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Detection of transmissible viral proventiculitis in Iraq

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Abstract
In this study, avian transmissible viral proventiculitis virus was detected for the first time in Iraq. Thirty samples were collected from chickens in different areas of Iraq. Conventional reverse transcriptase - polymerase chain reaction technique (RT-PCR) by amplification of VP1 gene was used to amplify extracted nucleic acid. Nine samples out of thirty samples were positive by using conventional RT-PCR technique. Histopathological examination was performed for collecting infected proventriculi after post mortem examination. Histopathological examination revealed severe destruction of glandular epithelium and necrosis of glandular epithelium with diffuse mononuclear cells and lymphoid apoptosis together with hyalinization of some mucosal folds and ulceration of mucosal layer with blood vessels congestion along with mild epithelial hyperplasia.

Keywords: Transmissible viral proventiculitis virus, histopathology, PCR

Introduction
Transmissible viral proventriculitis (TVP) is a recognized cause of production losses in broiler chickens and it has been reported in broiler breeder and commercial layer hens [1].
Lesions in the proventriculus have been reported worldwide in broilers suffering from diseases under names such as malabsorption syndrome, feed passage syndrome runting or stunting syndrome, and infectious or Transmissible proventriculitis (TP) [2]. Studies have suggested that TP has a complex aetiology with several clinical and pathological expressions. It was noted that enlargement of the proventriculus associated with hyperplasia of the glandular epithelium, fibrosis, and oedema reported in North America is different from the swelling of the proventriculus due to a massive infiltration of lymphoid cells in glandular tissue and mucosa described in Holland [2].
Infectious bursal disease virus (IBDV) has been associated with TP, although recent studies on its role indicated that the disease could occur in the absence of IBDV [3, 4].
Many agents have been implicated with proventriculitis either alone or in combination. They may be infectious or dietary agents. These include reovirus (5), IBDV [6, 7], adenovirus [8], infectious bronchitis virus (IBV) [9], clostridium [10], biogenic amines [11], but in case of Transmissible viral proventriculitis (TVP), the experimental studied suggested etiological agent of the disease, Chicken proventricular necrosis virus (CPNV), isolate R11/3, was isolated from transmissible viral proventriculitis-affected chickens and was determined to be the likely etiology of this disease [12]. CPNV was identified as a birnavirus on the basis of virion size and morphology (icosahedral, approximately 75 nm in diameter, nonenveloped); a genome comprising bisegmented, double-stranded RNA (approximately 3.8 and 3.4 kilobase pairs); and nucleotide sequence analyses [12].

The aim of the study: detection of causative agent of transmissible viral proventiculus for the first time in Iraq by using conventional RT-PCR and histopathological examination.

Materials and methods
1. Collection of samples: thirty samples were collected from chickens in different areas of Iraq. Samples were selected from middle and southern governorates, origin: Turkey, Bulgaria and Belgium as the followings:
Twenty six samples were selected from broiler aged (11-31) days old.
Four samples were selected from layer aged (55-191) days old.

2. RNA kit extraction: (Intron - Korea): the kit was used for extraction of RNA from infected samples with Proventricular necrosis virus.
3. Primers specific for VP1 gene of proventricular necrosis virus were manufactured by (Alpha DNA, Montreal, Quebec) and were imported by URUK Center.
Forward primer: 5-CGTAGACCTCGTCCTTCTGC-3
Reverse primer: 5-GGGCGTAACCATTCAGATA-3
4. Reverse transcriptase: cDNA Kit (reverse Transcription System, Promega Co, USA).
5. Agarose: Promega, USA, concentration 1.5%, voltage 100, time 60 minutes.
6. ladder: 100 bp, Promega, USA.
7. Buffer: TBE (Tris borate EDTA), 1X, promega, USA
8. Master Mix: Taq Green Master Mix, Promega, USA

Methods

1. **Preparation of samples**: The Proventriculi were cut into two parts: the first part was prepared for amplification by RT-PCR; the proventriculi were ground for extraction of RNA.
   The second part of samples was put in 10% formalin for histopathological examination.
2. **Extraction of nucleic acid**: RNA was extracted from prepared samples by RNA extraction kit.
3. **Amplification of extracted nucleic acid** by conventional RT-PCR:
   A-Preparation of complementary DNA: Viral complementary DNA was prepared by using reverse transcriptase reaction kit.
   B-Amplification of preparation of complementary DNA: The conventional RT-PCR was run according to [12] as the followings:

1- Denaturation at 95 °C for 3 min.

2- a-Denaturation at 95 °C for 1 min.
b-anealing at 55 °C for 1 min.
c-extension at 72 °C for 1 min.
} 32 Cycles
3-extention at 72 °C for 5 min.

Then were cooled at 4 °C.

4. Gel electrophoresis for separation of amplified cDNA: 1.5% of agarose was used and the method of gel electrophoresis was conducted according to [13] method**,** then 1.5 µl of ethidium bromide (10mg/ml) solution was added and poured in gel chamber until the agarose solidify, finally the amplified DNA was loaded into the agarose wells. 10µl of loaded amplified DNA was added to each lane of gel Agarose and 10µl of marker (1000 bp) was added to first lane of electrophoresis. The amplified DNA was separated by electrophoresis at 100 volts for 60 minutes.
5. Histopathological examination was done for collecting infected proventriculi according to [14]. Samples were fixed in buffered 10% formalin for 24 hrs. and tissues were embedded in low- melting point paraffin then sectioned at 5 *M*m thickness and stained with hematoxylin and eosin.

Results

1. Results of post mortem examination

Post mortem examination for infected proventriculus revealed dilatation of the wall with congestion and hemorrhage as seen in the figure1.1, 1.2:

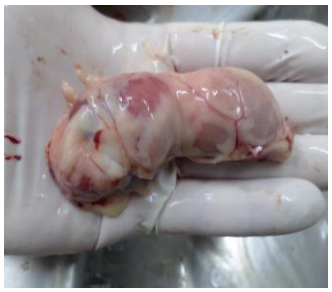


Fig.1.1

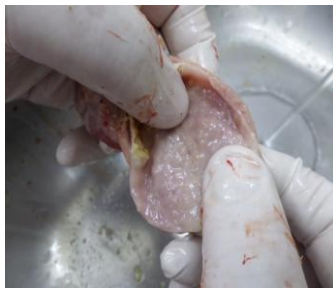


Fig.1.2

Fig 1.1, 1.2: post mortem examination for infected proventriculus of broiler chicken aged 24 days revealed dilatation of proventriculus and hemorrhage.

2. Results of RT-PCR technique

Ten samples (infected proventriculi) were positive with age range from (11-24) days from 3 different farm in Iraq out of thirty samples of broiler chicken using conventional RT-PCR technique as seen in the figure 2.1, 2.2:

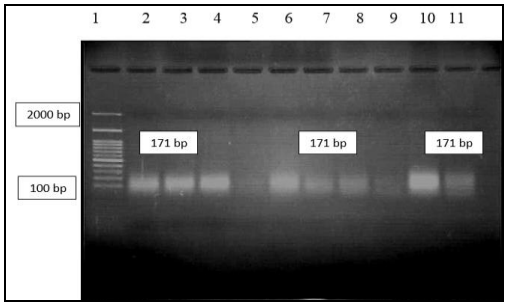


Fig 2.1: explain positive result of RT-PCR Amplification Product of VP1 (171 bp) of proventricular necrosis virus for nine of collected samples of broiler chickens. Lane 1: marker (100-2000) bp, lane 2, 3, 4: broiler chicks aged 11 days old (Hussein raheem farm), lane 6, 7: broiler chicks aged 22 days old (Hassan lifta farm), lane 8, 9, 10, 11: broiler chicks aged 24 days old (Muhmoud thwid farm).

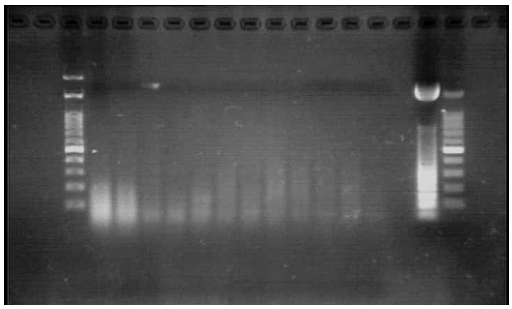


Fig 2.2: negative result of RT-PCR Amplification Product of VP1 (171 bp) of proventricular necrosis virus for other collecting samples of broiler chickens and layer.

3. Results of Histopathological examination for collecting infected proventriculi

The histopathological examination showed diffuse mononuclear cells infiltration in Lamina propria and submucosa consisting mainly of lymphocyte and plasma cells some of mucosal glands dilated with mucin secretion along with severe destruction of glandular portion. The histopathological examination also showed presence of apoptosis in the lymphoid tissue and cystic distention of some mucosal glands with severe necrosis of glandular epithelium that contains numerous necrotic debris and blood vessels congestion also lymphoid apoptosis with mild epithelial hyperplasia and slight cellular infiltration of inflammatory cells in the connective tissue as seen in the following figure 3.1-3.15:

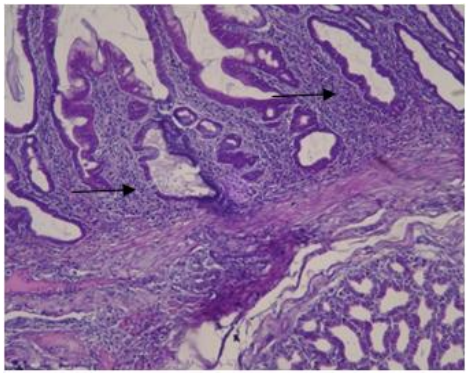


Fig 3.1: the predominant finding was diffuse mononuclear cells (⟶) infiltration in Lamina propria and submucosa consisting mainly of lymphocyte and plasma cells some of mucosal glands Dilated with mucin secretion other appeared atrophy(H and E:40X)

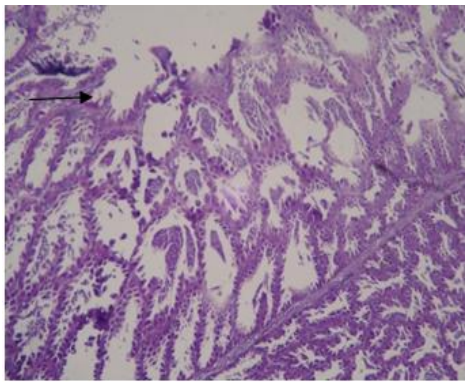


Fig 3.2: severe destruction of glandular portion (⟶) shows destructive lesion in the glandular tissue of proventiculus characterized by sloughing of glandular epithelia with debris in the lumen of glandular tissue (H and E 10 X)

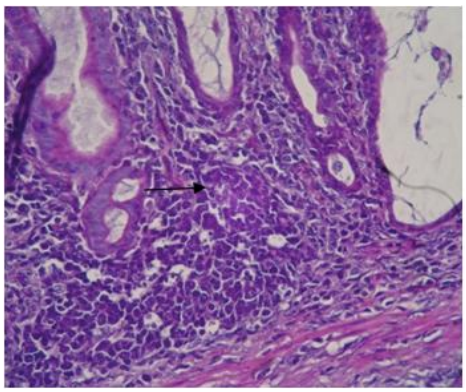


Fig 3.3: This section show presence of apoptosis in the lymphoid tissue characterized by space formation containing apoptotic debris (H and E 40X)

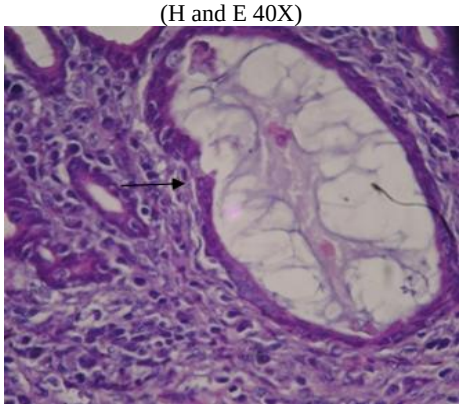


Fig 3.4: show cystic distention of some mucosal glands with thin epithelial lining together with laminar mucin secretion (⟶) (H and E 40X)

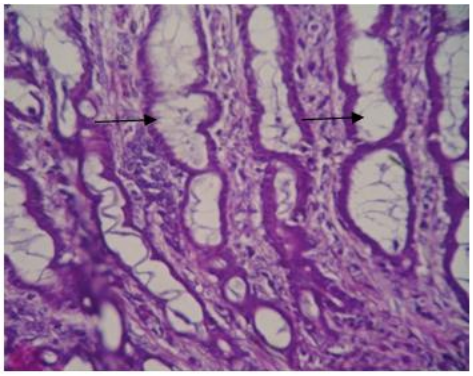


Fig 3.5: tortuous appearance of mucosal folds with mucin strands (⟶) in their lumen and mild cellular proliferation (40 X).

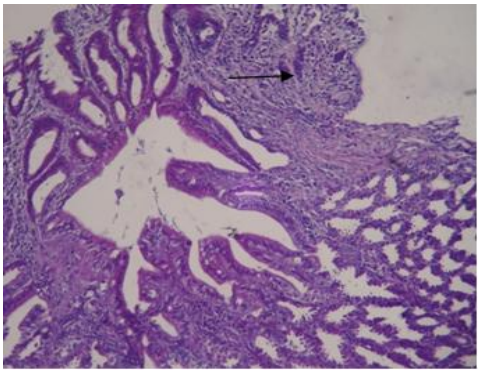


Fig 3.6: Focal mucosal ulceration accompanied with cellular infiltration (⟶) together with degenerative changes in glandular portion (H and E: 10X).

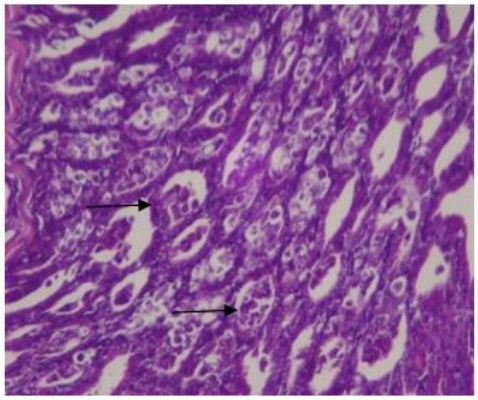


Fig 3.7: Sever necrosis of glandular epithelium that contains numerous necrotic debris (⟶) (40X).

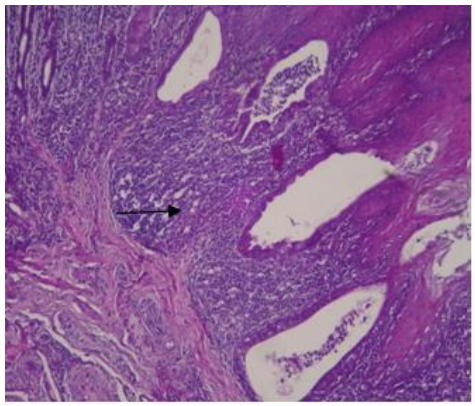
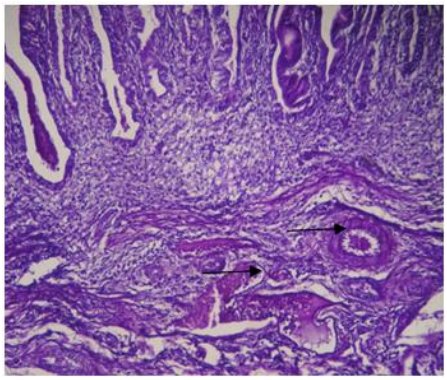


Fig 3.8: Diffuse mononuclear cells infiltration with lymphoid apoptosis together with hyalinization of some mucosal folds and some mucosal glands dilated and containing inflammatory cells (⟶). (H and E: 20X).



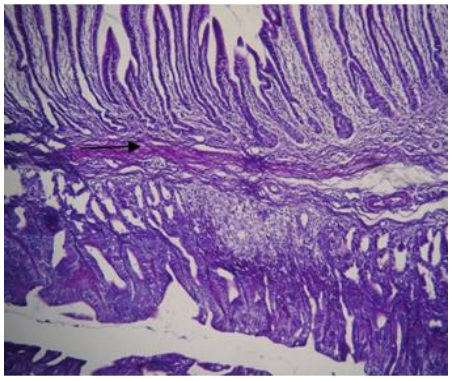


Fig 3.9-10: Show focal necrosis of glandular tissue mainly in deep portion (3.10:10X) together with massive mononuclear cells infiltration consist mainly of foamy macrophage with blood vessels congestion (⟶) associated with fragmentation of muscular layer (3.11:40X) (H and E).

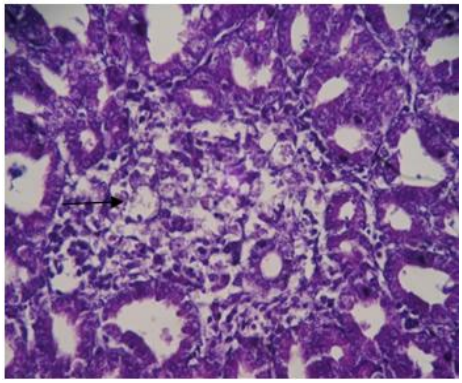


Fig 3.11: This section showed focal mononuclear cells aggregation (⟶) seen between degenerative glands (H and E: 40X)

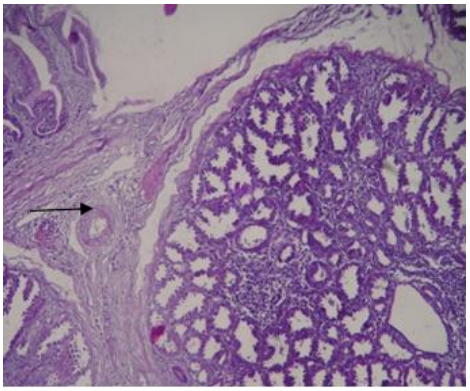


Fig 3.12: Revealed focal ulceration of the mucosal layer (⟶)accompanied with moderate cellular infiltration in an adjacent mucosal folds that extends to mascular coat accompanied with multifocal mononuclear cells aggregation seen between degenerated proventricular glands with evidence of cystic dilation of other glands (H and E: 10X).

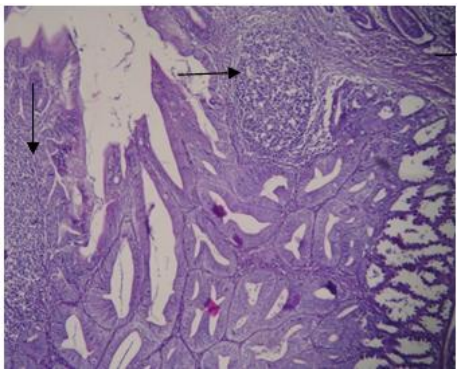


Fig 3.13: Lymphoid apoptosis with diffuse cellular infiltration (⟶) in Lamina propria and submucosal layer (H and E: 20X).

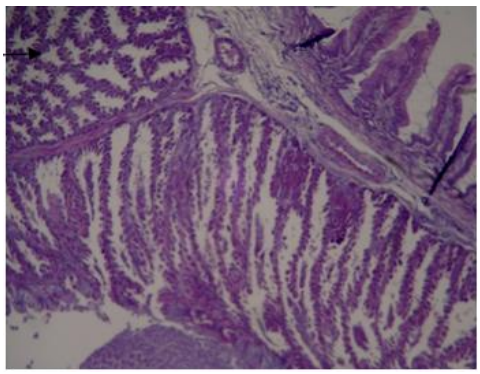


Fig 3.14: mild epithelial hyperplasia with slight cellular infiltration of inflammatory cells (⟶) in the connective tissue (H and E: 20X)

Discussion

The proventricular necrosis virus was detected for the first time in Iraq, the virus was detected in broiler with dilatation of proventriculus and hemorrhage and was not detected in layer due to few samples were collected from layer, the present results are inagreement with [15] which explained that the virus occurs commonly in broiler and related to fragility of proventriculus, impairment of growth and impairment of digestion but the virus was detected by [1] in both broiler and layer. The RT-PCR technique was suitable to detect the virus in agreement with [16]. The RT-PCR technique was sensitive to detect proventricular necrosis virus and rapid tool for detection of the virus therefore by using conventional RT-PCR technique, nine samples with age range from (11-24) days out of thirty samples were positive and it's the most susceptible age to TVP infection by amplification of VP1(RNA directed RNA polymerase) [2]. The histopathological changes of examined proventriculi were severe destruction of glandular epithelium along with necrosis of glandular epithelium that contain numerous necrotic debris with diffuse mononuclear cells infiltration which is in agreement with studies of [17, 18] and lymphoid apoptosis together with hyalinization of some mucosal folds and ulceration of mucosal layer with blood vessels congestion also mild epithelial hyperplasia with slight cellular infiltration of inflammatory cells in the connective tissue were detected inagreement with [1]. The main histopathological changes were marked necrosis and degeneration of the proventricular glandular epithelium along with infiltration of lymphocytes which are inagreement with study of [19].

Conclusions

In this study, the causative agent of transmissible viral proventiculus was detected for the first time in Iraq in broiler

chicks. The conventional RT-PCR technique was suitable to detect causative agent of transmissible viral proventiculus. The histopathological changes of infected pronentriculi were diffuse mononuclear cells infiltration in Lamina propria and submucosa consisting mainly of lymphocyte and plasma cells and some of mucosal glands dilated with mucin secretion along with severe destruction of glandular portion. The histopathological examination also showed presence of apoptosis in the lymphoid tissue and cystic distention of some mucosal glands with severe necrosis of glandular epithelium that contains numerous necrotic debris and blood vessels congestion.

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