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SOME CAUSES OF CHICKEN'S GROWTH RETARDATION IN SHARKIA, EGYPT

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ABSTRACT

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Over six month period from July to December 2013, investigation of poor performance problem was carried out among meat type chicken populations that routinely immunized against Newcastle, Infectious bursitis, Infectious bronchitis and Avian Influenza at Minia El-kameh, Sharkia governorate, Egypt. Seventy stunted birds of different breeds aged between 20 and 40 days old were selected from eight private farms. Post mortem examination incriminated probable infection of Reo virus and *E. coli*. Total of 350 organs (provintireculus, pancreas, small intestine, thymus and heart) sampled for REO and 420 (liver, spleen, lung, joint, bile, heart blood) for *E. coli* isolation. Results showed that 35 (10%) were positive REO virus identified by agar gel precipitation test and 34 (8.1%) *E coli* serotyped O_{55}, O_{78}, O_{119} and O_{125}. One hundred and forty day old chicks were deployed to conduct two experimental infections of the isolated virus and bacteria via different routes to evaluate its effect on chicken's performance and histological changes. In each experiment chickens were divided into 3 groups, 1 and 2 were infected with *E. coli* O_{78} or REO while third group remained uninfected, served as control. Intramuscular infected groups recorded the highest mortalities and lower body weight comparing with oral and ocular route. Reisolation and histopathological changes confirmed pathogenicity of *E. coli O* and REO virus respectively. It is recommended that further studies to be done on REO diversity and molecular characterization in Egypt whilst adoption of vaccination programs against Reo virus and *E. coli* infection especially for breeder hens considered an utmost need to avoid serious economic losses.

Key words: *REO virus – E. coli O78 – Performance – Histopathology*

INTRODUCTION

Escherichia coli is a commensally intestinal bacteria that commonly used to monitor resistance to therapeutically valuable antimicrobials in poultry (Jakobsen *et al.*, 2010). Simultaneously, *E. coli* is as a major pathogen of widespread importance in commercially produced poultry contributing to significant economic losses (Hammerum and Heuer, 2009). A wide range of *E. coli* serogroups including O_2 and O_{78} which are among most frequent have been isolated from poultry lesions due to colibacillosis (Dho-Moulin and Fairbrother 1999, Ewers *et al.*, 2003 and Jamalludeen *et al.*, 2009). Colibacillosis is one of the most important diseases of the poultry industry around the world causing either directly due to increased mortalities or indirectly by reduction of weight gain, high feed conversion rate and carcass condemnation (Saif 2008). It is seen clinically as acute colisepticemia, subacute fibrinopurulent serositis and as chronic granulomtous disease in viscera. In nearly all cases the disease is believed to arise by extension of inhaled litter dust contaminated with feaces to the lower respiratory tract and then to the blood stream. Thus Colibacillosis of domestic birds appears to be a respiratory tract disease that is maintained by carrier status of *E. coli* within the intestinal tract (Barnes 1994 and Ogunbanwo *et al.*, 2004).

REO virus infections in poultry are prevalent worldwide. It has been isolated from chickens showing a wide variety of clinical signs including arthritis/tenosynovitis, malabsorption syndrome, pericarditis, myocarditis and immunosuppression (Mc Nulty 1993). Enteric REO virus strain (ERS) was isolated and identified from broilers in Poland showing high mortality. Usually this strain isolated from birds with malabsorption syndrome but on studying its pathogenicity and dissemination in specific pathogen free (SPF) chickens, it was able to induce a high mortality, tenosynovitis and malabsorbtion syndrome. Also commercial broilers with maternally derived antibodies against REO virus showed a growth retardation of 35 and 25% in broilers inoculated at day old and 7 days old, respectively, (Van Loon *et al.*, 2001). Upon screening for REO in the field, it was observed that ERS are

present in Netherlands, Belgium, Ireland, United Kingdom, Spain, Germany, Italy, USA, Argentina, United Arabic Emirates, South Africa, Philippines and Indonesia (Van de Zande and Lin 2005).

The study targeted virus and bacteria that may be responsible for stunting of the meat type broilers to be isolated and identified for minimization of economic losses risk as a result of deaths and poor performance.

MATERIALS and METHODS

Samples and specimens: Among 6 months period (July – December 2013) five to seven birds of different breeds and ages that showing uneven growth were collected from 8 private broiler farms at Minia El-Kameh, Sharkia governorate for laboratory examination. Clinical and postmortem findings were recorded after humanly scarification for each bird. Organs such as proventirculus, pancreas, small intestine, thymus, heart, lung, liver, spleen and joint in addition to heart blood and bile were harvested for viral and bacterial isolation.

Isolation, **Identification and serotyping of bacteria:** According to the standard bacteriological methods, blood, bile and organs (liver- spleen- lung-joint) were cultured on nutrient broth (BioMerieux), MacConkey's and Levins Eosin Methylene Blue agar (Oxoid). Isolates were identified by using *E. coli* diagnostic O antisera (Animal Health Research Institute, Dokki - Egypt) and slide agglutination test (Ewing 1984).

Isolation and Identification of virus: Small section of provintireculus, pancreas, small intestine, thymus and heart was treated with antibiotic solution (Penicillin 10000 IU/ml, Streptomycin 10 mg/ml, and Gentamycin 0.25 mg/ml) and homogenized. 0.1 ml of the homogenate was inoculated in Embryonated chicken eggs (ECE) via yolk sac resulting in embryonic death 3-5 days post inoculation then identified by agar gel precipitation test using standard REO antigen and antisera obtained from Serum and Animal Health Research institute Dokki – Egypt.

Experimental birds: One hundred and forty apparently healthy day old chicks, Hubbard breed obtained from commercial hatchery (Cairo Poultry Company) rose hygienically in isolated floor pens, fed on un-medicated growing ration feed and water was provided ad libtum.

Challenge: Two experimental infections were conducted in chicken groups. First one was carried out by inoculation of isolated *E. coli* O_{78}, K_{80} suspension via different routes with 1×10^8 cfu/ml while second experiment was done by using isolated REO virus that titrated in ECE and the titer was expressed as 50% embryo infected dose $0.2mlx10^{4.8}$.

Histopathology: According to the standard procedures, tissues of dead birds from REO challenged groups were fixed in 10% formalin, embedded in paraffin wax, processed to hematoxylin and eosin (HE) stained sections and microscopically examined.

Experimental design: 140 day old chicks were divided into 3 main groups. Chickens of the first group (60) were subdivided equally into A1, A2 and A3 infected with $1mlx10^8$cfu of *E. coli* O_{78} at 14 days old via oral, ocular and imtramuscular (I/M), respectively. Chickens of the second group (60) similarly subdivided into B1, B2 and B3 but infected with $0.2mlx10^{4.8}$ of REO virus at day old. Chickens of third group were (20) remain uninfected control. Mortalities, Body weight (BW) and body gain (BG) were recorded and calculated for three weeks post infection. Blood and organs were collected for reisolation and histopathology (Table 1).

Statistical analysis: Data were collected, organized and analyzed using one-way analysis of variance (ANOVA) through the general linear models (GLM) procedure of the Statistical Package for Social Sciences version 22.0 (SPSS for Windows 22.0, Inc., Chicago, IL, USA). Duncan multiple range test were used to separate means at $p < 0.05$.

RESULTS

Seventy chickens aged between 20 and 40 days were randomly selected from ten farms in Minea Elkameh at Sharkia governorate that had a problem of uneven growth and mortality. Examined bird showed feather deformity, depression, droopy wings, diarrhea, pasty vent and mild respiratory manifestation. Post mortem findings showed anemic carcass, proventriculitis, pericarditis, air saculitis, peritonitis, perihepatitis, pancreatic atrophy, enteritis and the intestine filled with gases and contained undigested food. Bacteriological isolation showed that out of 420 specimens (heart blood, bile, liver, spleen, lung and joint) 34 was *E. coli* positive serotyped pathogenic O_{55} (9), O_{78} (13), O_{119} (4) and O_{125} (8). Viral isolation on embryonated chicken eggs (ECE) of 350 tissue samples (proventriculus, pancreas, small intestine, heart and thymus) showed that 35 were REO virus positive identified by agar gel precipitation test (Table 2).

Experimental infection with isolated *E. coli* O_{78} ($1 ml\times10^8$ cfu) via different route at 14 days old showed that I/M yield 15% mortality in addition to significant decrease of body weight 652.3 ± 1.74^c, 1090.3 ± 1.55^c and 1175.3 ± 4.15^d at 1st, 2nd and 3rd week post infection, respectively, and cumulative body gain 836.5 ± 3.87^e in comparison with not only control group but also oral and ocular infected groups. Postmortem examination revealed pericarditis,

perihepatitis, enteritis and peritonitis. *E. coli* O_{78} reisolation was 29.5%, 35%, 27% and 25.1% from air sac, heart, liver and spleen respectively, from infected groups.

Both oral and ocular infection with isolated REO virus with 0.2 ml $\times 10^{4.8}$ at day old caused 5% mortality while I/M infection induced 35% mortality. Macroscopic findings showed hydro pericardium, enlarged liver with necrotic foci, discolored enlarged spleen and enlarged proventriculus. Statistical analysis elaborated that there was a significant decrease of body's weight and gain of infected groups with REO virus comparing with control group while there was no significant difference between oral, ocular and I/M at 1st, 5th and 6th weeks post infection as well as cumulative body gain. Moreover 2nd, 3rd, 4th week post infection with REO virus showed significant difference between body weight of I/M group 606.2 ± 1.04^{e}, 299.6 ± 1.08^{e} and 230.7 ± 0.71^{c} respectively, and other infected groups (Table 3). Histopathology findings of infected birds showed leukocytic aggregation of in submucosa and infiltration among compound gland of the proventriculus (Fig. 1,A,C), mild interstitial and perivascular leukocytic infiltration of with vacuolar degeneration of the pancreas (Fig. 1, B), focal necrosis and hemorrhages of liver (Fig. 1, E), necrosis of the heart muscle fibers and replacement with reticular cells and atrophy the intestinal villi and partial to complete desquamation (Fig. 1, D).

Table 1: Experimental Design

Grp	No of bird	infection		Performance parameter			Other parameters	
		Dose	Route	Age	BW	BG		
A1	20	***E. coli O78*** 1ml $\times 10^{8}$cfu	Oral	14days old	21, 28, 35d	14-35d	Mortality	Re isolation
A2	20		Ocular					
A3	20		I/M					
B1	20	***REO*** 0.2 ml $\times 10^{4.8}$	Oral	Day old	7, 14, 28,35d	0-35d	Mortality	Histopathology
B2	20		Ocular					
B3	20		I/M					
C	20	-	-	-				

Table 2: Results of viral and bacterial isolation and identification from sampled farms

IA	Organ / Breed	Ross (11)	Hubbard (40)	Sasso (19)	*E.coli* Serotyping		
					Isolates	No	%
E. coli O78	**Liver**	1/11	5/40	2/19	O_{55}	9	26.5
	Spleen	0/11	3/40	2/19	O_{87}	13	38.2
	Lung	2/11	4/40	2/19	O_{119}	4	18.4
	Joint	0/11	0/40	0/19	O_{125}	8	11.8
	H. blood	1/11	7/40	2/19			
	Bile	0/11	3/40	0/19			
REO	**Proventriculus**	1/11	4/40	2/19	-	-	-
	Pancreas	2/11	5/40	3/19	-	-	-
	S. intestine	0/11	3/40	2/19	-	-	-
	Thymus	3/11	5/40	4/19	-	-	-
	Heart	1/11	0/40	0/19	-	-	-

IA*=Infective agent*

Table 3: Mean body weight of chicken post infection with E. coli & REO

Gr*	7days PI	14days PI	21days PI	28days PI	35days PI	Comulative B gain*	Mortality	
							No.	%
A1	-	338.9±0.63[a]	660.5±0.85[b]	1094.6±0.99[b]	1221.3±0.60[b]	882.4±0.77[c]	-	
A2	-	338.9±0.63[a]	654.4±1.51[c]	1094.6±0.99[b]	1195.7±11.52[c]	856.8±11.26[d]	-	
A3	-	338.9±0.63[a]	652.3±1.74[c]	1090.3±1.55[c]	1175.3±4.15[d]	836.5±3.87[e]	3/20	15
B1	90.1±0.57[b]	240.1±1.74[b]	319.7±4.89[d]	628.7±1.25[d]	1090.5±0.88[e]	1047.9±0.91[b]	1/20	5
B2	90.1±0.57[b]	241.7±1.98[b]	325.4±1.18[d]	627.2±0.70[d]	1090.6±1.22[e]	1050.3±1.33[b]	1/20	5
B3	84.8±0.26[b]	230.7±0.71[c]	299.6±1.08[e]	606.2±1.04[e]	1091.9±0.92[e]	1051.3±1.01[b]	7/20	35
C	161.4±50.09[a]	338.9±0.63[a]	679.7±0.67[a]	1121.3±0.60[a]	1626.1±1.03[a]	1584.7±1.05[a]	-	

***Group* A** *infected at 14 days old with E. coli O_{78},**Group* B** *infected at day old with REO, **Group C** uninfected*
PI=*Post infection*

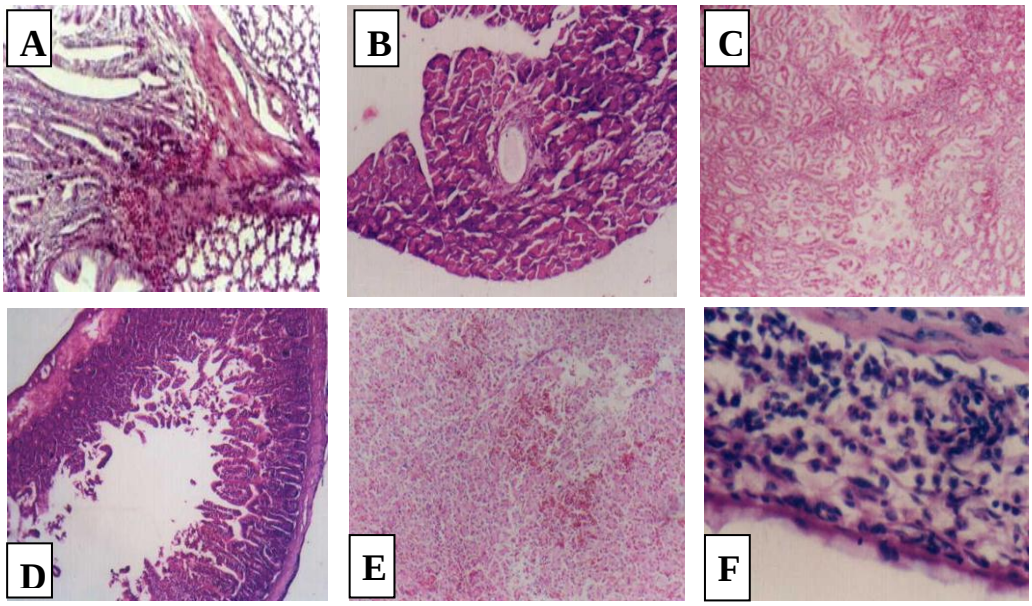

Fig. 1: Histopathological changes in REO infected chickens

A - Proventriculus: aggregation of leukocytes in submucosa H&E x 150.
B - Pancreas: mild interstitial and perivascular leukocytic infiltration H&E x 120.
C - Proventriculus: disseminated leukocytes infilteration among compound gland H&E x 150.
D - Small intestine: atrophied intestinal villi with partial desquamation H&E x 120.
E - Liver: focal necrosis and hemorrhages H&E x 120.
F - Heart: replacement of necrotic muscle fibers with proliferated reticular cells (intensive of heterophils) H&E x 150.

DISCUSSION

E. coli O_{78} infection in this study agreed Haritova *et al.* (2011) who noted that clinical signs of the infected chickens included depression, anorexia, increased water consumption, watery yellow-green diarrhea with dehydration and decreased mobility. Post-mortem findings show catarrhal enteritis, pericardis, perihepatitis and peritonitis in untreated and infected chickens. Reisolation percentage of *E. coli O_{78}* from different tissue spleen, lungs and heart was 20%, 60% and 20% respectively, at 25 days post intra-tracheal infection. Un like Dheilly *et al.* (2011) who stated that mortality 13/60 (21.7%) with very severe air saculitis and peritonitis lesions and several perihepatitis lesions were observed and mean body gain over 3 weeks post air sac inoculation with

E. coli O_{78} was 232.2 gram. *E. coli* O_{78} re-isolation from the internal organs was 21/59 of infected groups and the rate were 34%, 30.5%, 25.4% and 27.1% from air sac, pericardium, liver and spleen, respectively. Mahmoud and Edens (2005) recorded that enteropathogengic *E. coli* induced mortality 35% and significant reduction of the mean body weight 1.8 kg with bad feed conversion rate 2.1 comparing with uninfected group at 42 days old. Oliveira *et al.* (2010) that reported morbidity and mortality were 95% and 70% respectively, among chickens challenged with 0.2ml of a 3h grown culture of 5.0×10^8 cfu/ml of *E. coli* O_{78} via left air sac inoculation and postmortem findings was acute coli septicemia. While Peighambari *et al.* (2000) mentioned that intranasal infection of chickens with *E. coli* O_{78} alone showed 18% pericarditis with total score lesions 6% with no deaths. The variance of mortality, body gain and recovery rates probably raised from differences of age, breed and immune status of susceptible birds, characterization of the pathogenic *E. coli* and route of experimental infection other than stress factors.

Regarding REO virus infection, unlike our results Saskia and Eva-Maria (2007) mentioned that high mortality (79%) was seen within 7 days after IM inoculation while only one bird died at 10 days after oral inoculation but similarly to our findings of poor growth and helicopter chickens (mal-position of the feathers) were seen from day 4 and chickens stayed small until the end of the experiment in both groups. On the other hand Awandkar *et al.* (2012) reported that no mortality resulted from I/M infection with malabsorptin syndrome REO virus at 3days old but all birds were susceptible and developed the disease. The variation in mortality may be attributed to REO virus virulence of different serotypes and susceptibility of birds due to maternal antibodies. Our postmortem findings agreed with Saskia and Eva-Maria (2007) where the liver was enlarged with multiple white to yellow foci, spleenomegaly with discoloration and hard consistency and hydropericardium and Nili *et al.* (2007) recoded that the intestines of the stunted chicks were pale and dilated with gaseous and watery contents. Enlarged proventriculus (5.35%) and pancreas atrophy (3.5%). The intestinal contents of the stunted chicks showed poorly digested food materials. Parallel to our results Songserm *et al.* (2002) recorded that the mean body weights of the oral infected groups were significantly lower than that of the control groups and there was no significant difference in weight gain depression between the REO inoculated groups of the two lines. At day 7 and 14 PI, a few broilers of each MAS inoculated group showed retarded feathering. Nili *et al.* (2007) recorded that there was a significant difference in live body weights between the treatment groups with and the controls on 8, 12, 14, 20, 30, and 35 days post inoculation. Their histological examinations revealed that enlarged proventriculi showed lymphocyte infiltration and dilatation of the glandular acini. Pancreatic histological changes were degeneration, vacuolation, loss of zymogen granules of acinar cells, and fibrosis. Moreover Songserm *et al.* (2002) noted that vacuolar degeneration was present in the intestinal villi day 2, 7 and 14 post infections with REO virus. At day 7 and 14 PI, lymphoid, macrophage and granulocytic infiltration into the lamina propria, cystic formation of crypts of Lieberkühn and villus atrophy were present in the small intestine. Saskia and Eva-Maria (2007) noted that multifocal or confluent necrosis with or without evidence of heterophils infiltration in the liver and spleen of infected groups. These findings agreed with ours.

It concluded that pathogenic *E. coli* O_{78} and Reo virus are a serious threat for poultry industry. Both microbes are responsible for economic losses due mortalities and retardation of growth among meat type broilers. Prevention strategies should be adopted against especially in breeder flocks to produce maternal immunity capable to protect their progenies against *E. coli* and REO consequently enhancement of its performance.

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بعض أسباب تأخر نمو الدجاج فى الشرقية مصر

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خلال فترة سته شهور من يوليه إلى ديسمبرعام ٢٠١٣ أثناء فحص مشكلة تقزم ووفيات فى مزارع دجاج اللحم فى مركز مينيا القمح شرقية والتى حصنت روتنيا ضد مرض النيوكاسل والجامبورو والألتهاب الشعبى المعدى والانفلونزا. تم تجميع ٧٠ دجاجة مصابة بالتقزم من مختلف القطعان عمرها يتراوح بين ٢٠ إلى ٤٠ يوم وبالفحص العينى كانت تعانى من إسهال أصفر وعدم أنتظام نمو الريش مع وجود التهاب بريتونى والتهابات أغشية القلب والكبد والأمعاء مع تورم المعدة الغدية ووجود بقايا طعام غير مهضوم بالأمعاء. وقد تم عزل فيروس الريو من الأعضاء المصابة بنسبة ١٠% بأستخدام بيض الدجاج المخصب وتأكيده بأختبار سيرولوجى (ترسيب الأجار) كما تم عزل الميكروب القولونى بنسبة ٨.١% وتحديد العترات الضارية سيرولوجيا (4) O_{119}, (13) O_{78}, (9) O_{55} and (8) O_{125}. ولتأكيد ضراوة الميكروبات المعزولة تم اصابة مجموعات من الدجاج بطرق مختلفة واثبتت التجربة بأن الحقن العضلى سبب نفوق فى الدجاج المعدى تجريبيا وكذلك سجل أقل أوزان حية طوال التجربة، تم أعادة عزل الميكروب القولونى O_{78} من المجموعات المصابة كما اكد الفحص الهستولوجى لأعضاء الدجاج المصاب بأنه فيروس الريو. أنه من الضرورى تطبيق البرامج الوقائية لحماية القطعان فى الأمهات من هذين المرضين اللذين يؤثران على صناعة الدواجن .