

Molecular Characterization and Hemato Biochemical Studies of Reovirus in Ismailia Farms

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Abstract

The avian reovirus (AVR) induces various manifestations in chickens. They are associated with disease conditions including malabsorption syndrome and tenosynovitis. RT-PCR is a rapid, sensitive and specific diagnostic test for ARV detection and avoiding economic losses. Three broiler poultry farms in Ismailia with reovirus like symptoms were screened for Reoviruses in SPF embryonated chicken eggs. In these farms, there were 20-25% morbidities at 24th day of age and 3-4% morbidities at 14th day of age, and 5-6% mortalities at 24th day, while 2-3 % is at the 14th day. Three isolates of Reovirus were obtained and characterized and identified by RT-PCR. Electrophoretic pattern showed a specific band at 399 bp positive at lane 1, 3 and negative at lane 2. Results of haematological investigation showed low levels of total erythrocytic count (TEC), haemoglobin (Hb), and packed cell volume (PCV) but significant increase in level of total leukocytic count (TLC), absolute lymphocyte count (ALC) and absolute monocyte count (AMC) in infected chickens compared to apparently healthy. Biochemical estimation revealed marked elevation of total serum proteins, AST; ALT. Pathological changes in internal organs of the infected chicks were also described. The proventriculus revealed marked hyperplasia of lymphoid aggregates and the small intestine revealed marked loss of villi with replacement by fibrin, necrotic debris, high numbers of macrophages and lymphocytes. All these alterations were suggested to interfere with the immunity and normal digestive processes resulting in poor weight gain and stunting of chicks.

Key words: Reovirus- PCR- sequence- chicken- hematobiochemical alteration-Pathology

Introduction

Reoviruses belong to the genus Orthoreovirus, in the family Reoviridae. Virus particles measure 70 nm to 80 nm, are non-enveloped and have icosahedral symmetry with a double-shelled arrangement of surface protein. The virus contains double-stranded ribonucleic acid which has ten segments. The genome can be separated into three size classes, namely: L (large), M (medium) and S (small). Protein coding assignments of all ten genome segments of strain S I 133 have been determined (**varela and Benavente, 1994**). Both vertical and horizontal transmission of avian reoviruses is recognised. Egg transmission has been confirmed after experimental

infection (**Vander Heide et al., 1974, Menendez N.A. et al., 1975, Al-Mufarred et al., 1996**), but the rate of transmission is probably very low in nature. The transmission of the infection to susceptible chickens is realized horizontally. The vertical route of transmission is also proved. Reoviruses could persist in infected birds for more than 40 weeks. Avian reoviruses are ubiquitous among poultry flocks. Although infection is usually present without disease, reoviruses may occasionally be involved in several disease syndromes of which viral arthritis/tenosynovitis in chickens is the most important, particularly in broiler breeds. Diagnosis depends on detection of the virus in clinical samples, although the presence of the virus does not necessarily confirm that this is the cause of the disease, except where reoviruses are detected in affected joints. Serological tests are usually difficult to interpret in view of widespread and frequently harmless reovirus infection. Many vaccines are based on the S1133 strain isolated in the United States of America, but these may not be effective against antigenic variants (**Varela and Benavente, 1994**).

The stunting syndrome in broilers is associated with a reovirus infection but according to some studies, the role of the reovirus is probably secondary. It is characterized by a considerably reduced live weight in affected birds and a various degree of uniformity in the flock varying from 5-10% to 40-50%, usually seen after the age of 14 days.

Reoviruses are highly resistant to a number of environmental factors such as temperature, pH etc. Reoviruses are shed with faeces and could contaminate the egg shells. The polymerase chain reaction (PCR) as a diagnostic technique is generally known as a very sensitive, specific, and rapid tool for detection of viruses. It has been shown that reverse transcription (RT)-PCR can be used in the detection of avian reovirus (**Lee et al., 1998; Liu et al., 1999a, 2004; Bruhn et al., 2005**). However, conventional PCR tests can be hampered by the high risk of contamination by previously amplified materials.

Haematological examination of infected birds shows lower levels of haemoglobin (Hb), total erythrocyte count (TEC) and total leucocyte count (TLC) as compared to normal. Absolute lymphocyte count (ALC), absolute monocyte count (AMC) and absolute eosinophil count were also found lower than normal as stated by (**Muhammad et al., 2014**). A marked elevation of total serum proteins with corresponding increase of serum albumin level in infected chickens was observed, (**Singh et al., 2005**). All these changes may interfere with normal digestive processes and normal body functions resulting in poor weight gain and retarded growth or stunting chicks.

Histopathological changes due to reovirus infection including viral arthritis / tenosynovitis, stunting syndrome, respiratory disease, enteric disease, immunosuppression and malabsorption syndrome (**Rosenberger and Olson 1997, sterner et al, 1989, Van der Heide, 1996, 2000**). Pericarditis, myocarditis and immunosuppression also occur (**Mc Nuly 1993**).

This study aimed to detect the degree of spreading of AVR infection between some poultry farms in Ismailia governorate using the real –time RT-PCR as a rapid, sensitive and specific test. Also, the changes in hematological and some biochemical parameters of infected chickens were investigated as indication on the effect on and immunity, and for avoiding the economic losses by dealing with such parameters.

Materials and Methods

Isolation of Reovirus:

The broiler ARV field strain was isolated from ten farms, ten samples from farm. The observed symptoms were diarrhea, malabsorption, respiratory and intestinal disorders. The organs (intestine, proventriculus, lung, liver and spleen) were frozen-thawed for 2-3 times samples which collected from sick chickens (slaughter chickens) were homogenized in sterile phosphate-buffered saline (PBS, pH 7.2) to give a 20% suspension (w/v). After centrifugation at 3000 \times g for 20 min, the supernatants were filtered through 0.2 mm syringe filters. The filtered suspension was injected into the allantoic sac of 9-day-old chick embryos (0.2 ml/embryo). Embryos were candled daily for 7 days and the amino-allantoic fluids of the infected embryos were collected for further passage in the embryos of chickens and specific pathogen free (SPF). (**Schat and Purchase, 1989**). After third passage embryo showed stunting in growth.

RNA extraction: RNA extraction from samples was performed using the QIAamp viral RNA Mini kit (Qiagen, Germany, GmbH). Briefly, 140 μ l of the sample suspension was incubated with 560 μ l of AVL lysis buffer and 5.6 μ l of carrier RNA at room temperature for 10 min. After incubation, 560 μ l of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 60 μ l of elution buffer provided in the kit.

Oligonucleotide Primers:

supplied from (**Metabion Germany**) are listed in **table (1)**.

Table (1). Primers sequences, target genes, amplicon sizes

Primer	Target gene	Primer sequence (5'-3')	Length of amplified product (bp)
REO-F	S2	CCC ATG GCA ACG ATT TC	399 bp
REO-R		TTC GGC CAG GTC TCA AC	

PCR amplification: Was done according to (Bruhn et al 2005) Primers were utilized in a 25- µl reaction containing 12.5 µl of Quantitect probe rt-PCR buffer (QIAGEN, Gmbh), 1 µl of each primer of 20 pmol concentration, 0.25 µl of rt-enzyme 4.25 µl of water, and 6 µl of template. The reaction was performed in a Biometra thermal cycler. Reverse transcription was applied at 50 °C for 30 min, a primary denaturation step was done at 95 °C for 5 min, followed by 35 cycles of 94 °C for 30 sec., 55 °C for 45 sec. and 72 °C for 45 sec. min. A final extension step was done at 72 °C for 10 min.

Analysis of the PCR Products:

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applchem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A 100bp DNA ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Hematological and biochemical studies:

1-Haematological studies:

Blood samples were taken from diseased and apparently healthy birds (10 samples of each) two samples one without anticoagulant for serum separation and other one with anticoagulant for evaluation of red blood corpuscles (RBCs $10^6/\text{mm}^3$), heamoglobin (Hbgm/dl), packed cell volume (PCV %) and blood indices (mean corpuscular volume MCV fl, mean corpuscular haemoglbin MCH pg and mean corpuscular haemoglobin concentration MCHC %), total leukocytic count (WBCs $10^3/\text{mm}^3$), and differential leukocytic count were determined according to routine haematological examination and standard blood smear (Jain., 2000) .

2-Serum biochemical parameters:-

The level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, uric acid, creatinine, were estimated by using auto analyzeospital Hitachi 912 in Suez Canal University Hospital. Estimation of globulin was difference between total protein and albumin (Kaneko et al., 1997).

3-Statistical Analysis:

Data collected from haematological and serum biochemical results of different groups of chickens were statistically analyzed for the mean and standard deviation of analysis were performed according to (**Sndecor and Cochran.,1982**).

4-Pathological examination:

Postmortem examination was done immediately after slaughtering and tissue specimens from liver, kidney, lung, proventriculs and intestine were collected and fixed in 10% neutral buffered formalin. They were routinely processed by standard paraffin embedding technique. Section at 4 micron, stained with Hematoxylin and Eosin (**Bancroft and Gamble 2002**)

Result and Discussion

The clinical signs were characterisitic for reovirus infection in the present study. Affected chickens showed depression. Respiratory disorders, weight loss and diarrhea. The demonstrated morbidity did not involve all chickens in the farms under study. . Reovirus infection caused 30% morbidity and 20% mortality in Israeli poultry farms and the disease first appeared at 10 days of age and persisted in an affected Flock until six weeks of age. A reliable reverse transcriptase polymerase chain Reaction (RT-PCR) method was developed to detect ARV contaminations in poultry vaccines. Because ARVs exhibit diversity and heterogeneity in their genome (**Liu et al., 1999a, 2004**), in recent years, several attempts have been made to detect ARV in chicken tissues or cultured cells by conventional RTPCR (**Lee et al., 1998; Liu et al., 1999a, 1999b, 2004; Bruhn et al., 2005**). Reovirus infection caused 30% morbidity and 20% mortality in Israeli poultry farms and the disease first appeared at 10 days of age and persisted in an affected Flock until six weeks of age. In the present study reovirus infection caused 20-25% morbidities at 24th day of age and 3-4% morbidities at 14th day of age, and 5-6% mortalities at 24th day, while 2-3 % is at 14th day. So, the mortality rate at the younger ages seemed to be non-significant in the field of poultry industry. Rapid diagnostic detection of reovirus is particularly important to the poultry industry in order to prevent spreading of the disease and to limit economic losses. PCR has in addition benefits of being cost effective, time saving, specific and sensitive; furthermore, it has been used for screening and surveillance of poultry flocks (**Pang. et al 2002**). In this study, two out of three isolates from Ismailia farms of Reoviruses were characterized and identified by RT-PCR.. Electrophoretic pattern showed a specific band at 399 bp positive at lane 1, 3 and negative at lane 2 as shown in (**fig.1**). Haematology or blood study is an essential medical science applied to the diagnosis and treatments of various disorders related to blood, our haematological results as shown in **table (2)** exhibited significant reduction ($p<0.05$) in RBCs ,Hb and PCV and decrease in (MCV and MCH) in both ages as compared to apparently healthy control

chickens these findings illustrated anaemic changes (microcytic hypochromic anaemia) which may be caused by malabsorption syndrome, our results come agree with (Singh et al., 2005), (Nilli et al., 2007) and (Rani et al., 2011). Total leukocytic count (TLC), absolute lymphocyte count (ALC), and absolute monocyte count (AMC) significantly increased ($p < 0.05$) in infected chickens 14 days and 24 days old as shown in **table (3)** such findings are partially in agreement with (Rani et al., 2011) who reported leukocytosis with monocytosis but lymphopenia had occurred. Leukocytosis could be due to many reasons including stress and infection, (Otto et al., 2006). Significant increase of monocytes in response of invading micro-organisms, inflammation by migration into the tissues and necrotic materials, (Seller et al., 2010). The lymphocytes increased number was seen during viral infection, they can also play part in immunological defense mechanism (Young and Heath, 2000). A marked elevation of total Serum proteins with corresponding increase in albumin in 14 days old infected chickens (**table 4**) which come in agreement with (Singh et al., 2005). The increased level of total proteins in 14 days old chickens may be contributed to diarrhea catarrhal enteritis which is sign of illness that lead to dehydration and relative increase of total proteins (Dustan, 2009). Some plasma biochemical parameters have been examined in stunted broiler chickens by some researchers. (Sinclair et al., 1984) reported that there were no significant differences between stunted and non-stunted birds in the concentration of plasma proteins. Concerning liver enzymes the elevation of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in both ages parallel the magnitude of hepatocellular damage (Kaneko et al., 1997).

Gross Pathological examination of suspected cases showed that the liver slightly enlarged with multiple white small foci, the kidneys were swollen and pale with few small foci. The lungs were dark-red suggesting pneumonia. Mild catarrhal enteritis was also found in the small intestine.

Histopathological Examination:

The livers of the chicks showed congestion and dilatation of central and portal veins, the blood sinusoids were occasionally dilated and congested (**Fig. 3**). Fibrin thrombi were frequently observed in the lumen of portal vein (**Fig. 4**). The perivascular interstitial was expanded by edema admixed with fibrin and moderate numbers of heterophils, lymphocytes and macrophages (**Fig. 5**). (Saskia and Eva-Maria, 2007) noted that multifocal or confluent necrosis with or without evidence of heterophils infiltration in the liver of infected birds. These findings agreed with ours Liver of the chicken is considered to be one of the target organs for reovirus infection (McFerran et al, 19976). In this work, Lesions increased in Infected chickens at 14th day than 24th day. Occasionally, focal areas of hemorrhages (**Fig. 6**) and aggregates of lymphocytes and macrophages displaced the hepatocytes (**Fig. 7**). There were multifocal to coalescent areas of lytic necrosis (**Fig. 8**) replaced by eosinophilic

cellular and karyorrhectic debris and infiltrated by heterophils, lymphocytes, plasma cells and macrophages (**Fig. 9**). Occasionally, hemorrhagic necrosis was seen (**Fig. 10**). Rarely, the vacant space of necrotic hepatocytes was replaced by edema and erythrocytes. Moreover, the portal areas were expanded by moderate numbers of inflammatory cells mainly lymphocytes and fewer macrophages. The bile ducts revealed mild biliary hyperplasia with eosinophilic debris in the lumen and periductal fibrous tissue proliferation admixed with small numbers of lymphocytes and macrophages, these lesions were due to infected organisms which found with damaged tissues as a result of immunosuppression of the bird. (**Sharma et al., 1994; Pertile et al., 1996; Sanchez-Cordon et al., 2002**).

The examined kidneys revealed congestion of the renal blood vessels and intertubular capillaries with occasional fibrin thrombosis of some blood vessels. The interstitium of renal cortex was expanded by aggregates of lymphocytes. Multifocal coagulative necrosis of some tubular epithelium characterized by loss of cellular details with hypereosinophilic cytoplasm and pyknosis of nuclei was observed (**Fig. 11**). Occasionally, there was lytic necrosis of tubular epithelium replaced by eosinophilic cellular and karyorrhectic debris and infiltrated by erythrocytes, heterophils, few lymphocytes, plasma cells and macrophages (**Fig. 12**). Additionally, homogenous eosinophilic casts were observed in the lumen of some renal tubules.

The lung showed congestion of pulmonary blood vessels with occasional thickening of the wall due to fibrous tissue proliferation. Fibrin thrombi were frequently seen in the lumen of blood vessels. Multifocal, perivascular and interlobular septa were markedly expanded by fibrin, edema, hemorrhages and lymphocytes (**Fig. 13**). Foci of aggregates of lymphocytes, macrophages, fibrin and erythrocytes were occasionally seen in the Para bronchial wall (**Fig. 14**). Lesions in birds at 14 th day were increased than birds at 24 th day .(**Jones and Georgiou ,1984**), suggested that the age associated susceptibility may be related to the inability of young birds to develop an effective immune response . Reoviruses are suggested to have an immunosuppressive activity which is most probably caused by lymphodepletion mediated by factors released by macrophages (**Sharma et al, 1994,; Pertile et al, 1996; Sanchez-Cordon et al, 2002**).This immunosuppression could facilitate growth of bacteria and parasites and allow other viruses to co-infect the bird. The interatrial septa were also expanded by edema admixed with mononuclear inflammatory cells. The bronchial mucosa exhibited hyperplasia of lining epithelium with increased numbers of goblet cells (**Fig. 15**). Furthermore, subpleural edema admixed with fibrin, hemorrhage, and lymphocytes were also detected.

The proventriculus revealed marked hyperplasia of lymphoid aggregates (**Fig. 16**). Multifocal, glandular cells exhibited coagulative necrosis characterized by loss of cellular details with hypereosinophilic cytoplasm and pyknosis of nuclei (**Fig. 17**).

Additionally, there is no general agreement regarding proventricular lesions in Reovirus. (page et al, 1981, Bracewell and Randall ,1984,Shapiro and Nir ,1995) . While (Kouwenhoveretal, 1978) reported proventricular lesions in Reovirus .However, (Bayyan et al., 1995) believed that proventriclitis is not a consistent finding in Reovirus

The small intestine revealed marked loss of villi and occasionally crypts (**Fig. 18**) with replacement by fibrin, necrotic debris, large numbers of macrophages, and lymphocytes (**Fig. 19**). (Moreover Songserm et al., 2002) noted that vacuolar degeneration was present in the intestinal villi at day 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, these results agreed with ours. Adjacent less affected villi were fused multifocal eroded or ulcerated and the remaining lamina propria was expanded by edema admixed with fibrin, lymphocytes and macrophages (**Fig. 20**).In this work the infected birds had severe intestinal lesions at day 14 th because of the presence of more differentiated epithelia . The intestinal lumen contained abundant fibrin and necrotic debris admixed with sloughed epithelial cells,few macrophages and lymphocytes.(Haffen et al.,1989) have suggested that the cells of the lamina propria, or mesenchyme, can exert considerable influence on the differentiation of enterocytes. Damage to the lamina propria as a result of infection could interfere with the differentiation of the enterocytes with a resultant reduced absorptive capacity of the gut.



Fig 1: RT-PCR results for Reo virus: Lane 1: Positive control, Lanes 1,3Positive reo samples. Lane 2 negative reo sample. M= 100bp Ladder.



Fig 2: Growth retardation in embryo injected by Reo virus after third passage.

Table (2):Haemogram in chicken infected naturally with Reo virus and apparently healthy. (n=10)

Groups Parameters	Infected	apparently healthy	Infected	apparently healthy
	14 days old		24 days old	
RBCS ($10^6/\text{mm}^3$)	2.52±0.24 ^b	5.64±0.81 ^a	3.7±1.16 ^b	6.31±0.32 ^a
Hb (g / dl)	6.50±1.6 ^b	9.26±0.24 ^a	7.3± 0.42 ^b	11.83±1.08 ^a
PCV %	20.17± 2.12 ^b	28.67±0.42 ^a	26.00±1.7 ^b	33.33±0.56 ^a
MCV f1	3.167±0.15 ^b	12.07±0.45 ^a	4.30±0.37 ^b	9.47±0.02 ^a
MCH Pg	1.47±0.29 ^b	3.47± 0.24 ^a	1.43±0.12 ^b	3.16±0.02 ^a
MCHC %	32.76±0.37 ^a	32.40±0.80 ^a	32.97±0.58 ^a	32.63±0.76 ^a

Means ± S.E with different superscript a, b within the same column are significantly different at $p < 0.05$

Table(3) Leukogram in naturally infected chicken with Reo virus and apparently healthy (n=10)

Parameters \ Groups	Infected	Apparently healthy	Infected	Apparently healthy
	14 days old		24 days old	
TLC (10 ³ / mm ³)	87.54±3.65 ^a	46.66±2.56 ^b	114.00±3.89 ^a	92.20±5.50 ^b
Heterophils (10 ³ / mm ³)	16.43±2.47 ^a	15.75±1.02 ^a	22.32±5.74 ^a	21.15±3.52 ^b
Lymphocytes (10 ³ / mm ³)	63.11±3.94 ^a	25.91±1.34 ^b	83.14±4.50 ^a	65.52±4.74 ^b
Eosinophil (10 ³ / mm ³)	1.33±0.18 ^a	1.1±0.14 ^a	1.40±0.05 ^a	1.2±0.09 ^a
Monocyte (10 ³ / mm ³)	6.67±0.7 ^a	3.9±0.06 ^b	7.14±0.45 ^a	3.33±0.28 ^b

Means ± S.E with different superscript a ,b within the same column are significantly different at p<0.05

Table(4): Serum biochemical parameters in chickens naturally infected with Reo virus apparently healthy. (n=10)

Parameters \ Groups	Infected	apparently healthy	Infected	apparently healthy
	14 days old		24 days old	
Total Protein (g /dl)	2.67±0.02 ^a	2.38±0.02 ^b	2.33±0.35 ^a	2.47±0.11 ^a
Albumin (g/dl)	1.42±0.20 ^b	1.07±0.01 ^a	1.18±0.06 ^a	1.25±0.02 ^a
Globulin (g/dl)	1.25±0.20 ^a	1.31±0.03 ^a	1.15±0.38 ^a	1.22±0.13 ^a
AST (U/l)	127.75±3.57 ^a	49.50±2.10 ^b	164.25±8.1 ^a	58.75±2.75 ^b
ALT (U/l)	46.70±0.94 ^a	23.90±0.01 ^b	45.44±0.47 ^a	23.27±0.13 ^b

Means ± S.E with different superscript a, b within the same column are significantly different at p<0.05

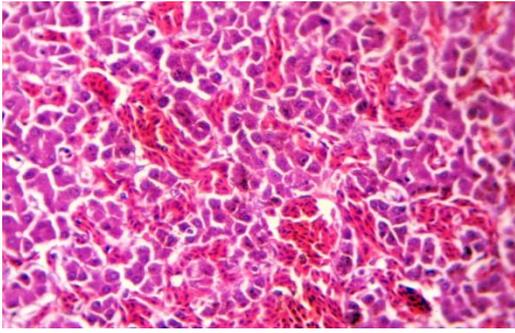


Fig. 3: Liver of chick, 24 days old, showing dilatation and congestion of blood sinusoids. H&E stain x 400.

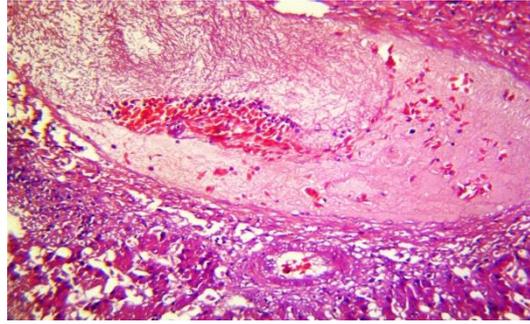


Fig. 4: Liver of chick, 14 days old, showing fibrin thrombus occlude the lumen of portal vein. H&E stain x 200.

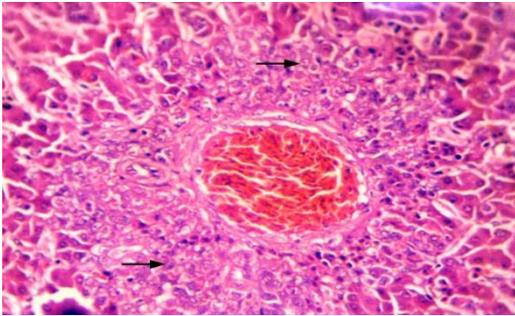


Fig. 5: Liver of chick, 24 days old, showing perivascular oedema admixed with fibrin and moderate numbers of heterophils, lymphocytes and macrophages (arrow). H&E stain x 400.

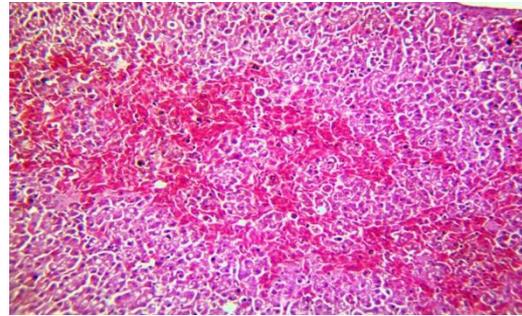


Fig. 6: Liver of chick, 14 days old, showing focal areas of hemorrhages. H&E stain x 200.

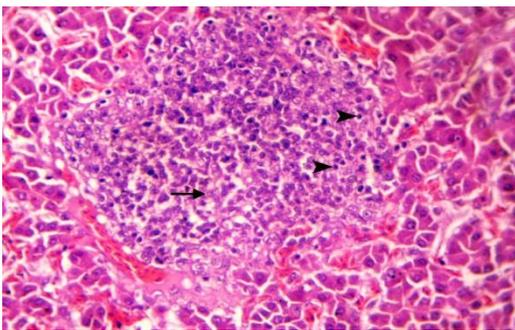


Fig. 7: Liver of chick, 24 days old, showing aggregates of lymphocytes (arrow head) and macrophages (arrow) displaced the hepatocytes. H&E stain x 400.

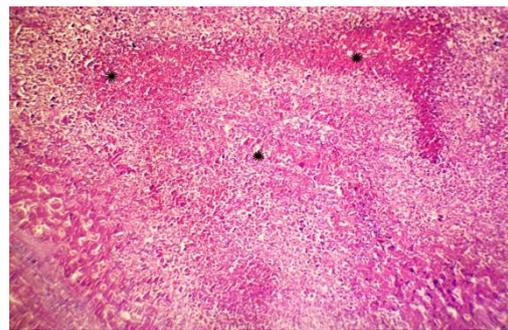


Fig. 8: Liver of chick, 24 days old, showing coalescent areas of lytic necrosis (asterisk). H&E stain x 100.

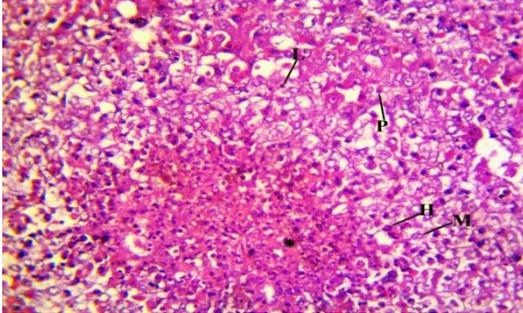


Fig. 9: Liver of chick, 24 days old, showing focal lytic necrosis replaced by eosinophilic cellular karyorrhectic debris (asterisk) and infiltrated by heterophils (H), lymphocytes (L), plasma cells (P) and macrophages (M). H&E stain x 400.

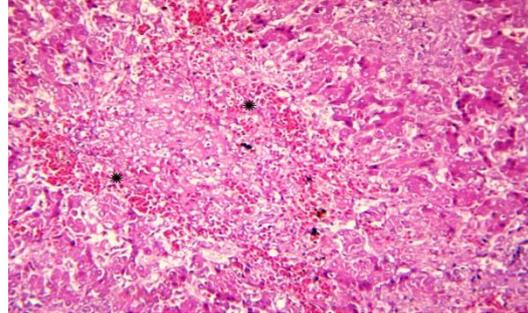


Fig. 10: Liver of chick, 24 days old, showing hemorrhagic necrosis (asterisk). and H&E stain x 200.

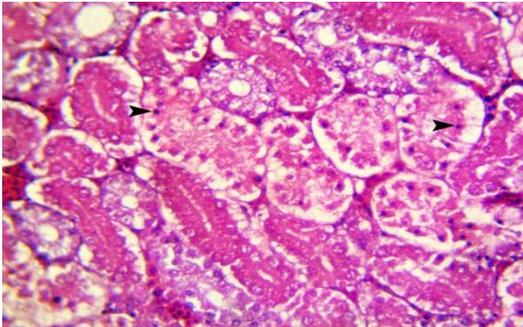


Fig. 11: Kidney of chick, 24 days old, showing coagulative necrosis of some tubular epithelium characterized by eosinophilic loss of cellular details with hyper-eosinophilic cytoplasm and pyknosis of nuclei (arrow head). H&E stain x 400.

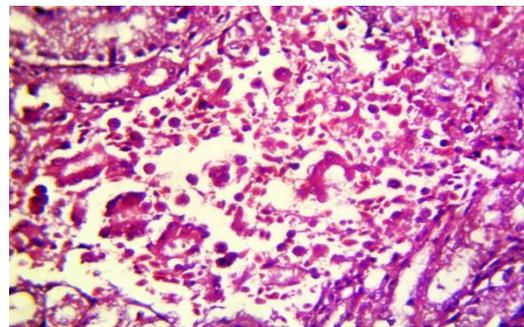


Fig. 12: Kidney of chick, 24 days old, showing lytic necrosis of tubular epithelium replaced by cellular and karyorrhectic debris, infiltrated by Erythrocytes and inflammatory cells H&E stain x 400.

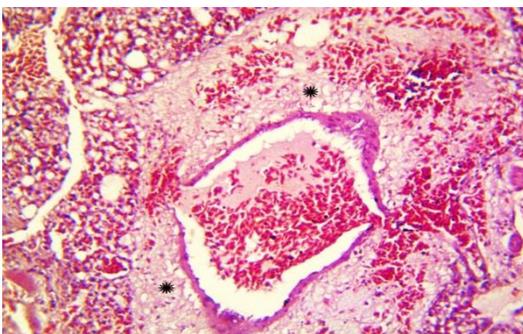


Fig. 13: Lung of chick, 14 days old, showing perivascular edema (asterisk) admixed with hemorrhages and low numbers of lymphocytes. H&E stain x 200.

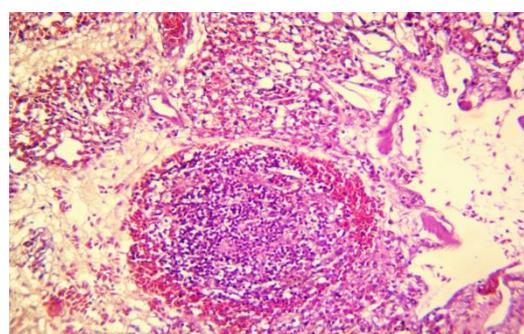


Fig. 14: Lung of chick, 14 days old, showing Focal aggregates of lymphocytes, fibrin, macrophages, fibrin and erythrocytes in the parabronchial wall. H&E stain x 200.

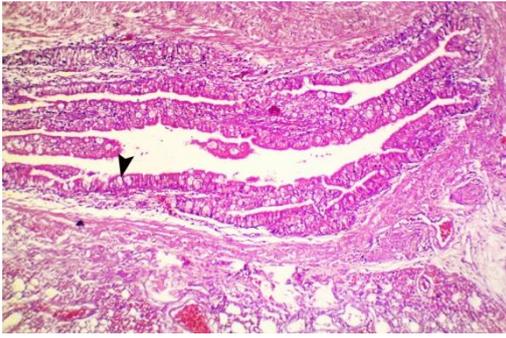


Fig. 15: Lung of chick, 14 days old, showing hyperplasia of lining epithelium of bronchial mucosa with increased numbers of goblet cells (arrow head). H&E stain x 200.

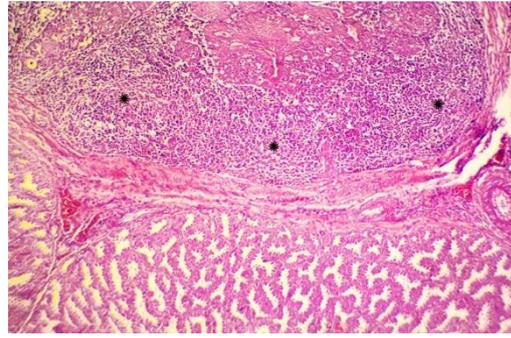


Fig. 16:) Proventriculus of chick, 24 days old, showing marked hyperplasia of lymphoid aggregates (asterisk). H&E stain x 200. cells

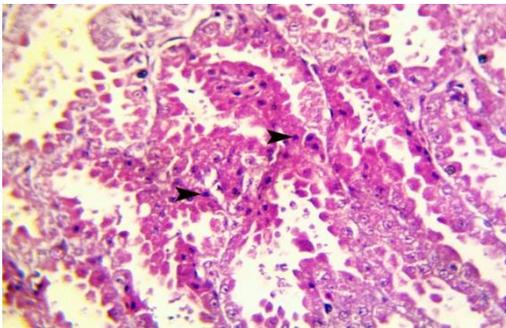


Fig. 17: Proventriculus of chick, 24 days old, showing coagulative showing marked necrosis of glandular cells characterized by loss of cellular details with hypereosinophilic cytoplasm and pyknosis of nuclei (arrow head). H&E stain x 400.

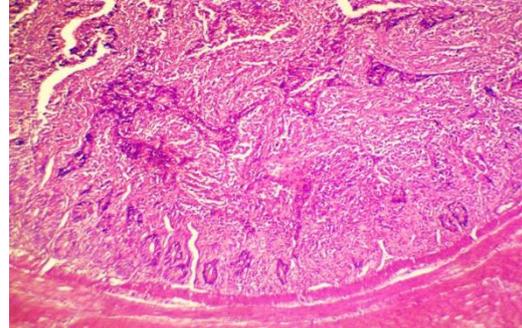


Fig. 18: Small intestine of chick, 14 days old, showing loss of villi and crypts. of H&E stain x 100.

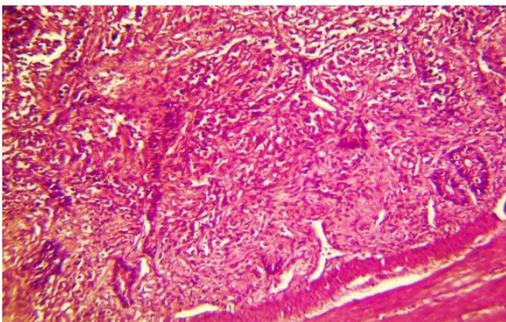


Fig. 19: Small intestine of chick, 14 days old, showing marked loss of villi and crypts replaced by fibrin debris high numbers of (asterisk) macrophages, and lymphocytes. H&E stain x 400.

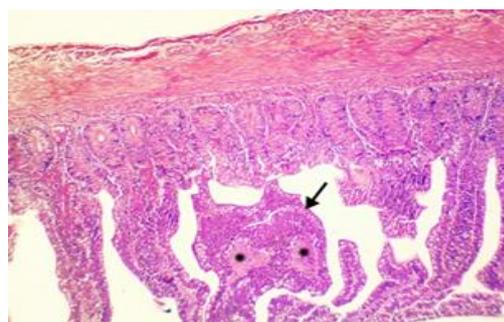


Fig. 20: Small intestine of chick, 14 days old, showing fusion, ulceration of the villi (arrow) and edema of lamina propria admixed with fibrin and mononuclear inflammatory cells. H&E stain x 100.

Conclusion

The isolation of reovirus from three farms in Ismailia province indicates its economic importance as one of the possible viral infections. The morbidity due to infection was noticed to be higher than mortalities and both increase with age. Haematological and biochemical changes in the examined birds might affect the immunity of chickens to reovirus infection, the histological lesions present in the proventriculus, small intestine and in the liver could interfere with the normal digestive processes resulting in poor weight gain and delayed or retarded growth.

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