

## تأثير الإصابة بمرض التهاب القصبة الهوائية والحنجرة على إنتاجية الفراخ البيضاء فرع لومان

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أصيبت أمهات بياضة سن ٣٤ أسبوعا من فرع لومان فى محافظة القليوبية بمرض التهاب  
القصبة الهوائية والحنجرة .

ولقد ظهرت أعراض تنفسية على الفراخ المصابة ، صعوبة فى التنفس مع مخاط دم من  
فتحتى الأنف ونقص ملحوظ فى إنتاج البيض والفقس أيضا نقص نسبيا فى الخصوبة وكمية  
الكثاكت الفاقه الغير طبيعية .

ولقد تمكن الباحثون من عزل الفيروس المسبب من العينات المأخوذة من الطيور  
المصابة وذلك بعد حقنها فى أجنة بيض حيث سبب الفيروس الأعراض الخاصة بهذا المرض  
فى الأجنة المحقونة وأيضا أمكن عزل الفيروس من العينات بعد حقنها فى خلايا الأنسجة  
نوع CER

ولقد أمكن إصابة طيور سن ١٦ أسبوعا صناعيا بالفيروس المعزول مع عزل الفيروس مرة  
ثانية من الطيور المصابة وحدث تغيرات خاصة فى خلايا الجهاز التنفسى بعد ١٤ ساعة  
من العدوى .

ولقد أمكن التعرف على أجسام مناعية خاصة فى أمصال الطيور التى تماثلت للشفاء  
وذلك باجراء التجارب السيرولوجية مثل تجربتى ال  
SNT ، AGPT

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## **AN OUTBREAK OF INFECTIOUS LARYNGEOTRACHEITIS (ILT) AMONG LAYING HENS: INFLUENCE ON THE FLOCK PRODUCTIVITY**

(With 5 Tables & One Fig.)

By

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### **SUMMARY**

An outbreak of infectious Laryngeotracheitis (ILT) occurred among a flock of laying hens (Lohmann's Mothers) of 34 weeks old at Kalama, Qalyobia governorate.

Affected birds manifested symptoms of respiratory distress, gasping and bloody exudates from the nasal passages together with a significant decrease in egg production and hatchability and a non significant decrease in fertility and abnormal dead embryos.

ILT virus could be isolated from samples collected during the course of the disease (nasal exudates as well as tracheal swabs and lung tissues). The isolated virus when inoculated in chicken embryos produced pock lesions on the CAM'S and it could be propagated in CER cells producing multinucleated giant cells and intranuclear inclusion bodies.

Susceptible birds of 16 weeks old could be experimentally infected with the isolated virus with re-isolation of ILT virus. Intranuclear inclusion bodies could be seen in epithelial cells lining the respiratory tract as early as 14 hours post-infection.

Specific antibodies could be detected in sera of recovered birds by using both the agar gel precipitation (AGP) and neutralization (SNT) tests.

### **INTRODUCTION**

MAY and TITSLER (1925) were the first who recorded the occurrence of ILT in the United States. Since then, the disease was reported from other parts of the World such as Canada (GWATKIN, 1925), Holland (VAN HELLSBERGEN, 1929), United Kingdom (DOBSON, 1935), Australia (SEDDON and HART, 1936), Sweden (MAGNUSSON, 1940) and Poland (MAREK, 1948). At the present time, the disease occurs all over the world especially where intensive poultry production exists. Thus, with the revolution in poultry production in Egypt during the last fifteen years and with the importation of chicks for rearing and laying, the disease showed itself for the first time in Egypt during 1982-1983 (TANTAWI, *et al.* 1983).

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Generally, workers concentrated on the isolation and identification of the virus causing the disease and/or the production of the disease experimentally in susceptible birds. Hence, the other manifestations of the disease such as mortality, drop in egg production, hatchability ... etc. in affected flocks or farms are not thoroughly studied or even neglected.

Thus, the purpose of the present work is to study some parameters such as egg production, fertility, hatchability and mortality in a farm at Gualyobia Province comprising c. 4500 Lohmann's mothers of 34 weeks old struck by the virus of ILT during 1983 and to find the influence of the disease on such parameters.

### **MATERIAL and METHODS**

#### **1) Birds:**

A farm of Lohmann's birds at Gualyobia Governorate was chosen for this study. It was formed of eleven separate compartments each comprising 400 hens and 40 cocks.

#### **2) Samples:**

26 tracheal swabs, 36 tracheal tissues, 20 tracheal exudates and lung tissues were collected from naturally infected birds and prepared for virus isolation as usual.

#### **3) Virus isolation:**

##### **A- Inoculation of chicken embryos:**

Samples were inoculated on the chorioallantoic membrane (CAM) of 10-12 days old embryonated chicken eggs. The inoculated eggs were incubated at 37°C with daily examination for a period of 4-5 days.

##### **B- Tissue culture:**

Monolayers of CER cells were infected with the samples and then incubated at 37°C for a period of 7 days with daily examination for the presence of cytopathic effect (CPE).

#### **4) Titration of the virus isolate:**

Ten fold dilutions from the isolated virus were prepared in sterile saline solution. Five embryonated chicken eggs (10-12 days old) were inoculated with a dilution and each embryo received 0.2 ml via the CA route. Infected eggs were kept under observation for 8 days and the resulting pock lesions counted. The titer was calculated according to REED and MUENCH (1938).

#### **5) Serological tests:**

The following tests were carried out on the virus isolates as well as on convalescent sera:

##### **A- Serum neutralization test (SNT):**

According to HITCHNER, *et al.* (1958).

##### **B- Fluorescent antibody technique (FAT):**

The indirect method was used as described by MEBUS, *et al.* (1971).



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### C- Agar gel precipitation test (AGPT):

Following the technique of WOERNLE (1963).

### 6) Experimental infection:

A group of twenty susceptible birds of 16 weeks old were used for this purpose. Each bird received 0.5 ml. containing  $10^{5.2}$  PFU/0.1 ml. of the isolated virus intranasally and another dose (0.5 ml) containing the same titer was given intratracheally. The birds were kept under observation for a period of three weeks. Clinical signs and/or deaths were recorded and specimens were collected for virus isolation. Ten birds of the same age were not infected and kept for the same observation period as controls.

### 7) Statistical analysis:

According to MORONEY (1977).

## RESULTS

### 1) Epidemiological studies:

An outbreak characterized by respiratory distress, gasping and bloody exudates from the nasal passages together with a significant decrease in egg production and hatchability as well as an insignificant decrease in fertility and abnormal embryos occurred among a flock of Lohmann's laying hens of 34 weeks old. The flock consisted of 11 separate compartments, each of 400 hens and 40 cocks. Nine of these compartments manifested the above mentioned symptoms and the other two which were away from these nine compartments showed only mild respiratory symptoms.

The disease appeared on the 9<sup>th</sup> week of production in the nine compartments except in one compartment (No. 8) where it started a week earlier. The course of the disease was 4-5 weeks causing a death rate of 18% and a drop in egg production of 13-15%. Results of this study are presented in Figure (1) and Tables (1 and 2).

### 2) Virus isolation in Chicken Embryos:

Inoculated samples produced the following lesions in infected embryos: Pock lesions on the CAMs which had depressed necrotic centres and being apparent after 3 days postinoculation. The embryos were stunted and showing swelling all over the body. One could also notice intranuclear inclusion bodies in the proliferated tissues of the CAMs.

### 3) Virus growth in tissue culture:

When the isolates were inoculated on CER cells, CPE could be seen after 24 hours in the form of giant cell formation (syncytium) and then detachment of the whole sheet after 3 days. Stained cells, revealed the presence of Cowdry type A intranuclear inclusions.

### 4) Virus titration in chicken embryos:

The isolated virus had a titer of 6.2 pock forming units (PFU) when titrated in chicken embryos.



### 5) Experimental infection:

Birds experimentally infected with the isolates manifested characteristic symptoms which were: moist rales with coughing and gasping accompanied by blood stained mucous from the mouth in fifteen out of twenty and ten out of twenty died (50%). The course of the disease and severity of lesions varied among birds. Surviving birds showed milder symptoms and recovered within two weeks. At autopsy affected birds manifested an inflamed bloody trachea as the only characteristic lesion. Smears from the tracheal epithelium of naturally and experimentally infected birds showed Cowdry type A intranuclear inclusions.

### 6) Serological tests:

#### A. Serum neutralization test:

There was a marked reduction in the number of pock lesions on the CAMs of embryos inoculated with isolate-hyperimmune serum (kindly supplied by Dr. A.A. SAMY) mixtures in comparison with those only inoculated with the isolates.

#### B. Fluorescent antibody technique (FAT):

Viral antigen was detected in infected tracheal and not in uninfected control smears.

#### C. Agar gel precipitation test (AGPT):

A clear sharp precipitin line appeared after 24-48 hours with infected samples and not with control ones. In addition, sera from convalescent birds contained specific antibodies against ILT virus detected by both the AGP and SN tests.

### 7) Statistical analysis:

#### A. Analysis of the total No. of eggs during the various periods:

The following table was constructed, from which there was no difference between the total No. of eggs during the various periods since F calculated (2.953) was less than F tabulated (19.44).

#### B. Analysis of the fertility during the various periods:

The following table was constructed from which one can't find any significant difference between the various periods since F calculated (1.373) was less than F tabulated (19.44).

#### C. Analysis of the hatchability during the various periods:

The following table was constructed where the differences are not significant since F calculated (1.25) was less than that tabulated (19.44).



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Table (1)  
Fertility, hatchability, dead embryo and abnormal embryo percentages of the farm infected with ILT virus

Age of mother in weeks	Period	Total no of eggs	Non fertile		Fertile		Unhatched		Dead embryos		Hatched		Abnormal embryos	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
29-33	Before infection	11166	3281	29.7	7860	71.0	476	6.8	1530	19.6	2862	73.6	125	2.1
34-38	During infection	13858	3401	24.5	10457	75.4	509	5.5	906	8.3	8842	84.5	200	2.5
39 end	After infection	9281	2475	27.3	6798	72.6	829	15.0	876	13.9	5144	71.1	140	3.4

. = Average.

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Table (2)  
 Cumulative results showing the fertility, hatchability and abnormal embryo percentages in a farm infected with ILT virus

Age of mother in week	Period	Rate of fertility (%)	Rate of hatchability (%)	Rate of dead embryos (%)	Rate of abnormal embryos (%)
29	Before infection +(5)	66.0-75.0 @(71.0)	67.7-81.1 @(73.6)	14.6-25.3 @(19.7)	1.3-3.1 @(2.1)
34	During infection +(5)	72.9-77.0 @(75.4)	79.5-89.8 @(84.5)	5.2-12.0 @(8.3)	1.9-5.2 @(2.5)
39	After infection +(24)	61.7-79.1 @(72.6)	42.1-99.0 @(71.1)	1.0-39.9 @(13.9)	0.0-9.5 @(3.5)

@ = Mean of the group.

+ = No. of the weeks for each period.

SOV	d.f.	SS.	M.S	F.
Total	34-1=33	573554234.20	---	
Between period	3-1= 2	91791215 .20	45895607.6	F=2.953
Within (error)	33.2=31	481763019.0	15540742.55	

$F_{0.05}$  Tabulated at d.f. (2,21) = 19.44

SOV	d.F	SS.	MS.	F
Total	33	280.74	--	
Between	2	22.85	11.425	1.373
Within	31	257.89	8.319	

$F$  tabulated  $_{0.05}$  (d.f. 2,31) = 19.44



## INFECTIOUS LARYNGEOTRACHEITIS AMONG LAYING HENS

SOV	d.f	SS.	MS.	F
Total	33	3785.3	--	
Between	2	282.73	141.365	1.25
Within	31	3502.57	112.986	

F tabulated  $0.05 (2,31) = 19.44$

## DISCUSSION

An outbreak characterized by respiratory symptoms, gasping and bloody exudates from the nasal passages together with a drop in egg production occurred among laying hens of 34 weeks old.

Samples collected from diseased birds were processed for the isolation and identification of the causative agent.

Specimens inoculated into chicken embryos produced characteristic pock-like lesions on the CAMs of infected embryos. These lesions has a somewhat larger size and depressed necrotic centres being apparently seen on the third day post-inoculation. In addition, the embryos were stunted with swelling and bloody inflamed trachea. TANTAWI, *et al.* (1983) got the same results with a field isolate of ILT virus but in a slightly shorter time being 48 hours in the present work.

When the isolate was inoculated onto CER cells, it produced the characteristic cytopathic changes in infected cells, i.e. syncytial cell formation and intranuclear inclusion bodies.

Moreover, the isolated virus produced a drop in pock count when inoculated into chicken embryos after being mixed with ILT specific hyperimmune serum. The viral antigen could also be identified by the FA and AGP tests. These results confirm that the isolate was an ILT virus (JORDAN, 1964). Once more, when the virus was inoculated into susceptible birds, the disease was reproduced experimently with the same symptoms and lesions as the natural infection.

Looking to fig. (1) we could notice the decrease in egg production during the infection period. Moreover, table (1) revealed that the farm passed by three successive periods: one before the occurrence of the outbreak, a second during the outbreak and a third after the outbreak. Statistical analysis of the data to clarify the influence of the disease on the productivity of the flock revealed the following facts: there was no effect with respect to total no. of eggs, fertility and hatchability. This was after estimating the F value for the parameters and found that its value is less than the tabulated one. However this must not mask the fact that the isolated agent was an ILT virus which may be characterized by a much weaker virulence thus not significantly affecting the total productivity of the flock apart from a drop in egg production.



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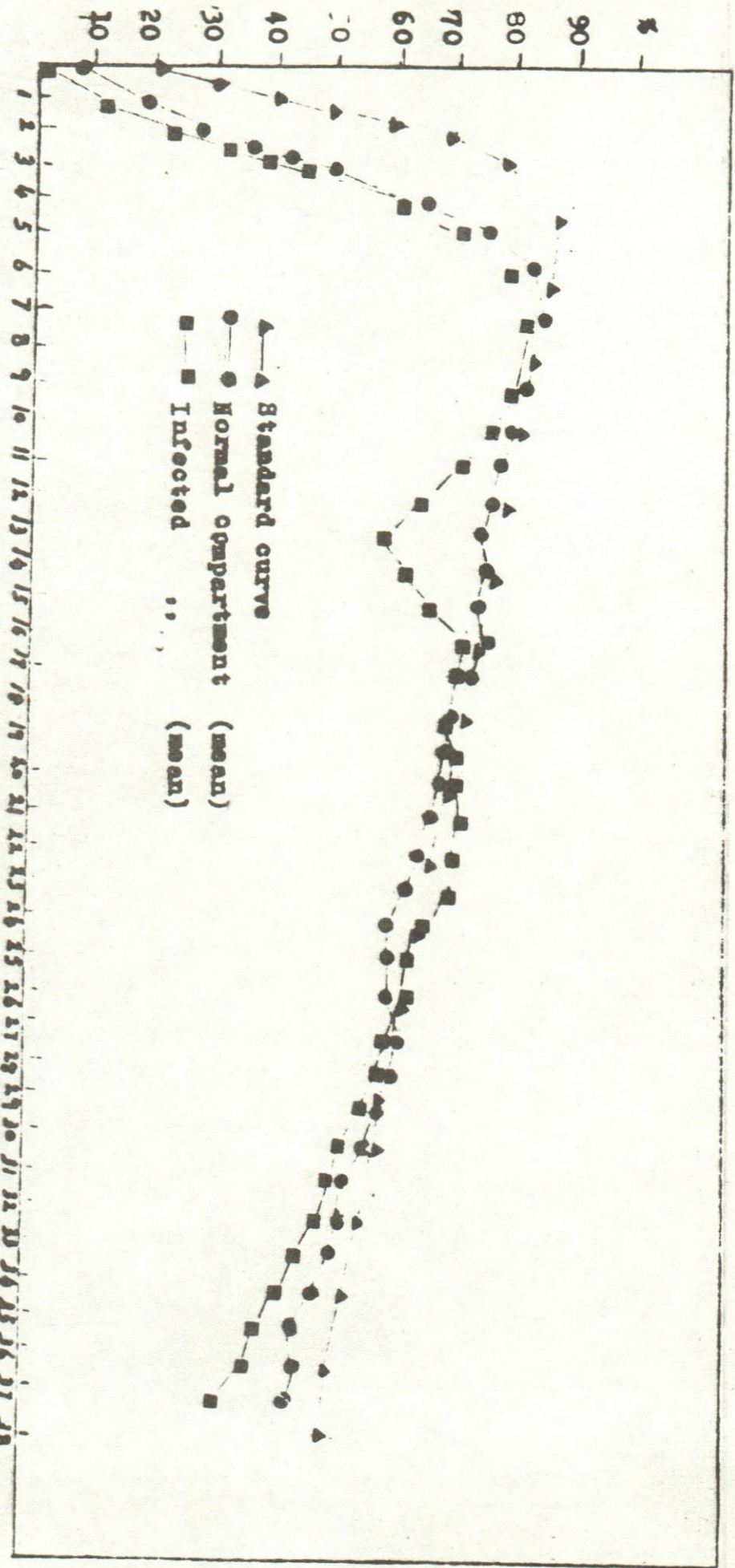


FIG. (1) The mean of egg production in all infected compartments comparing with the two normal ones.





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