Experimental infection on the locally isolated avian infectious laryngotracheitis virus

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Summary

The aim of this study was to evaluate virulence of local isolated avian infectious laryngotracheitis virus in experimentally infected chicken. Forty 10 week old chickens were used for the experimental infection with the locally isolated infectious laryngotracheitis virus. Chickens were divided into three groups, the first group consisted from 20 chickens infected with isolated infectious laryngotracheitis virus ($2\times10^{4.16}$ TCID 50/50 µl) via eyes and mouth drops (one drop for each). The second group consisted of 10 chickens (non-infected) and left in contact with infected group inoculated with maintenance media (Minimum essential medium) on their eyes, to observe if the infected group can spread the virus. The third group consisted of 10 chickens (non-infected) were left as a control group separated from other groups, inoculated with maintenance media (Minimum essential medium) on their eyes. Clinical signs and mortality were examined daily up to 12 days post infection. The main clinical signs were depression coughing and gasping with mild conjunctivitis and no mortality. Enzyme linked immunosorbent assay (ELISA) test was conducted on the collected sera of chickens before and after experimental infection with isolated virus. The results of ELISA test was negative for all groups of chickens before experiment and positive results for infected group with titer approximately ranging from (2534-7910); Measure of central tendency and dispersion were used with mean (4874.75) and stander error (355.96\ 13.6%); while negative results for contact group and control group. Eighteen chickens (10 weeks old) separately were divided into three groups (infected, contact and control) treated as mention above and were used for histopathological examination; the chickens were killed, two in each group at 24 hr., 48 hr. and 72 hr. post infection. The histopathological changes on trachea and larynx were intracellur inclusion bodies formation detected at 72hr., post infection for infected group only.

Keywords: Infectious laryngotracheitis virus, Field isolates, Experimental infection.

Introduction

Infectious laryngotracheitis virus is an enveloped virus with a double stranded DNA genome and it is the member of the family Herpesviridae and subfamily Alphaherpersvirinae (1 and 2). The ILT virus causes respiratory ocular signs in chickens, pheasants and peafowls in the world (3). The ILT virus is transmitted via nasal and ocular secretions and lead to respiratory manifestations (mild to severe form). The severe form of ILT results in suffocation and bloody respiratory mucus secretion and consequently the mortality rate up to 70% (4). The mild form of the ILT is manifested by depression; drop in egg production and emaciation (5). The main characteristic feature of infection with ILT virus is inclusion bodies in epithelial cells; the presences of inclusion bodies are for a few days at the early stage of infection before death of epithelial cells. When the necrotic epithelial cells are detached from the trachea, bloody mucus was observed (6). This study aimed to investigate virulence and histopathological changes of local isolated avian infectious laryngotracheitis virus in experimentally infected chickens.

Materials and Methods

Fifty eight (58) layers chickens 10 weeks of age were supplied from local farm at Al-Taji (Hussan Ali farm) and were divided into two groups (40) and (18), the latter used for histopathological examination, before the experiment chickens had been isolated for 3 weeks at poultry diseases laboratory\College of Veterinary Medicine-University of Baghdad, to make sure the clearance from other infections.

Experimental infection of chicken with local isolated virus at poultry diseases laboratory\College of Veterinary Medicine-University of Baghdad.

Forty chickens at 10 weeks old were used for the experimental infection with the isolated ILT virus. Chickens subdivided into three groups, first group consisted from 20 chickens were infected with ILT virus isolate ($2\times10^{4.16}$ TCID 50/50 µl) via eyes and mouth drops (one drop for each). Second group consisted from 10 chickens in contact with infected group inoculated with maintenance media (Minimum essential medium) on their eyes, to observe if the infected group can spread the virus. The third group consisted from 10 chickens were left as a control group separated from other groups, inoculated with maintenance media (Minimum essential medium) on their eyes. Chickens were examined daily up to 12 days post infection for Clinical signs and mortality.

ELISA (ProFlock®) for the detection of Infectious laryngotrachieitis antibodies in serum imported from Synbiotics Corporation\ U.S.A. test was conducted for collected sera of chickens before and after experimental infection. Whole blood was collected in a covered test tube (without anticoagulant); after collection the blood was left undisturbed at room temperature for 15 min. to clot. The clot removed by centrifuging at 1000 RPM for 10 minutes. Following centrifugation, the supernatant (serum) was immediately transferred into clean polypropylene tube using Pasteur pipette. The serum samples were cooled to 2 ºC then transported to -20 ºC till used.

Eighteen chickens separately were divided into three groups (infected, contact and control) treated as mention above were used for histopathological examination, two chickens were killed in each group at 24 hr., 48 hr., and 72 hr., post infection, and then the samples (trachea and larynx) were collected. The samples of experimentally infected chickens were fixed in buffered 10% formalin for 24 hrs. and tissues were embedded in low-melting point paraffin then sectioned at 5 mm thickness and stained with hematoxylin and eosin (7).

Results and Discussion

The main clinical signs were depression coughing and gasping with mild conjunctivitis and no mortality. These signs are the most characteristic of mild form (8). However most birds showed mild edema and congestion of conjunctiva (6). This explains why the contact group wasn't infected by the virus.

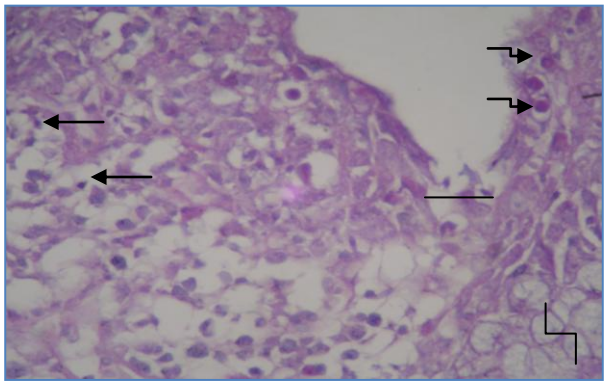
ELISA test was negative for all groups before experimental infection and positive for infected group only. The antibodies titer approximately ranged from (2534-7910); Measure of central tendency and dispersion were used with mean (4874.75) and stander error (355.96\ 16.3%); while negative results for contact group and control group as seen in (Table, 1).

Table, 1: Detection of ILT virus antibodies by ELISA test for infected group 12 days post infection.

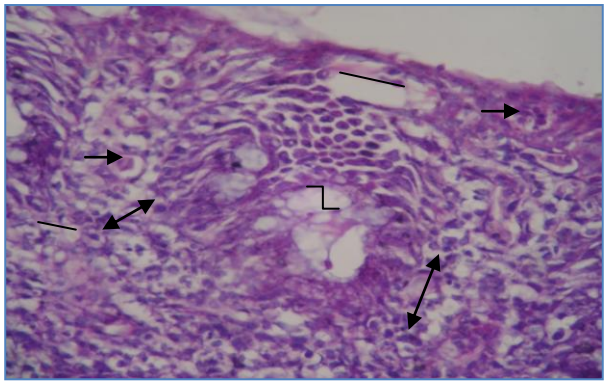
Sample No.	Titer	Sample No.	Titer
1	5030	11	5112
2	6172	12	4672
3	4022	13	6750
4	7605	14	5004
5	3220	15	6521
6	5601	16	2534
7	2623	17	4945
8	2800	18	3750
9	3456	19	3863
10	7910	20	5901
			S.E 355.96
			Mean±4874.75

The result of ELISA test of experimental infection agreed with others (9 and 10) who explained that antibodies to ILT were detected after 10-14 days but in contact group the result was negative because the probability that the dose of virus exposure was insuffecient to induce antibodies or cause viral infection.

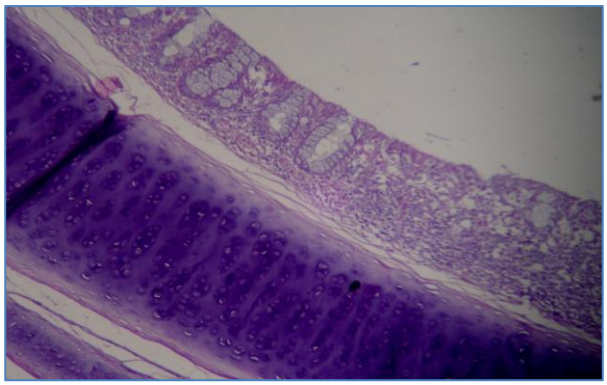
The histopathological changes were detected after 72 hr. Post infection in infected group only. This changes showed intracellular inclusion bodies formation, lymphocytes infiltration, goblet cells hypertrophy and hyperplasia with epithelial and subepithelial vacuolation (Fig. 1 and 2) in compare with contact (Fig. 3 and 4) and control groups (Fig. 5 and 6).



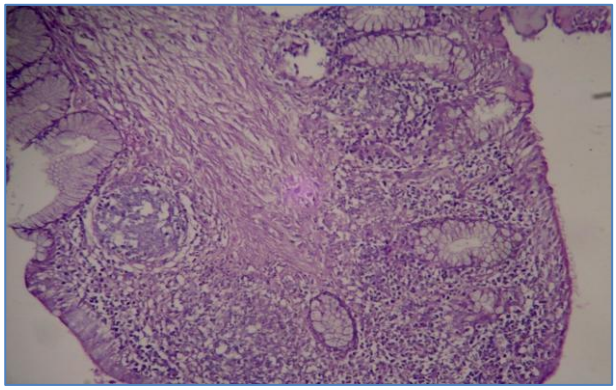
Figure, 1: Histopathological section in infected trachea shows vacuoltion in the epithelial cells (———), hyperplasia and hypertrophy in goblet cell of mucosal gland (⌙), lymphocytes infiltration (⟵) and containing intranuclear inclusion bodies (⟶) 72 hrs. post infection (H and E) (400X).



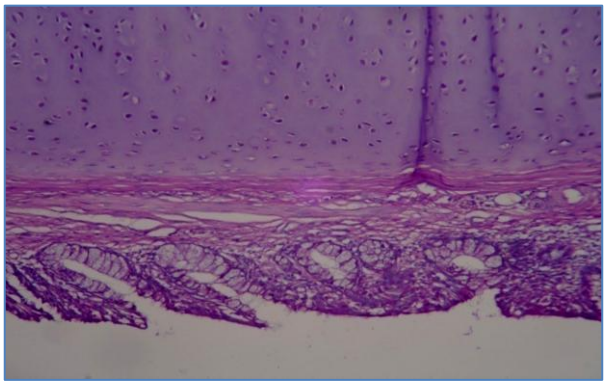
Figure, 2: Larynx shows focal vacuolation of epithelial and subepithelial layer (———), epithelial cell infiltration (⟷), goblet cell hypertropy of mucosal gland (⌙) and intranuclear inclusion bodies (⟶) 72 hrs. post infection (H and E), (400X).



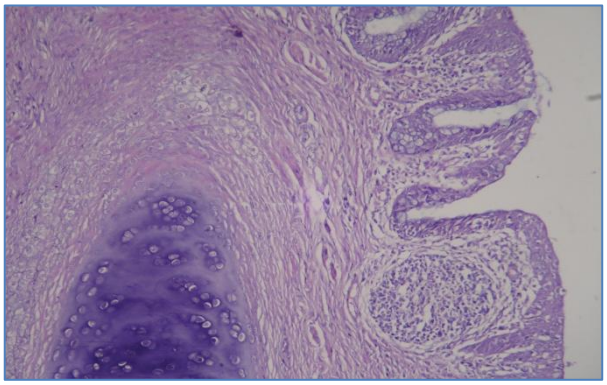
Figure, 3: Contact trachea shows normal tissue, 72 hrs. post infection, (H and E) (100X).



Figure, 4: Control larynx shows normal tissue, 72 hrs. post infection (H and E) (100X).



Figure, 5: Control trachea shows normal tissue, 72 hrs. post infection (H and E) (100X).



Figure, 6: Control larynx shows normal tissue, 72 hrs. post infection (H and E) (100X).

The histopathological changes were goblet cell hypertrophy, lymphocyte infiltration, vacuolation of epithelial and subepithelial layer and intranuclear inclusion bodies in agreement with (8 and 11). Inclusion bodies were present after 72 hr. in corresponding with (12) who explained that Inclusion bodies are usually present in the early stages of infection, 1 to 5 days post infection, and will disappear as infection progresses as a result of necrosis and desquamation of epithelial cells.

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الخمج التجريبي للعترة المحلية لفايروس التهاب الحنجرة الرغامي المعدي في الدجاج

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الخلاصة

استعملت 40 دجاجة بعمر 10 أسابيع لغرض الإصابة التجريبية بفايروس التهاب الحنجرة والرغامي المعدي المعزول 50/50 ($2\times10^{4.16}$ TCID مل) بالتقطير بالعين والفم. إذ أُصيبت المجموعة الأولى المؤلفة من 20 دجاجة، المجموعة الثانية المؤلفة من 10 دجاجات (غير مصابة) ملامسة للمجموعة المصابة أما المجموعة الثالثة المؤلفة من 10 دجاجات تركت كمجموعة سيطره (غير مصابة) تم تقطيرها بالوسط الزرعي الخاص بالخلايا عن طريق الفم والعين. لوحظت الأعراض السريرية والهلاكات على مدى 12 يوم من إصابة الدجاج بالفايروس واهم ما لوحظ على الدجاج هو الإجهاد، السعال، اللهاث مع التهاب بسيط لملتحمة العين و بدون هلاكات. أجري فحص الانزيم المناعي الممتز على الامصال التي جمعت من أفراخ التجربة قبل الإصابة وبعدها بفايروس التهاب الحنجرة والرغامي المعدي المعزول. إذ كانت نتيجة الفحص سالبة لكل مجاميع التجربة قبل الإصابة بالفايروس وموجبة فقط للمجموعة الأولى التي أصيبت بالفايروس بمعيار يتراوح من (2534-7910) واستعملت مقاييس التمركز والتشتت بمعدل (4874.75) وخطأ قياسي (13.6\355.96%) في حيث كانت سالبة للمجموعة الثانية والثالثة بعد الإصابة. أستخدمت ثماني عشرة دجاجة بعمر 10 أسابيع بصورة منفصلة حيث عوملت كما في التجربة أعلاه لغرض إجراء الفحص النسيجي المرضي تم قتل دجاجتان من كل مجموعة بعد 24 ساعة، 48 ساعة و 72 ساعة من الاصابة بفايروس التهاب الحنجرة والرغامي المعدي المعزول. إذ كانت التغيرات النسجية المرضية للأعضاء التي جُمعت من (الحنجرة والرغامي) هي تكون أجسام نووية ضمنية وخلايا عملاقة بعد 72 ساعة من الإصابة بالفايروس المعزول لمجموعة الإصابة فقط.

الكلمات المفتاحية: التهاب الحنجرة الفيروسي، العزل الحقلي، الخمج التجريبي.