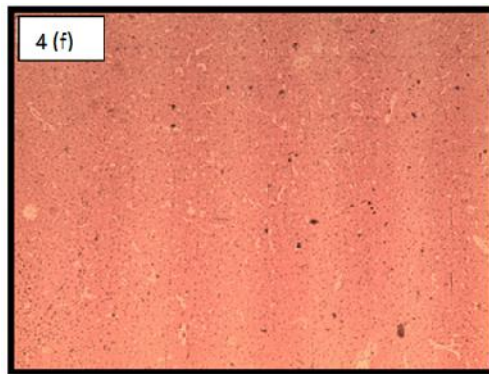


Fig. (4f): The *NDV* infected and IgS treated group shows normal liver (H&E X10).

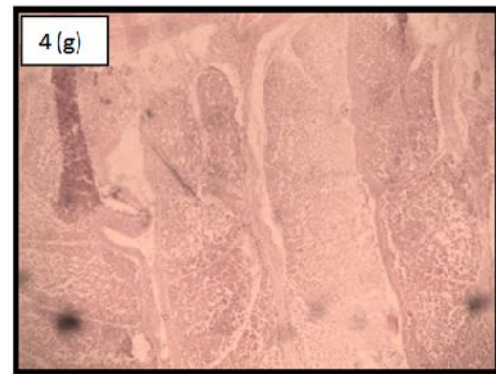


Fig. (4g): The *NDV* infected and IgS treated group shows normal bursa (H&E X10).

Discussion

The treatment of infectious diseases are increasingly complicated by the development of antibiotic resistant organisms. The emergence and rapid dissemination of new pathogens and the growing incidence of opportunistic infectious agent identification and commercialization of new antimicrobial agents has always been a time consuming and expensive process. Development of new strategies to control diseases are therefore clearly warranted.

Bovine immunoglobulins (Igs) are a revolutionary new protein source that is based on Igs derived from bovine serum. Bovine serum is rich in naturally occurring bioactive proteins such as immunoglobulins and peptides which boost immunity and accelerate lean tissue healing in animals. The effect of specific and non-specific gamma-globulins in prophylaxis or treatment against certain pathogens in animals and human had been studied by many investigators (Walser and Brumer, 1967; Corbella, 1970; Signorini, 1970; Penhale *et al.*, 1971; Logan *et al.*, 1974; Cadman *et al.*,

1994; Takemura *et al.*, 2004; Cook *et al.*, 2007; Kyoung-Sik *et al.*, 2009; Yuzhuet *et al.*, 2009; Tamilzarasan *et al.*, 2009 and Yegani and Korver, 2010). Their usage in the controlling of *E.coli* infection in avian species was already studied by (Awaad, 1975; Youssef, 1976; Kutkat, 1988; Kariyawasam *et al.*, 2004; Youssef *et al.*, 2005; Awaad *et al.*, 2008 and Hassanain *et al.*; 2008).

In the 1st experiment the effect of bovine immunoglobulins on the treatment of experimentally infected broiler chicks with *E.coli* (table 3 and figure 1) was investigated. It was clear that the birds treated with bovine immunoglobulins after enteropathogenic *E.coli* serogroup *O*1 infection showed less clinical signs and mortality rate of 13% with 100% morbidity as compared with infected untreated group that showed marked clinical signs with 40% mortality and 100% morbidity. These findings are in accordance with results dedicated by Youseif *et al.*; (2005) who compared the avian IgY, bovine IgG, avian IgM and bovine IgM against experimentally induced coli septicemia in

chickens and found that bovine (calf) IgG rich fraction was the best one in prophylaxis and therapy of chicken colibacillosis. **Awaad (1975)** on evaluating the therapeutic effect of gamma-globulins in chickens infected with *E.coli* reported that the best obtained results were from usage of bovine gamma-globulins (gammabov) or specific avian IgY rich fraction (gamma-av IgY rich fraction). Also mentioned that, when these preparations were administered orally the best results were obtained when specific gamma-av IgY rich fraction was used. **Awaad *et. al.*; (2008)** mentioned that the usage of the probiotic *Pedio-coccus acidilactici* and/or bovine IgG-rich fraction in either prophylaxis or therapy of chicken coli septicemia revealed significant reduction in both clinical and pathological pictures provided that results of prophylactic study were much better than therapeutic one. **Hassanain *et. al.*; (2008)** proved the effectiveness of bovine IgG-rich fraction in both prevention and control of *E.coli* O78 infections in chickens. The results obtained by **Youssef (1976)**, **Kutkat (1988)** and **Kariyawasam *et. al.*; (2004)** partially agreed with our results where they studied the effect of immunoglobulins in prophylaxis but not in therapy. **Take-mura *et. al.*,(2004)** found that purified IgG from cows immunized with ferric citrate receptor; FecA further decreased the growth of 14 *E.coli* serotypes from intramammary infections. **Kyoung-Sik *et. al.*,(2009)** mentioned that ovine immunoglobulins rich fraction had an antibacterial and binding activity to pathogenic whole cell antigens, lipopolysaccharide (LPS) and staphylococcal enterotoxin B in vitro.

Histopathological findings in sacrificed birds showed severe degenerative changes in livers of infected untreated group (Fig. 2a and b), with severe congestion, mononuclear infiltration with compensatory air capillaries in the lung (Fig. 2c and d). The heart showed edema, mononuclear infiltration, myocarditis and pericarditis (Fig. 2e). The bursa showed depletion with cystic formation and heterophylic infiltration (Fig. 2f). These findings accord with those

of **Cheville and Arp (1978)**, **Taylor and Francis (1982)** and **Pourbakhsh *et al.* (1997)**. The Igs treated group showed only degenerative changes in liver in the 2nd week post treatment (Fig. 2g and h) which became milder on 3rd week examination (Fig. 2i). The negative control group showed normal histopathological findings at all examined weeks (Fig. 2j and k).

In experiment 2; bovine Igs proved to be effective in treatment of challenged chickens with *NDV* (Table 4 and Figure 3). Challenged treated group with bovine immunoglobulins showed marked decrease in mortality (occurring within 2 days post inoculation) and clinical signs and challenged birds returned to their normal feed and water consumption with normal vitality (even birds showed nervous manifestations like torticollis and tremors improved) within two weeks post inoculation of immunoglobulins. While in challenged non treated group; the severity of the clinical signs increased. The mortality reached 13% and 100% in the two groups respectively. This result accords with findings of **Yuzhu *et. al.*, (2009)** who studied the effect of specific immunoglobulin (IgY) against viral infection with porcine transmissible gastroenteritis (TGEV) in piglets and found that the mortality was dramatically reduced by the oral administration of IgY from egg yolk of white leghorn hens. **Ikemori *et. al.*, (1992)** reported on significance efficacy of usage of chicken egg yolk Igs from hens immunized with bovine rotavirus (BRV) for protection against homologous BRV in calves.

The histopathological findings in the *NDV* infected untreated group was severe pneumonia in lung (Fig. 4a and b) with degenerative changes in liver (Fig. 4c and d) and severe bursitis with depletion in bursa (Fig. 4e). These findings accord with results of **Cheville *et al.* (1972)** who reported that infected birds with two U.S. viscerotropic *ND* isolates, (Texas 219 and Florida Largo) showed marked lesions of lungs seen with both examined viruses and the former produced congestion and edema of the parabronchi while the latter gave more exten-

sive lesions consisting of hemorrhage and erythrophagocytosis in alveolar areas of the parabronchi with edema, cell infiltration, and increased thickness and density of the air sacs. **Stevens et al., (1976)** summarized the microscopic picture of *NDV* as regressive changes found in lymphopoietic system characterized by disappearance of lymphoid tissue, hyperplasia of mononuclear phagocytic cells in various organs, especially in liver, that may take place in subacute infections. Necrotic lesions were found throughout the spleen. They also added that focal vacuolation and destruction of lymphocytes might be seen in the cortical areas and germinal centers of the spleen and thymus. Moreover; there was marked degeneration of lymphocytes in the medullary region of cloacal bursa.

On the other hand; *NDV* infected and Igs treated group showed normal liver (Fig. 4f) and normal bursa (Fig. 4g).

Conclusions

Bovine Igs are a cheap natural environmentally friendly product that had a beneficial effect on the treatment of bacterial infections in poultry.

Bovine Igs can make a valuable contribution to flock health and safety of poultry products as food in the domain of treatment from poultry diseases.

Bovine Igs improve growth and performance of birds.

Bovine Igs are a technology having the potential to be used as a viral disease treatment strategy.

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التأثير العلاجي للأجسام المناعية غير المتوافقة في السيطرة على عدوى الميكروب القولوني و عدوى ميكروب النيوكاسيل في الدجاج.

د. محمد محمود سامي^١، ا.د. أحمد عيسى محمد سعد^٢، ا.د. زكريا عبد الوهاب حسانين^١، د. عبدالله عبدالظاهر^١، ا.د. محمد حسين عواض^٣، ا.د. حاتم حسين بكري^٢

١- معهد بحوث صحة الحيوان – الدقي

٢- كلية الطب البيطري – جامعة بنها

٣- كلية الطب البيطري – جامعة القاهرة

الملخص العربي

اجريت هذه الدراسة لتسليط الضوء على استخدام الاجسام المناعية غير المتوافقة من الابقار للسيطرة على عدوى الميكروب القولوني و عدوى ميكروب النيوكاسيل في الدجاج. تمت تجربة الاجسام المناعية للابقار في علاج عدوى الميكروب القولوني و النيوكاسيل في الدجاج. أظهرت النتائج ان الاجسام المناعية للابقار لها تأثير فعال في علاج الدجاج من عدوى الميكروب القولوني و النيوكاسيل.