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ROLE OF IBIS IN TRANSMITTING REOVIRUS INFECTION AMONG POULTRY FARMS

(With 6 Figures)

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تم فحص عدد ١٣ عينة أظهرت ثلاثة منها ورم فى الركبة، ووجد فى حالة واحدة بطش حمراء فى البنكرياس. بالفحص الفيروسى تم عزل فيروس الريو. تم العزل فى بيض مخصب عن طريق حقن البيض بالحقن على الغشاء. ظهرت بثرات بيضاء على الغشاء. وتجمعات نووية داخل الخلايا على الزرع النسيجى. الفيروس مقاوم للكلوروفورم والاثير ودرجة حرارة ٥٦ م لمدة ساعة. تم حقن ٢٠ كتكوت تحت جلد القدم عمر ٣ أسابيع وتم العزل والفحص الباثولوجى لهذه الكتاكيت وظهر ورم فى الرجل بعد ٣،٤،١٢،١٥ يوم بعد الحقن. بالفحص الباثولوجى أظهر عتامة فى الغشاء المحيط بالقلب والتهابات فى عضلة القلب. والفحص الهستوباثولوجى تهتك وتنكرز فى خلايا الكبد وتغيرات فى البنكرياس والغدة الهضمية.

SUMMARY

Out of thirteen Ibis examined, 3 cases showed swelling of the digital flexor and metatarsal extensor tendon, and one case has red patches on the surface of pancreas. Virological examination revealed reovirus resembles that in cage bird. Experimental infection of 3 weeks old 20 chickens through foot pad inoculation was done. Histopathological and virological examination of the inoculated chickens were described. The results revealed diarrhea at 3rd day PI and swollen legs at 4th, 12th and 15th day PI. Isolation by egg inoculation (CAM) showed pock lesions in one case and tissue culture (vera) revealed syncytia. Virus resisted ether,chloroform and it was heat stable. Pathological lesions were pericarditis, myositis, hepatic vaccuolar degeneration and necrosis in addition to lesions in pancreas and proventriculus.

Key words: *Ibis, reovirus, poultry farms.*

INTRODUCTION

Ibis in Egypt has a close relation with agricultural land and farmer, it is called farmer's friend. Giza zoo is considered as a perminant habitat for it. So research works are needed to know the role of Ibis as a carrier or reservoir of infection for domestic poultry and zoo birds. Soad and wafaa (2003) isolated some bacterial microorganisms (Pseudomonas aeruginosa, E.coli, Salmonella enterica typhimurium, Proteus vulgaris and P.haemolytica) from Ibis.

Avian Reoviruses are members of the Orthoreovirus genus in the reoviridae family. They can be differentiated in commercial poultry by antigenic configuration, pathotype, relative pathogenicity, growth in cell culture, sensitivity to trypsin and host specificity (Jones *et al.*, 1994) and (Jones and Georgian, 1984). Concerning the economic importance of Reovirus,Pass *et al.* (1982) reported the economic significant of viral arthritis (VA) and malabsorption syndrom (MAS) in broiler breeder pullets and hens.

In young meat type chickens, economic losses related to reovirus infections are frequently associated with increased mortality viral arthritis/tenosynovitis (Rosenberg and Olson, 1997), loss in weight gain poor feed conversion, uneven growth rates and reduced marketability of affected birds (Dobson and Glisson, 1992). Reoviruses are prevalent world wide in chickens,turkeys and other avian species.Viral arthritis is observed primarily in meat type chickens but can be found in lighter breeder (Jones and Onunkwo, 1978 and Schwartz *et al.*, 1976) and in turkeys (Alafaleg and Jones, 1989, Back and Nagaraja, 1996 and Nersessian *et al.*, 1986) in the digestive and respiratory tract of clinically normal chickens and turkeys Kawamura *et al* (1965) and Wooley *et al.* (1972). Van der heide (1996) estimated that greater than 80% of reoviruses isolated from chickens were apathogenic. Reovirus can be isolated also from pigeons (McFerran *et al.*, 1976) and from diseased pigeons, pheasants, parrots and exotic avian species (Gough *et al.*, 1988). Reoviruswas also isolated from the liver of an African grey parrot (Graham, 1987), from American wood cock (Docherty *et al.*, 1994), from Bob white quail (Ritter *et al.*, 1986) and from pheasants (Mutlu *et al.*, 1998). Cadman *et al* (1994) detected antibodies to avian reovirus by ELISA in Ostriches from Zimbabwe. The purpose of this study was to investigate the epidemiology of reovirus in Ibis and the propability of being a reservoir and carrier of the disease.

MATERIALS and METHODS

1- History and PM examination:

Thirteen Ibis Farm Abu Rawash, Giza. were examined, 3 cases only showed swelling of the digital flexor and metatarsal extensor tendon. One case has red patches on the pancreas.

2- Egg inoculation:

Collected pancreas,liver and synovial fluid of each case was inoculated in 5 eggs per each 10 day old embryonted chicken eggs via chorioallantoic membrane.

3- Agar gel preciptodion test:

Harvested and allantoic fluid were added. Frozen thawed 3 times and put in agar gel made by 1.2 purified agar in phosphate buffer pH 7.2. Refrence sera which taken from Dr.Attia Sami, Virology Department Cairo, Univ. put in the center allantoic membrane in prepheral well and examined for 3days for precipitation line.

4- Tissue Culture:

Vero cell line were inoculated with inoculum (synovial fluid and pancreatic emulsion) and examined for 5 days for cytopathic effect and syncytia.

5- Heat stability:

Viral isolates were divided into 5 tubes each then incubated in water bath at 56°C for 5,10.15, 30 min. and 1 hour. Control tube kept in room temperature.Treated and control samples were checked for infectivity in tissue culture.

6- Chemical test:

a- Ether sensitivity:

It was carried according to the method described by Andraws and Horsman (1949). Two ml of the virus was prewarmed in water bath 37°C and 0.8 ml of chemically pure diethylether was added. The tube was agellated and incubated at 37°C for 30 minutes then the aqueous layer was collected for the residual virus to be titrated in tissue culture.

b- Chloroform sensitivity:

The technique used was that described by Feldman and Wang (1967). One part of chemical purified chloroform was fixed with 19 parts of tissue culture containing the virus to have final dilution of 5% chloroform. The mixture was left at room temperature for 10 min.with continuous vigorous shaking. The mixture was centrifuged at 3000rpm for 5 min.and aqueous layer was collected and the residual virus was collected and titrated in tissue culture.

7- Virus neutralization:

Virus was titrated 4 well per each dilution 0.0025 serum (Virology Department, Cairo University), the same amount was added to each well which contained 0.0025 of virus.Incubation for 1 hour and MEM was added 0.050 in each well (alphaprocedure). The difference between virus titration only and virus serum titration was calculated as neutralization index.

8- Experimental design:

Three weeks old 20 chickens were inoculated via foot pad, other 8 chickens kept as control. Four sacrificed birds were examined at 2,4,12 and 15 days post inoculation (PI) for:

1- Postmortem examination.
2- Diagnosis by agar gel precipitation test.
3- Histopathological examination.

9- Histopathological examination:

Samples from tendon, pancreas, liver and heart were fixed in 10% neutral buffered formalin.The specimens were treated chemically then sectioned at 5um and stained with haematoxylin and Eosin (Culling 1963).

RESULTS

Viral isolation:

One case of Ibis from three cases of swelling legs showed whitish pock lesions on CAM. Death of embryos occured within 3-5 day PI. One line of precipitation appeared against the positive serum by agar gel.

Tissue culture:

In case of the first passage, cell rounding occurred.
By the second passage syncytia appeared.
Third passage showed necrobiotic changes.

Virus titration	Ether	Chloroform
$10^{-4.75}$	$10^{-4.75}$	$10^{-4.75}$

Thermostability:

Virus was stable at 56°C for 1 hour.

Neutralization index:

Index	2

Clinical signs& Post-mortem findings:

At 2nd day PI one dead bird showed diarrhea,at 4th day PI 3 birds showed tenosynovitis,swollen legs (Fig. 6) with greenish diarrhea appeared at 12 and 15 day PI. Liver appeared pale at 3rd day PI,other cases showed congestion at 4th day PI.

Virus reisolation:

100% of pancreas was positive untill 9th day PI but 25% of liver was positive.

3-Histopathological findings:

At 2nd and 4th day PI, the liver showed severe congested blood vessels (Fig. 1) in addition to severe vaccuolar degeneration (Fig. 2) of the hepatocytes. Focal coagulative necrosis was observed. The proventriculus showed hyperplasia and metaplasia of the lining epithelium with cellular degenerative changes. While the heart at 2nd day PI revealed focal pericardial and myocardial oedema with degeneration in the myocardial muscle fibers. Heart lesions became severe at 4th day PI as pericarditis (Fig. 3), pericardial oedema with myositis and heterophilic infiltration between the myocardial fibers (Fig. 4). At the 15 day PI the lesions became less in heart, liver and proventriculus while the pancreas showed vaccuolar degeneration of the acinar cells of the exocrine tissue (Fig. 5) in addition to cellular necrotic debris.

DISCUSSION

Reovirus is very common among domestic poultry and other avian species. Inoculum was prepared from synovial fluid, pancreas and liver of infected Ibis and inoculated in embryonated chicken eggs via chorioallantoic membrane. Pock lesions were developed in addition to embryo mortality within 3-5 day PI and stunting of survival embryo. These results agreed with Mustaffa and Spradbraw (1971) and Bains, *et al.* (1974).

The infected CAM homogenate were tested by AGPT using Reovirus S1133 antiserum. Isolates that produced pathological lesions in chicken and gave precipitation line were propagated for the passage in vero cell. Syncytia formation followed by detachment from monolayer cell sheet. The choice of vero cells for reovirus isolation was manipulated with Sahu and Olson (1975), Tantawi *et al.* (1984), Shapouri *et al.* (1996) and Samah (1997).

Reovirus in Ibis resemble that in cage bird. Trials of reisolation proved that the best organ for reisolation is the pancreas.

Symptoms and postmortem lesions resembled that described by Sterner (1989) and Van der heid (1996). The histopathological lesions involved myocarditis and heterophilic infiltration between the myocardial fibers as mentioned by Kerr and Olson (1969) and Olson and Solomon (1968). Lesions in liver as vaccuolar degeneration and necrosis were also observed by Gouvea and Schnitzer (1982).

These lesions might be due to the replication of the virus in the cytoplasm consuming the cellular elements and cause irritation and cell damage.

In conclusion, Ibis could be a carrier and a reservoir for avian reovirus,so control measures are needed to prevent spreading of infection among poultry farms and zoo birds.

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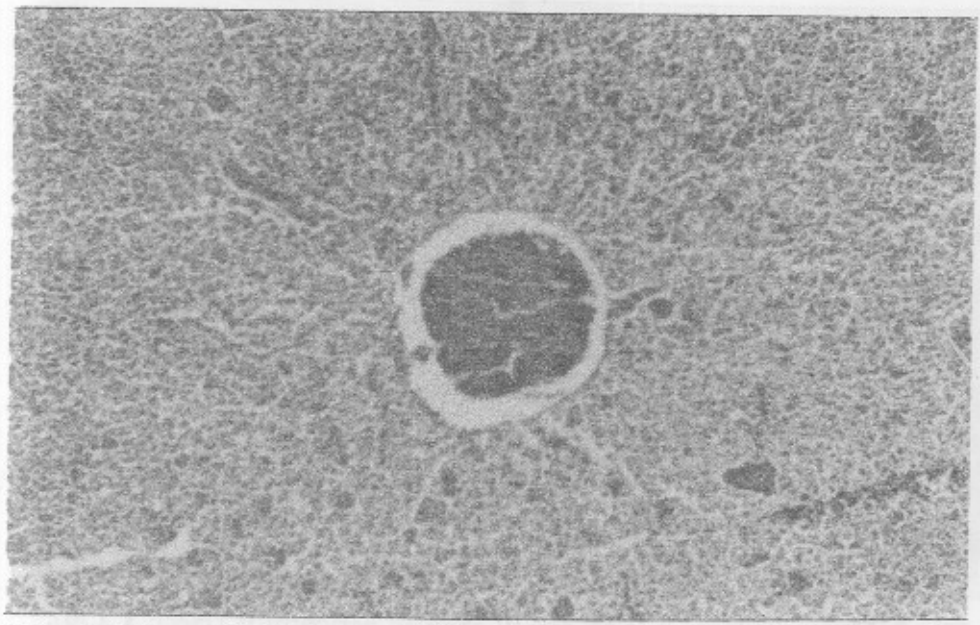

Fig. 1: Liver of chicken at 2nd day PI showed congested blood vessels (H&E x 100).

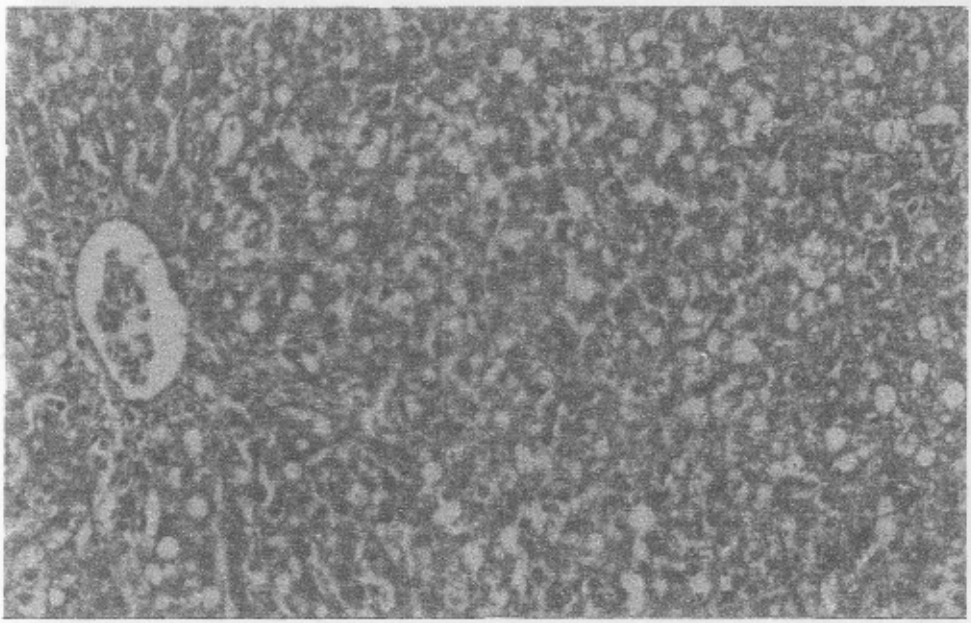

Fig. 2: Liver of chicken at 2nd day PI showed vacuolar degeneration and necrosis (H&E x 250).

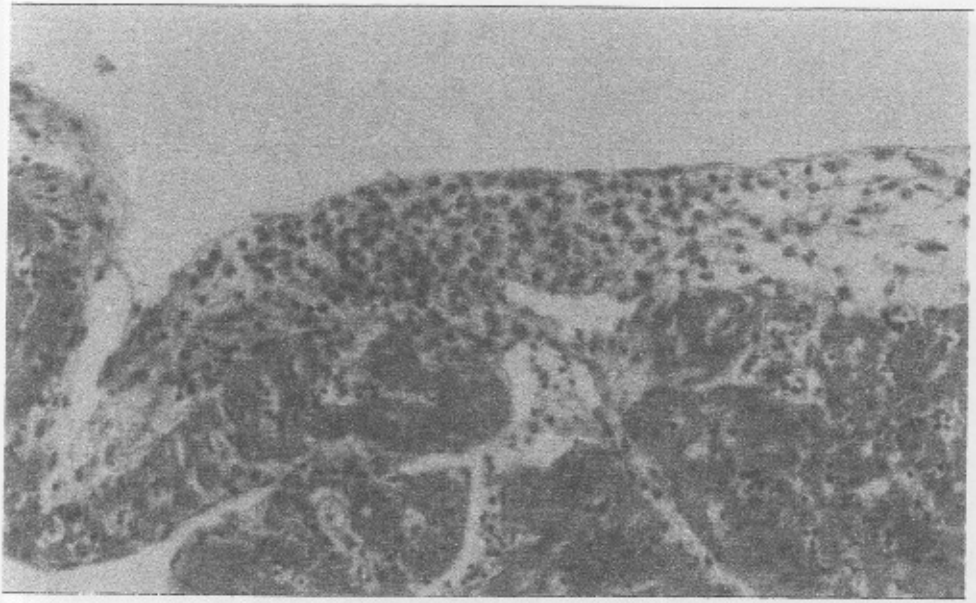

Fig. 3: Heart of chicken at 4th day PI showed pericarditis (H&E x 250).

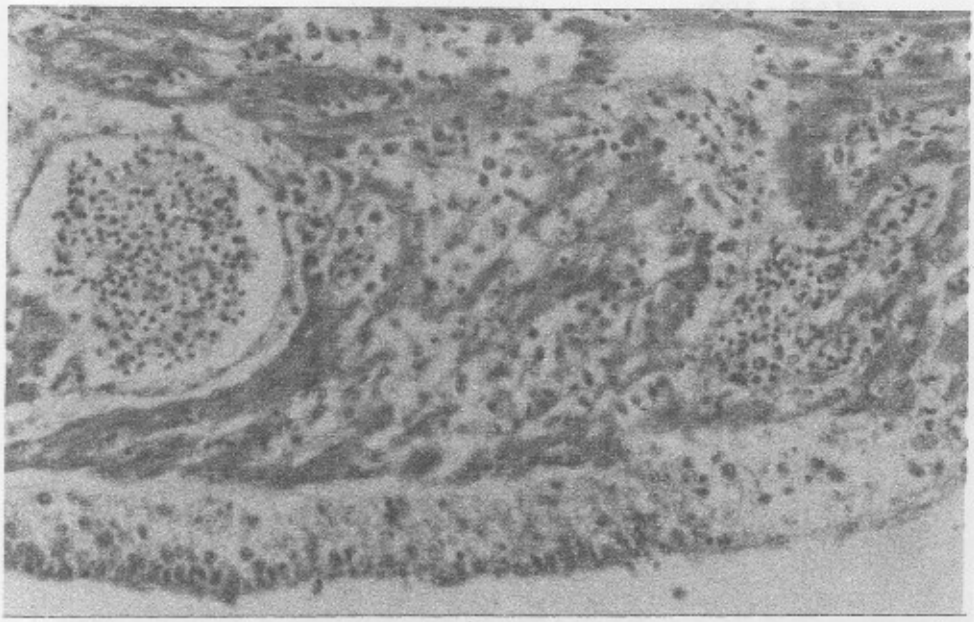

Fig. 4: Heart of chicken at 4th day PI showed myositis with heterophilic infiltration (H&E x 250).

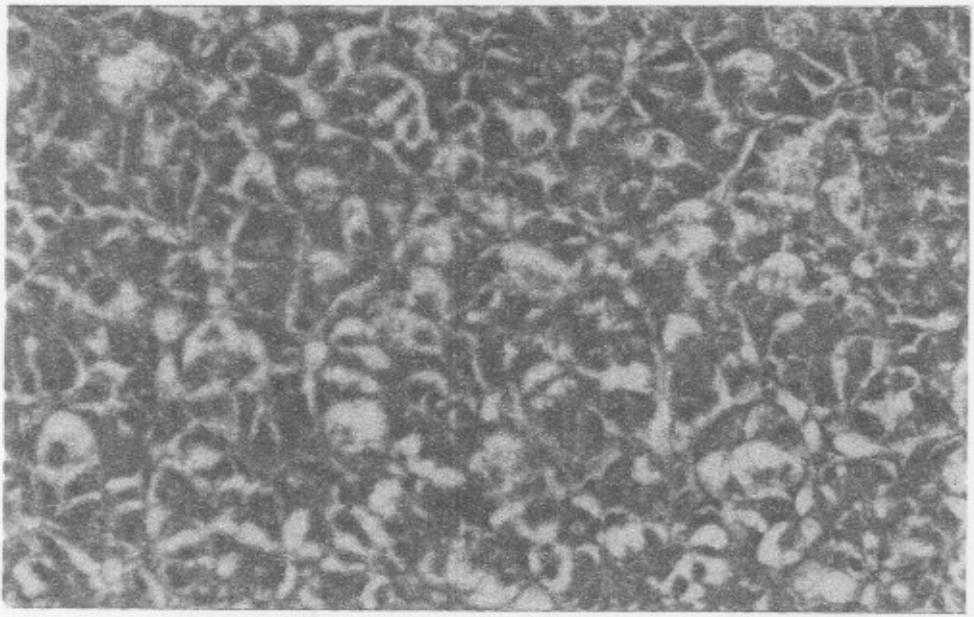

Fig. 5: Pancreas of chicken at 15 day PI showed vacuolar degeneration of acinar cells (H&E x 400).

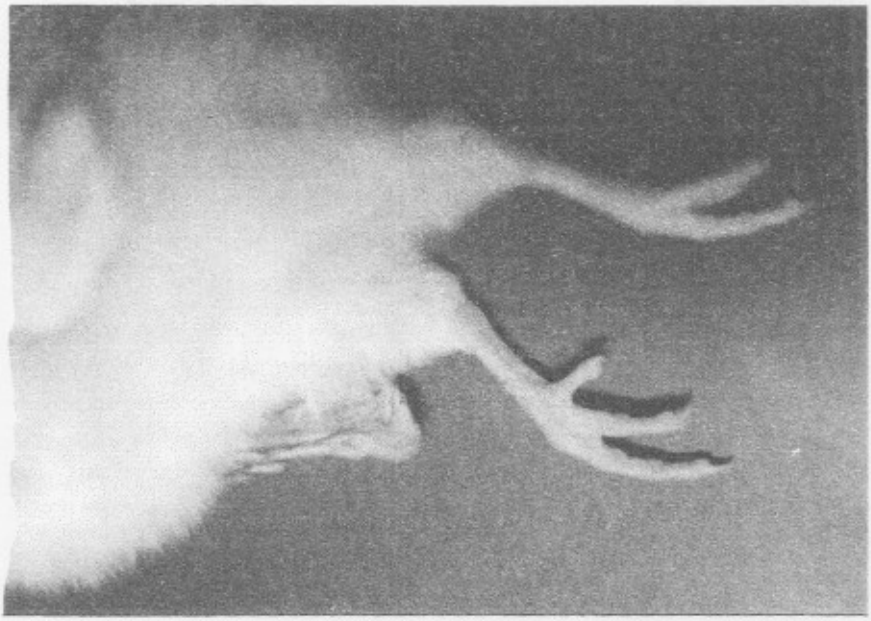

Fig. 6: Leg of inoculated chicken at 4th day PI swelling.