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NATURAL OUTBREAK OF INFECTIOUS LARYNGOTRACHEITIS IN BROILER CHICKENS IN SAUDIA ARABIA

(With One Table and 3 Figure)

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تفشى وباء مرض الحنجره والقصبه الهوائيه المعدى فى دجاج التسمين

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تم تشخيص وبائين لمرض التهاب الحنجره والقصبه الهوائيه لأول مره فى قطعان دجاج التسمين عند عمر ٣ - ٤ أسابيع فى المنطقه الغربيه - بالمملكه العربيه السعوديه . سجلت معدلات الاصابه والنفوق وكذلك الأعراض الاكلينيكيه المميزه والآفات التشريحيه - تم الكشف عن الاجسام الضمنيه المميزه فى مسحات القصبه الهوائيه المصبوغه بالجيمسا وكذلك المثبته بالفورمالين. عزل الفيروس على أغشية (CAM) وكذلك وفيات فى الاجنه . تم التعرف على الفيروس سيرولوجياً باستخدام اختبار الترسيب فى الآجار. تم وصف التغيرات الهستوباثولوجيه فى القصبه الهوائيه فى الحالات المصابه . كما تم عمل المقارنه المرضيه بين الفيروسات المعزوله من الوبائين فى أجنة البيض .

SUMMARY

Two natural outbreaks of infectious laryngotracheitis (ILT) were diagnosed for the first time in 3-4-week-old broiler chickens in western area of Saudia Arabia. The morbidity and mortality rates as well as the characteristic clinical signs and postmortem lesions were described. The specific intranuclear inclusion bodies were detected in direct Giemsa stained tracheal smears and formalin fixed tracheal tissue sections. The virus was isolated on chorioallantoic membrane (CAM) of chicken embryo which produced typical plaques on CAM & embryo deaths, then identified serologically by agar gel precipitation test. Histopathological examination of naturally infected tracheal tissues were described. Comparison of pathogenicity between ILT field virus isolates in chicken embryos was reported.

Keywords: *Natural outbreak, infectious laryngotracheitis, broiler chickens, Saudia Arabia.*

INTRODUCTION

Infectious laryngotracheitis (ILT) is an acute repiratory disease of chickens caused by an alphaherpes virus. It is an economically important disease of chickens which has been recognized in

many countries *(HANSON and BAGUST, 1991)*. Nowadays ILT is a major menace to most countries with developing and developed poultry industries *(BIGGS, 1982)*. Affected chickens present severe respiratory symptoms and occasionally suffocate, low weight gain, increase untnriftiness and heavy mortality particularly in mature chickens *(SEDDON and HART, 1935; CURTIS and WALLIS, 1983; TRIPATHY and HANSON, 1989)*.

The present paper describes two natural outbreaks of ILT in 3-4 week-old broiler chickens in Saudi Arbia, isolation and identification of the virus, and pathological lesions in naturally infected chickens. Comparison of pathogenicity was made between ILT-field virus isolates in chicken embryos.

MATERIALS and METHODS

Embryonated chicken eggs (ECE):

Fertile eggs were obtained from Known ILT nonvaccinated commercial private farm previously tested and proved negative to ILT-precipitatining antibodies.

Eggs were incubated at 37 °C for 11 days and then used for virus isolation, titration and pathogenicity tests.

Reference ILT-virus and antiserum:

Both ILT-precipitating antigen and ILT-chicken hyperimmune serum were supplied from Central Veterinary Laboratory, New, Wybridge, Surrey, UK.

Reovirus, Infectious bronchitis (IB), adenovirus (CELO) chicken hyperimune sera:

Known positive reovirus, IB adenovirus antiserum and normal chicken-serum were from our laboratory and used in agar gel precipitation (AGP) test as control.

Collection and processing of specimens:

3-4 week old diseased and dead chickens with history of respiratory distress were submitted to Veterinary Laboratory-Jeddah. The Larynx & trachea were collected from naturally infected chickens in a sterile petridish and then removed to a microbiological safety cabinet for further processing.

Slide-smears were prepared from tracheal mucosa and stained with Giemsa and examined microscopically *(ARMSTRONG, 1959)*.

The larynx and part of tracheal tissues were stored at 20 °C till used for virus isolation, while another part of tracheal tissue were fixed in formalin and processed for histopathological examinations.

Virus isolation:

10% W/V suspensions of laryngeal and tracheal tissues were prepared with physiological buffer saline and centrifuged at 3000 rpm for 15 minutes. The supernatant fluid was collected, bacteria free suspensions were prepared by adding antibiotics (penicillin & streptomycin) and used as virus inoculum.

ECE, 11 day-old were inoculated on the chorioallantoic membrane (CAM) with 0.1 ml virus inoculum. Inoculated embryos were reincubated at 37 °C and candled daily for 7 days. Embryos died within first 24 hours post inoculation (PI) were considered non-specific. All embryos died 2-7 days PI were subjected for gross and micro-pathologi-

cal examination. The remaining survivor embryos at the end of observation period were chilled and examined as above.

Infected CAMs with plaques were homogenised centrifuged, and the supernatant fluid (as antigen) was tested in AGP test against reference ILT, reovirus, IB adenovirus and normal antisera. Control antigen was similarly prepared from uninoculated embryos and tested in parallel with infected antigens.

Agar gel precipitation (AGP) test:

Plates prepared with 1.5% Noble agar and 8% sodium chloride in veronal buffer (ph 7.2) were used. Infected CAMs prepared antigens were tested against reference ILT, IB, reovirus and adenovirus antisera as well as normal chicken serum as control. Precipitating lines were reaed within 24-48 hours after incubation at 37 °C. Positive AGP-test reached CAMs of the two virus isolates desigated ILT-M (from 1 st outbreak and ILT-W (from 2 nd outbreak) were selected for further studies (Virus titration and pathogenicity test).

Virus titration:

Serial ten-fold dilutions of ILT-M and ILT-W virus isolates at 2nd egg passage were inoculated on CAM of ECE (0.1 ml/egg and 5 eggs/dilution).

The virus titre was expressed as plaque forming units per ml (PFU/ml) produced by the virus on CAM. The fifty percent embryo infective dose per ml (EID 50/ml) was estimated after Reed and Muench, 1938.

Pathogenicity test:

A comparison was made between pathogenicity of ILT-M and ILT-W filed virus isolates in ECE after *IZUCHI and HASEGAWA, 1982.* Briefly, 20 ECE/isolate were inoculated in the allantoic cavity with 0.1 ml virus inoculum containing 10^3 EID 50/embryo and candled once daily for 7 days. The mortality index of chicken embryo (MICE) was calculated as cumulative number of dead embryos for 7 days PI/cumulative number of live embryos for 7 days PI.

RESULTS

Clinical and postmortem findings in naturally infected broiler chickens:

Two natural outbreaks (1 st and 2 nd) were observed in 22-day old and 28 day-old broiler chickens respectively. The 1 st outbreak was characterized by high mortality (18.2%) and high morbidity (55%).

The onset of the disease was associated with severe conjunctivitis, nasal discharge, severe respiratory distress with coughing, sneezing, gurgling and marked dyspnea. Some chickens exhibited the characteristic gasping respiration and bloody mucus exudate was expelled. In the second outbreak, the clinical manifestations were similar to that observed in the first outbreak but were less in severity, and low mortality (5.35%) and morbidity (38%).

The course of the disease in affected flocks was about three weeks comparison of daily mortalities between the two outbreaks is shown in (Fig.1).

Postmortem findings: In the first outbreak, severe-congestion of the conjunctival epithelium was observed.

Hemorrhages were most consistently in laryngeal & tracheal tissues and the tra-

cheal lumen was often filed with bloody stained exudate. In the second outbreak, a necrotic and caseous laryngitis was prominant and the tracheal lumen was filled with caseous yellowish exudate. Edema of conjunctival epithelium and infraorbital sinuses were observed in some cases.

Tracheal slide-smears strained with Giemsa revealed characteristic intranuclear inclusion bodies.

Histopathology:

The most characteristic changes of the tracheal mucosa collected from the 1 st outbreak were hypertrophy of the epithelial cells which lost their cilia. Focal and diffuse infiltration of lymphocytes, plasma cells, macrophages and histocyte cells were detected in lamina propria resulting in severe thickening of the tracheal mucosa. Congestion and hemorrhages of blood capillaries with edema were seen. Also degenerative changes, necrosis and sloughing of the mucosa cell layer with blood clot were seen in the lumen of tracheal tissues which fused to from syncytia (Fig. 2).

Characteristic intranuclear inclusion bodies were detected in the syncytia and epithelial cells of mucosa layer (Fig. 3).

Histopathological changes of the tracheal mucosa of the 2 nd outbreak revealed less congestion of blood capillaries in lamina propria, slight hyperplasia of epithelial cells which showing few intranuclear inclusion bodies. Embryo deaths occurred 2-7 days PI.

Infected CAMs with confluent plaques were positive in AGP-test against ILT-hyperimmune serum. One more lines of precipitin developed between infected CAMs and ILT-antiserum within 24-48 hours after incubation. Histopathological examination of infected CAMs revealed characteristic intranuclear inclusion bodies.

Virus titration:

Virus titre of ILT-M & ILT-W virus isolates at 2 nd egg passage in CAM was 1.6×10^6 PFU/ml & 7.6×10^5 PFU/ml respectively. The EID_{50} of ILT-M isolate was $10^{-5.7}$ while was $10^{-3.1}$ for ILT-W virus isolate.

Pathogenicity test:

Comparison of mortality indices of ECE between ILT-M ILT-W virus isolates is shown in Table.1

Virus isolation and identification:

Laryngeal and tracheal tissues taken from all naturally infected chickens yielded varying plaques on CAM and embryo deaths. The plaques could be observed as early as 48 hours PI on CAM, which varied from a few scattered foci to large numbers.

The plaques were circumscribed edematous swellings, greyishwhite, 3-6 mm in diameter with depressed central areas of necrosis. Plaques produced by virus isolates of the 1 st outbreak were larger in size than that produced by virus-isolates of the second outbreak.

DISCUSSION

For many years, ILT infection was reported in most countries as serious economic disease in chickens. Our diagnosis of ILT-infection was reported by several workers *(SEIFRIED, 1931; SEDDON and HART, 1935;*

RMSTRONG, 1959; SEVOIAN, 1960; OERNLE, 1966; GRAUSGRUBER et , 1967; PURCELL, 1971; EL-ZEIN et , 1979 and KALETA et al., 1981) hich based on characteristic clinical gns, postmortem findings, detection of ranuclear inclusion bodies in Giemsa ained-tracheal smears & Fixed rmalin-tracheal tissue as well as virus olation and serological identification AGP-test.

Although ILT affects all ages, charac-istic signs are observed in adult ickens (HANSON and BAGUST, 91). In the present study, two tpical T-outbreaks were seen in 3-4-week-d brioler chickens which represents the st paper on diagnosis of ILT infection young broiler chickens, at Western ea of Saudia Arabia. The postmortem well as histopathological examination the tracheal tissues from the 1st tbreak revealed cellular, vascular, and udative changes which indicate the ute form of ILT infection.

However, the pathological changes of cheal tissues from the 2 nd outbreak dicate exposure of the chickens to less ute form of ILT-infection. In general r results are agreed with previous udies (SEIFRIED, 1931; THORP and GRAHAM, 1951; COVER and BENTON; PURCELL, 1971; ODAGIRI, 1982; HAYASHI et al., 1985) on pathological lesions of ILT-infection. MICE of the ILT-M and ILT-W field virus isolates were 0.43 and 0.28 respectively indicating that both virus isolates were highly pathogenic for chickens (IZUCHI and HASEGAWA, 1982), and the ILT-M virus isolate was more pathogenic than ILT-W isolate which well correlated with the natural clinical picture of the two outbreaks. IZUCHI and HASEGAWA, 1982 demonstrated experimentallay good correlation between MICE and pathogenicity for chickens.

CONCLUSION

Natural ILT-outbreaks (acute and less acute) were reported for the first time in western area of Saudi Arabia and this area is considered as geographic area where the disease is endemic. Immunization of chicken flocks is recommended using ILT (ECE-Origin) vaccine at 17 day-old via drinking water-2 vials (1000 doses each) for 1000 birds-which provided satisfactory field protection against subsequent ILT-outbreaks.

Table.1: Comparison of mortality indices of ECE between ILT-M & ILT-W Field virus isolates

Virus isolate	Status of embryo	No.of embryos on days PI 1	2	3	4	5	6	7	Cumulative NO.of embryo	MICE
ILT-M	Dead	0	0	2	4	10	12	14	42	0.43
	Alive	20	20	18	16	10	8	6	98	
ILT-W	Dead	0	0	0	3	6	10	12	31	0.28
	Alive	20	20	20	17	14	10	8	109	

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Fig.(1): Comparison between daily mortalities of the 1st and 2nd ILT-outbreaks.

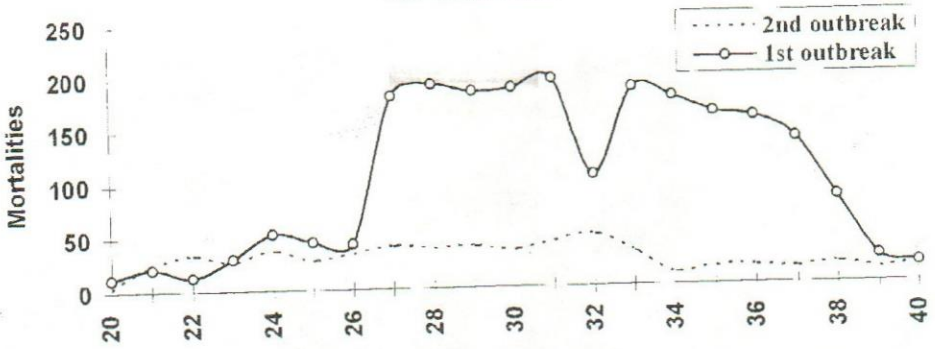

Fig. 1: Age of diseased birds in days

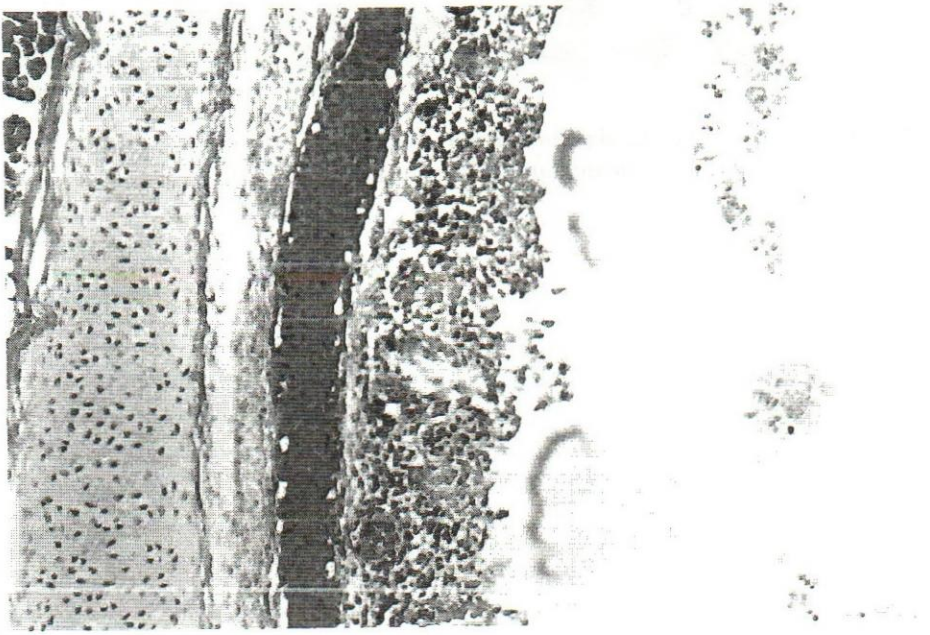

Fig.2: Tracheal tissues of naturally ILT-infected chickens, showing hyperplasia, sloughing of the epithelial mucosal cells with infiltration of inflammatory cells and severe congestion of blood capillaries. (H&E. X 40).

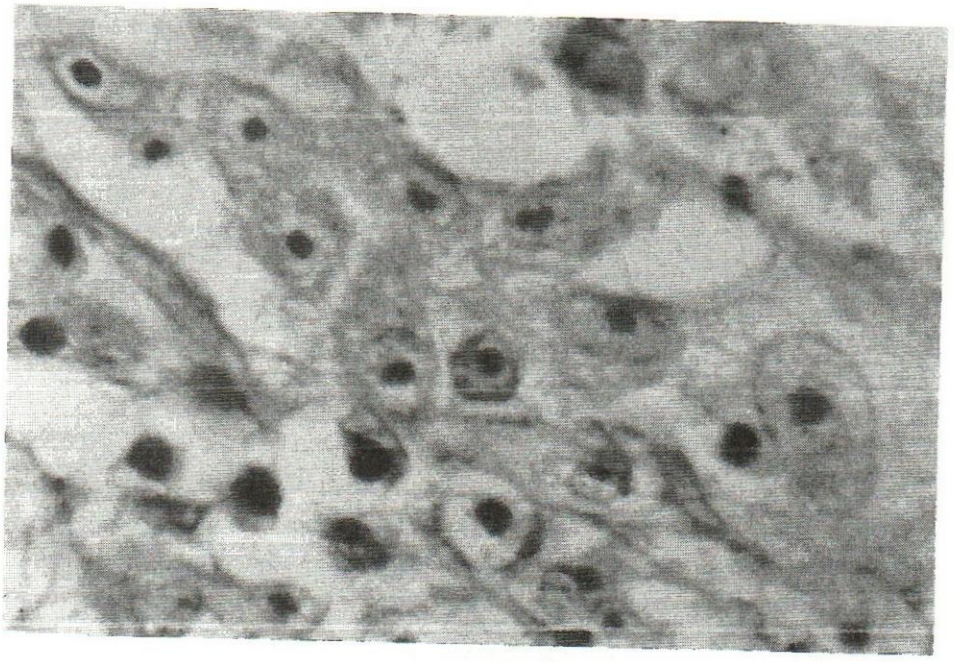

Fig. 3 Tracheal epithelial mucosa of ILT-naturally infected chickens, showing characteristic inttranuclear inclusion bodies. (H&E. X 1000)