PATHOLOGIC STUDIES ON HEMORRHAGIC ENTERITIS OF TURKEYS, WITH REFERENCE TO ISOLATION AND IDENTIFICATION

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ABSTRACT

Three turkey flocks (2000, 3000 and 2500 birds and 6, 8 and10 weeks old respectively were located in Sharkia and Ismailia governorates) showed depression, bloody droppings and 12-18% mortalities with negative response to antibiotic treatment. Bacteriological and parasitological examinations of diseased turkeys revealed negative results; but hemorrhagic enteritis virus (HEV) was isolated on fertile eggs. The isolated virus was identified using agar gel precipitation test (AGPT).The embryo lethal dose fifty (ELD50) of isolated virus was 10^5/ml.

Twenty healthy turkey poults, (6 week old) were orally infected by the isolated virus (0.1 ml of inoculum which contained 10^3 mean ELD50/bird. The experimentally infected turkeys showed depression, bloody droppings with 40% moralities, 6 day post-inoculation .The virus was re-isolated from the experimentally infected cases. The dead birds, from the natural and experimental infection were necropsied, and specimens from the intestine, liver, spleen, lungs and bursa of Fabricious were fixed in 10%buffered formalin. Paraffin sections of 5 μ thick were prepared and examined microscopically. Both the naturally and experimentally infected turkey poults showed pale carcasses. The mucosa of the small intestine was congested. The intestinal lumen was filled with reddish bloody content. The spleen was enlarged and mottled with 1 mm red and pale foci. The liver was enlarged. Microscopically, the small intestine showed hemorrhagic enteritis. The lamina propria showed hyperemic blood vessels and round cell infiltration. The hepatic parenchyma was congested and showed focal coagulative necrosis. The spleen showed intranuclear inclusion in the lymphoreticular cells beside depletion of lymphocytes from the white pulp. The bursa of Fabricious

showed mucinous degeneration of the epithelial lining beside depletion of lymphocytes particularly from the center of the follicles. The lungs showed congestion and desquamation of the epithelial lining of the bronchioles.

INTRODUCTION

Hemorrhagic enteritis (HE) is an acute viral disease of turkeys, 3 - 5 weeks of age and older. It was first observed in Minnesota by **Pomeroy and Fenstermacher (1937)**, and in Ohio by **Gale and Wyne (1957)**. Hemorrhagic enteritis virus (HEV) is related to Adenoviridae under the genus Aviadenoviruses **(Domermuth and Gross 1984)**. The mortality rate was high due to the immunosuppressive nature of HEV and subsequently the secondary bacterial infection. **Fenner et al. (1993)** mentioned that the infection becomes severe specially in the immunocompromised birds by other infections, like avian leukosis, infectious bursal disease or chicken anemia viruses. Early studies suggested that HEV replication takes place in the nuclei of the reticuloendothelial cells **(Domermuth and Gross 1984)**. Recently the infected cells were noticed in a variety of tissues including intestine, bursa of Fabricious, thymus, liver, kidneys, peripheral blood leukocytes, lungs and spleen **(Fitzgerald et al. 1992, Hussain et al. 1993, Trampel et al. 1992 and Saunders et al. 1993)**. HE is characterized by depression, bloody droppings and death. The mortality rates, in field outbreaks varied from less than 0. 1 % to over 60% and reached 80% in the experimental infection with most pathogenic strains **(Gross and Moore 1967)**. The mortality occurs 5 - 6 days after oral or cloacal inoculation or 3-4 days after intravenous inoculation **(Domermuth et al. 1972-a)**. The clinical disease usually persists in affected flocks for 7-10 days **(Pierson and Domermuth 1997)**. Intranuclear inclusions were observed in liver, bone marrow, peripheral blood leukocytes, lungs, pancreas, brain and renal tubular epithelium **(Hussain et al. 1993 and Meteyer et al. 1992)**.

The aim of this work was to study the pathologic changes induced by HEV in the naturally and experimentally infected turkey poults. Isolation and identification of the virus were considered.

MATERIAL AND METHODS

Flock history :

Three turkey flocks of 2000, 3000 and 2500 poults which were 6, 8 and 10 weeks old respectively, and located in Sharkia and Ismailia governorates were used. These flocks were fed on a commercial ration and exposed to routine vaccination program against viruses. The turkeys

showed depression, bloody droppings and blood was noticed on the skin and feathers surrounding the vent. The mortality rates varied from 12 - 18% with negative response to antibiotic treatment. Bacteriological and parasitological examination of the diseased turkeys revealed negative results. HEV was suspected.

Samples for Virus Isolation :-

Samples such as feces, kidneys, intestine, lungs, spleen, liver and bursa of Fabricious were collected from infected and dead poults. Such samples were transported in containers containing virus transport medium including high concentrations of antibiotics, phosphate buffered saline and Hanks salt solutions. The appropriate pieces of samples were pooled, minced, and grinned with sterile sand in a morter. Suspensions were prepared and exposed to centrifugation at 3000rpm/30 minutes. The supernatant was collected and used for virus isolation.

Samples for Serology :

Forty-two serum samples were collected from the infected and contact turkeys for measurement and detection of HEV antibodies by the use of micro-immundiffusion.

The standard antigen and serum were obtained from Animal Health Research Institute Dokki. Egypt

Isolation of HEV on Fertile Eggs :

Isolation of HEV from minced splenic tissue was performed according to **Ahmed and Sharma (1993)**. Pathogen-free turkey eggs were inoculated via the chorioallantoic membrane (CAM) route on day 14 of embryonation with 0.1 ml of 1 : 10 Wt/Vol. suspension of splenic tissue. The inoculated eggs were reincubated with periodical examination. The procedure was repeated for 3 successive passages and the 3rd serial passages virus was confirmed by AGPT.

Detection of HE viral antigen, in the pooled minced field tissue and embryonic harvested tissue, was determined by the use of micro agar gel precipitation test (AGPT) as described by **Domermuth et al. (1972 a & b)**.

Detection of the viral antibodies, in serum samples collected from both naturally and experimentally infected birds, was attempted by the AGPT (**Domermuth et al. 1972. - b**).

Experimental infection :

Twenty one day old, turkey poults were obtained from commercial hatchery and were seronegative for HEV. The turkeys were fed on balanced ration. Routine vaccination program was applied. Ten poults, 6 week old, were orally infected with 0.1 ml of inoculum which contained 10^3 mean ELD50/bird of the prepared crude PBS extract of the minced affected organs as spleen, intestine and liver according to **Gross and Moore 1967 & Harris and Domermuth (1977)**. The remaining poults were kept as control. All birds were kept under strict hygienic conditions with daily observation. Samples were collected for virus reisolation from infected poults.

Pathological studies:

The clinical signs and postmortem findings of both the naturally and experimentally infected Turkey poults were observed and recorded. Specimens were collected from the intestine, liver, spleen, lungs and bursa of Fabricious and fixed in 10% buffered formalin solution. Paraffin sections of 5 μ thick were prepared and stained with hematoxylin and eosin for microscopic examination (**Lillie and Fulmen 1976**).

RESULTS AND DISCUSSION

Clinical signs :

Both the naturally and experimentally infected poults showed depression, bloody droppings and soiled skin and feathers around the vents. The mortality rates were 12-18% among the naturally infected birds. On the other hand 40 % of the inoculated turkeys died 6 days Post-inoculation (PI) and the remaining birds were sacrificed 30 day PI.

Pathologic findings :

Postmortem finding of both the naturally and experimentally infected poults showed pale carcasses. The Small intestine was filled with reddish brown bloody content. The intestinal mucosa was congested. The Spleen was enlarged and mottled with 1mm red and gray foci; however the spleen was small in some cases. The liver was enlarged and contained pale whitish areas. The lungs were congested. Microscopically, the small intestine, particularly duodenum showed severe congestion of the intestinal mucosa beside mononuclear cell infiltration (Fig. 1). Desquamation of the villus epithelium beside congestion and extravasated blood were noticed in the lamina propria (Fig.2). Hemorrhagic enteritis could be seen and the lumen contained numerous eryth-

rocytes (Fig.3). The hepatic parenchyma showed focal coagulative necrosis, evidenced by pyknosis and more eosinophilic cytoplasm (Fig.4). The portal veins, hepatic sinusoids and central venis were congested. The surrounding hepatocytes were vacuolated (Fig.5). Hemorrhage was noticed among the necrotic hepatocytes with the presence of golden yellow pigments inside the kupffer cells or scattered among the hepatic cells. The spleen showed hyperplasia of white pulp in the early stage. Numerous intranuclear inclusion bodies were noticed in the lymphoreticular cells, (Figs.6 & 7). Lymphoid necrosis of the white pulp could be seen later on. The bursa of Fabricious showed depletion of lymphocytes from their follicles, (Fig.8). Vacuolation of the epithelial lining was noticed (Fig.9). The pulmonary blood vessels were congested specially around the secondary bronchioles (Fig.10). The tertiary bronchioles and air capillaries were filled with eosinophilic material, (Fig. 11).

Isolation of HEV on fertile eggs :

HEV was detected in the pooled collected samples, as white / colorless spots (pock lesions) distributed over the CAM, harvested from the inoculated eggs. The ELD50 of the isolate was 10^5 /ml.

Detection of the viral antigens :

The specimens, collected from both the natural and experimental infections as well as from the harvested infected CAMS, were positive for the HE viral antigens by the application AGPT (Fig.12).

Detection of the viral antibodies :

Application of AGPT was successful for screening and detecting the HEV antibodies in the serum samples, obtained from the naturally diseased and experimentally infected turkeys.

Our investigation revealed that the moralities were 12-18% and 40% among the naturally and experimentally infected turkey poults respectively. The clinical signs included depression, bloody droppings. Macroscopically, the carcasses of the dead poults were pale due to blood loss through the hemorrhagic enteritis. The small intestine was distended with bloody content. The spleen was enlarged, friable and marbled in appearance. Such findings suggested HEV infection. This was confirmed by the isolation of HEV on fertile eggs where pock lesions were encountered on the CAM. The viral replication on CAM of the inoculated embryo was confirmed by the positive AGPT. **Gross and Moore (1967)** mentioned that HE was characterized by depression, bloody

droppings and moralities which varied from less than 0.1% to over 60% and reached 80% in the experimental infection with most pathogenic strains. Microscopically, numerous intranuclear inclusion bodies were noticed in the lymphoreticular cells of the spleen. This lesion in addition to the hemorrhage enteritis were pathognomonic for the HE of turkeys. Similar findings were reported by **Calnek et al. (1997) and Jordan and Pattison (1996).** The presence of intranuclear inclusion bodies in the lymporeticular cells of the spleen could be attributed to the intranuclear replication of the causative adenovirus. **Jones et al. (1997)** mentioned that the nucleic acid of adenoviruses consists of single linear molecules of double stranded DNA, which replicates in the nucleus where a characteristic inclusion body is produced. Recently **Hussain et al. (1993) and Sounders et al. (1993)** noticed infected cells in a variety of tissues including spleen. Meanwhile the early studies by **Domermuth and Gross (1984)** suggested that HEV replicates in the nuclei of the reticuloendothelial cells. Our findings revealed that the spleen of infected birds was enlarged and marbled in appearance. However the spleen was small in some cases, this could be attributed to blood loss and subsequently splenic contraction **(Calnek et al, 1997)** beside the depletion of the lymphocytes from the white pulps. The intestinal lesions, in this study are in agreement with **Hussain et al. (1993) and Meteyer et al. (1992).** Depletion of lymphocytes from the follicles of bursa of Fabricious and white pulp of the spleen could be attributed to the immunosuppressive effect of group II avian adenoviruses that appeared to be lymphotropic and lymphocytopathic **(Fizgerald and Reed 1991 and Suresh and Sharma (1995). Jordan and Pattison (1996)** added that the virus is immunosuppressive and may predispose to outbreaks of colibacillosis. Our experimental infection was done by inoculation of 6 week old turkey poults as younger turkeys, under 4 weeks of age, are not susceptible as protected by maternal antibody **(Jordan 1990).**

Finally, it could be concluded that HE induced12-18% and 40% mortalities among the naturally and experimentally infected turkey poults. The pathognomonic lesions were intranuclear inclusion bodies in lymphoreticular cells of the spleen, and hemorrhagic enteritis in both the naturally and experimentally infected turkeys. The virus isolation and identification confirmed our findings.

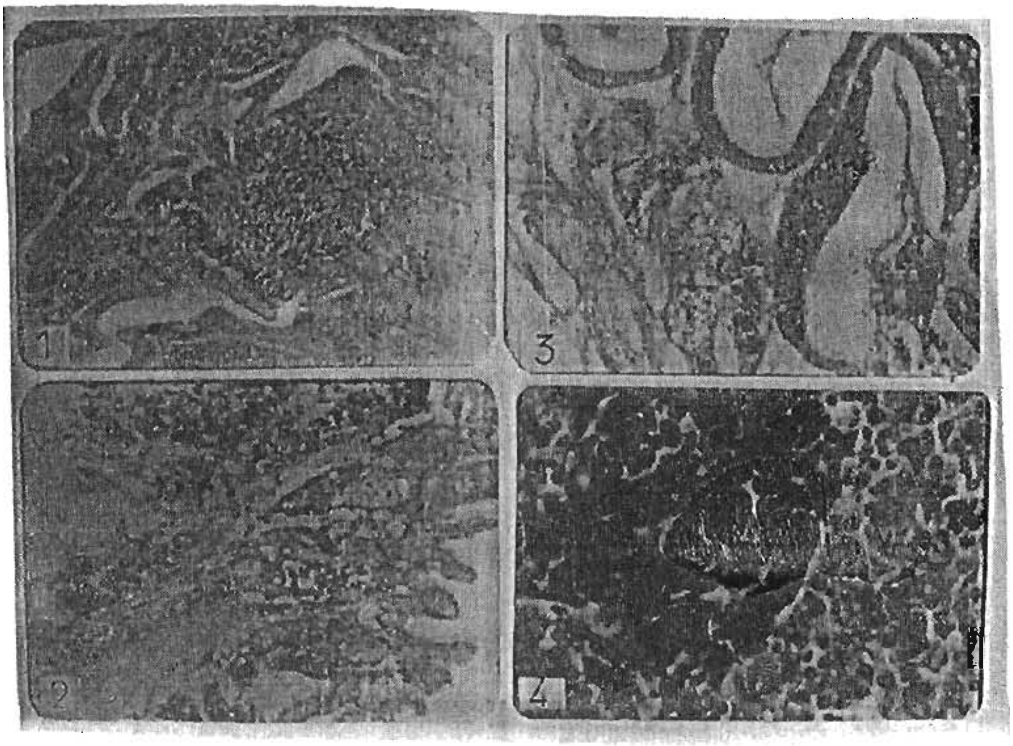

Fig. 1 : Intestine from the naturally infected poult, showing congestion of the intestinal mucosa beside mononuclear cell infiltration (H & E. x 300)

Fig. 2 : Intestine from the naturally infected poult, showing desquamation of the villous epithelium beside extravasated blood in the lamina propria (H & E. x 300).

Fig. 3 : Intestine from the experimentally infected poult, showing hemorrhagic enteritis. Numerous erythrocytes are seen in the lumen (H&E. x 300).

Fig. 4 : Liver from the experimentally infected poult, showing focal coagulative necrosis (H & E. x 1200)

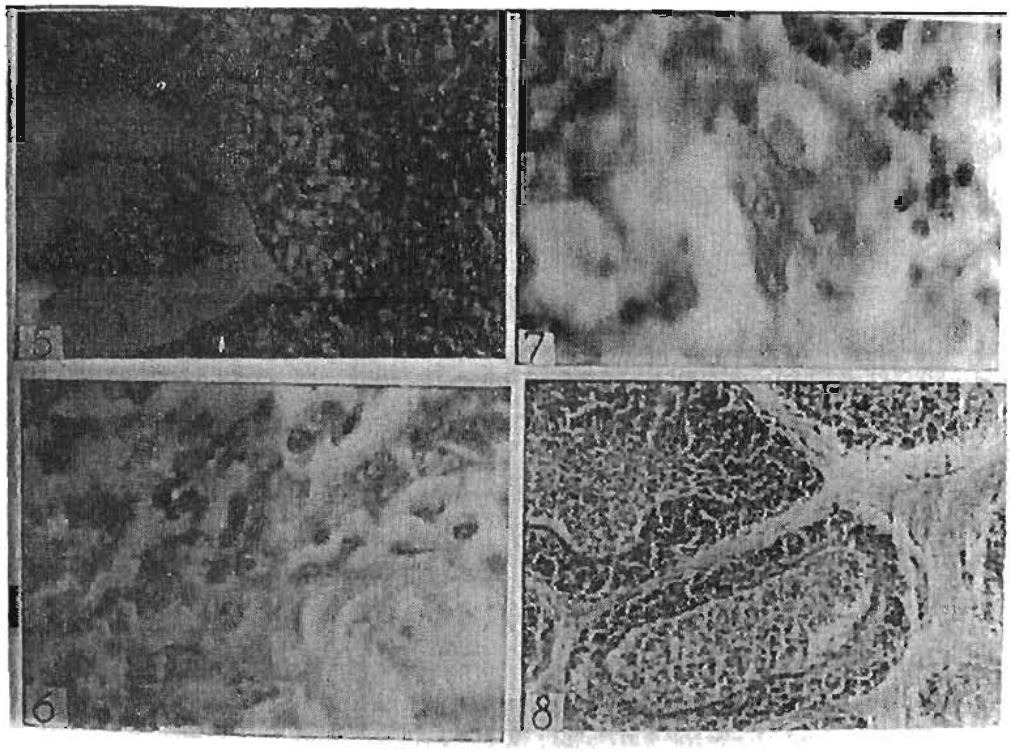

Fig. 5 : Liver from the experimentally infected poult, showing congestion of central veins and vacuolation of hepatocytes (H & E, x 1200).

Fig. 6 : Spleen from the experimentally infected poult, showing intranuclear inclusion bodies in lymphoreticular cells (H & E. x 1200).

Fig. 7 : Spleen from the naturally infected poult, showing intranuclear inclusion bodies in lymphoreticular cells (H & E. x 1200).

Fig. 8 : Bursa of Fabricious from the naturally infected poult, Showing depletion of lymphocytes from the center of the follicles (H & E. x 300).

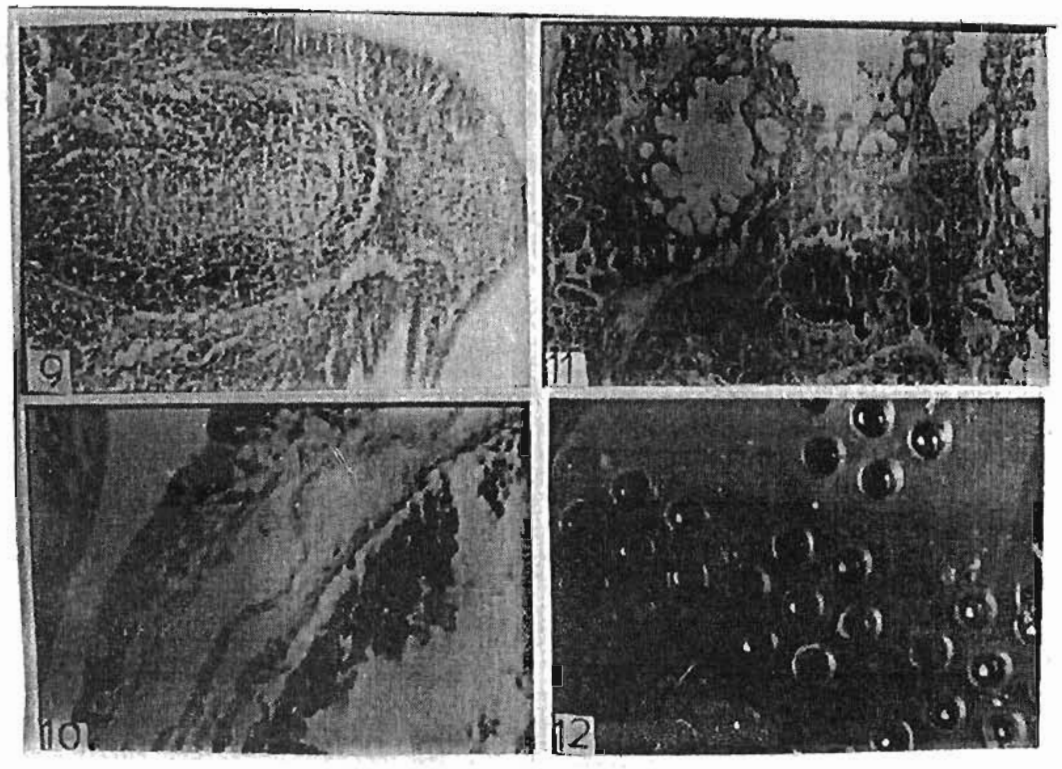

Fig. 9 : Bursa of Fabricious from the experimentally infected poult, Showing vacuolation of the epithelial lining beside depletion of lymphocytes from their follicles (H & E. x 300)

Fig. 10 : Lung from the experimentally infected poult, showing congestion of the peribronchial blood vessels (H & E. x 1200).

Fig. 11: Lung from the experimentally infected poult, showing eosinophilic material inside the tertiary bronchioles beside congestion of the pulmonary blood vessels (H & E. x 300).

Fig. 12 : Showing precipitin lines of identity between HEV Ag. against serum samples.

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الملخص العربى

دراسات باثولوچية على النزلة المعوية المدمة فى الرومى مع الإشارة إلى عزل وتعريف الڤيروس

المشتركون فى البحث

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تم إجراء هذه الدراسة على ثلاثة قطعان من الرومى ٢٠٠٠، ٣٠٠٠، ٢٥٠٠ طائر أعمارها ٦، ٨، ١٠ أسابيع على الترتيب فى محافظتى الشرقية والاسماعيلية، كانت الأعراض الإكلينيكية ممثلة فى الخمول والإسهال المدمم ونسبة النفوق تتراوح من ١٢-١٨٪ والتى لم تستجيب للمضادات الحيوية وكان الفحص البكتيريولوچى والطفيلى سلبى. هذا وقد تم عزل الڤيروس المسبب للنزلة المعوية المدمة على بيض الرومى المخصب وتم تعريفه بواسطة AGPT. وتم حقن الڤيروس المعزول فى ٢٠ طائر سليم عمرها ٦ أسابيع بالفم مما أدى إلى ظهور الخمول والإسهال المدمم و٤٠٪ نفوق وتم عزل الڤيروس مرة أخرى من الطيور المصابة تجريبياً. هذا وقد تم إجراء الصفة التشريحية لكل من الطيور النافقة من العدوى الطبيعية والتجريبية والتى تمثلت فى إحتقان فى الغشاء المبطن للأمعاء الدقيقة والتى كانت محتوياتها مدممة وكان الطحال متضخم وبه نقط حمراء وبيضاء قطرها حوالى ١ مللى وتم أخذ عينات للفحص الهستوباثولوچى حفظت فى فورمالين ١٠٪ والذى أوضح إلتهاب معوى مدمم فى الأمعاء الدقيقة مع إحتقان فى الأوعية الدموية وتخلل أنسجتها الخلايا الالتهابية وأوضح الكبد إحتقان فى الأوعية الدموية وتنكرز تخثرى وأوضح الطحال نقص فى عدد خلايا الليمفوسيت المكونة للب الأبيض مع وجود احسام إحتوائية داخل الأنوية وكان هناك تحول فى الخلايا المبطنة للبرسبا إلى خلايا كاسية مع نقص فى عدد خلايا الليمفوسيت المكونة لمركز الكرات الليمفاوية وكان هناك إحتقان فى الرئتين مع موت الخلايا المبطنة للشعيبات الهوائية.