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EXPERIMENTAL STUDIES ON AVIAN ENCEPHALOMYELITIS IN EGYPT

I. PATHOGENICITY OF A FIELD STRAIN IN DAY-OLD CHICKS AND IN CHICKEN EMBRYOS COMPARED WITH STANDARD VIRUS STRAIN

(With Two Tabes & 8 Figs.)

By

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دراسات تجريبية على إلتهاب الدماغ والنخاع الشوكـــــــــى
للطيـــور فـــى مصـــــــــــــر
١ ـ ضراوة عترة معزولة فى كتاكيت عمر يوم وكذلك فى أجنة الكتاكيـــت
بالمقارنة مع عترة معايـــــرة من الفـــــيروس .

ممدوح عفيفى ، أسامـــة الشاذلى ، مصطفى بسطامى ،الهام الخشـــــــــاب

تم عزل عترة فيروس مسبب لمرض الإلتهاب الدماغى والنخاع الشوكى فى الطيور فـــى بعض قطعان الدجاج فى مصر وقد تم إستخدام هذه العترة فى إجراء دراسة عن ضراوتها فـــى كل من كتاكيت عمر يوم وكذلك فى أجنة الكتاكيت . الطيور المصابة طبيعياً أظهرت أعراض مرضية وتغيرات عصبية التى تعتبر صفة مميزة للمرض ، وقد أعيد عزل الفيروس بنجاح فـــى اليوم العاشر بعد إستحداث المرض من الطيور التى ذبحت والتى نفقت . وقد ظهـــــرت الاستحالات فى العضلات وشلل الأرجل ونفوق الأجنة فى حوالى ٨٨ر٥٪ من الأجنة المحفوظـــة . وقد أظهرت الدراسة وجود إنتشار الفيروس المسبب لمرض الدماغ والنخاع الشوكى فى قطعان الدواجن فـــى مصـــر .

SUMMARY

A field strain of avian encephalomyelitis virus which was isolated from chicken flocks in Egypt was used to study the pathogenicity of the virus both in day-old-chicks and in chicken embryos. Infected chicks showed clinical signs and brain lesions characteristic of the disease. Reioslation of the virus was successful at the 10th day post infection both from killed and dead birds. Muscular dystrophy, leg paralysis and embryo deaths appeared in 88.5% of the inoculated embryos. The study proved the existance and the wide spread nature of avian encephalomyelitis virus infection among poultry flocks in Egypt.

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INTRODUCTION

Epidemic tremor or avian encephalomyelitis (AE) is one of many diseases that play an important role in retarding poultry industry, both through high embryo deaths and depressed hatchability as well as mortality in young chicks (TAYLOR, et al. 1955). The disease is naturally found in chickens (JUNGHERR, et al. 1956; IKEDA, et al. 1976 b and LUGINBUHL and HELMBOLDT, 1984), in Turkeys (HOHLSTEIN, et al. 1970) and pheasants (MATHEY, 1955 and VAN STEENIS, 1971), ducklings, turkey poults, young pigeons and Guinea fowls (VAN ROEKEL, et al. 1939).

In Egypt, a seriological survey was undertaken by AHMED, et al. (1975), indicating the existance of the infection in chicken flocks. Moreover, and according to the annual report of the research committee on poultry viral diseases of ecconomical importance in the farms of Egypt, AE virus was isolated from 7-10 day old broiler chickens ANON (1975). Accordingly, this study was carried out to study the prevalence of AE virus infection in chicken flocks in Egypt with trials at virus isolation and characterization and to study the pathogenicity of the isolated strain both for embryonated chicken eggs and for day-old-chicks compared with AE virus strian.

MATERIAL and METHODS

Materials:

Fertile chicken eggs:

They were obtained from non-vaccinated parent flocks against AE from Faculty of Agriculture and from private flocks. The eggs were incubated at 37.5-38°C untill inoculated at 5-7 days of age for propagation and titration of viral strains.

Three local virus strains were isolated from fifteen chicken flocks (approximately 3 week-old) obtained from different localities and showing clinical signs of depression, tremors of head and neck and ataxia characteristic of AE.

Experimental birds:

One-hundred and five, one-day native breed chickens obtained from a private flock were used for the experiment (I).

AE standard virus strain:

A reference strain of avian encephalomyelitis virus (Van Roekel egg-adapted strain) was received from Professor Dr. Kaleta, Poultry Disease Institute, Giessen, West Germany.

Methods:

Virus isolation: Specimens of brain and proventriculus of suspected cases (showing symptoms characteristic of AE) were divided into two parts; one part homogenized throughly and diluted 10% in sterile physiological saline, treated with 10.000 I.U penicillin and 10 mg streptomycin/ml. Samples were then inoculated into 6-day-old chicken

embryos via the yolk sac in a dose of 0.2 ml/egg. Embryos which died 24 hours post inoculation were regarded as non specific mortalities while those which died 2-10 days post-inoculation were tested by rapid slide haemagglutination test (ANON, 1971). Several serial passages were made in chicken embryos. Pathological changes in dead embryos were also recorded. The other parts of brain and proventriculus were preserved, fixed in 10% formol saline for histopathological examination.

Virus characterization:

The following methods were used: Heat stability (HESS and DARDIRI, 1968), Chloroform reistance (FELDMAN and WANG, 1961); Haemagglutination activity (ANON, 1971) and Cross neutralization test. The EID_{50} was calculated according to REED and MUENCH (1938) and the neutralizing index was then recorded.

Determination of virus infectivity end point (EID_{50}):

Titration of virus infectivity was carried out as ten-fold dilutions. Five chicken embryos were inoculated (0.1 ml/egg) dilution. The inoculated eggs were examined daily for 10 day. Virus infectivity of embryos that died or survived showing trypical lesions of AE was determined (ANON, 1971). The EID_{50} was calculated according to REED and MUENCH (1938).

Experiment I:

To study the pathogenicity of the isolated AE virus strain for chickens compared with standard AE virus strain (Van Roekel egg-adapted strain "VR-strain").

One hundred and five (105) one-day-old native breed chicken were used in this experiment. Bird were divived into 7 equal (1-7) groups consisting of 15 birds each. Groups (1-3) were infected on the first day of life with the isolated virus strain, both intracerebrally (I/C) (0.05 ml/bird) and subcutaneously (S/C) (0.01 ml/bird) as well as orally (0.1 ml/bird) using homogenized suspension of brain and gastro- interstinal tract of AE virus strain containing EID_{50} $10^{4.8}$/ml. Chickens of groups (4-6) were similarly infected via the same dose, using the reference VR-strain containing EID_{50} $10^{2.6}$/ml. Group 7 was left as non infected control. Chicken of all groups were vaccinated against Newcastle disease using B_1 vaccine intraocularly at the 6th day of age. Symptoms and/or deaths were recorded daily for 5 weeks. Two birds were killed at the 3rd, 7th, 14th, 21th and 35th days post-infection (P/I). Brain and proventriculus were collected, selected portions were fixed in formol saline, processed by conventional paraffin embedding technique, sectioned and stained by Haematoxylin and Eosin (H&E) for routine histopathological examination. Other portions were used for virus re-isolation.

Experiment II:

To study the pathogenicity of the isolated AE virus strain to embryonated chicken eggs.

Thirty-five embryonated chicken eggs, 5-7 day-old were inoculated with the isolated AE virus strain through three serial passages via the yolk sac, using mortality rate, pattern of embryonic deaths and clinical signs as well as gross pathological changes were studied and recorded.

RESULTS

Isolated virus strain:

Three virus isolates were isolated from chicken flocks, that showed clinical signs characteristic of AE. Only one isolate has been characterized and identified as AE virus. According to the physicochemical and biological characterization, the isolated strain showed heat stability, resistance to chloroform and faliure to agglutinate chicken, rat, sheep, horse and human O type R. B. Cs. It was neutralized by locally prepared antiserum (EL-KHASHAB, 1986) and standard antisera. Moreover, the standard strain was neutralized by hyperimmune serum against both local and standard antisera.

Histologically, brain of such birds (naturally infected birds), showed congestion of blood vessels both of brain and meninges, degenerative changes of neurons in the cerebellum and brain stem mainly in the form of chromatolysis mostly of central type, diffuse gliosis and necrosis of ganglionic cells with neuronophagia (Fig. 1) forming necrotic foci mostly found at the site of the nuclei of brain stem, ganglionic cells and the mollecular cell layer of the cerebellum and in different areas of the cerebral cortex. Perivascular lymphocytic cell infiltration which in many cases was formed of one cell layer of lymphocytes together with oedema of some glia cells were clearly observed (Fid. 2). The subepithelial area of the proventriculus (Muscularis mucosa) showed focal areas of lymphocytic cell aggregation.

Experiment I:

Clinical signs of AE observed in chickens experimentally infected both with standard and field strains of AE virus were clearly obvious at the 5th day P/I in nearly 50% of the inoculated birds of different groups. These include ruffling feathers, dulness, depression, weakened cry and diarrhea which was more severe in chickens infected with the field strain. Nervous signs represented by muscular incoordination, ataxia, tremors of head and neck and paralysis of leg and wings appeared 8-15 days P/I in 70-86% of infected birds in all groups. The highest mortality among birds was observed in those infected intracerebrally and the least in those infected subcutaneously (Table 1).

Reisolationof the virus was successful at the 7th day P/I for the groups infected with the standard AE strain and at the 10th day P/I for those infected with the field strain.

Histological examination revealed severe congestion of cerebral blood vessels associated with perivascular lymphocytic cuffs as early as one week P/I in groups infected

Histological examination revealed severe congestion of cerebral blood vessels associated with perivascular lymphocytic cuffs as early as one week P/I in groups infected with the standard strain and from the 10th day P/I in groups infected with the field isolate. Foci of ganglionic cell necrosis with neuronophagia and focal to diffuse gliosis (Fig. 3) appeared in the cerebrum and cerebellum and to a lesser extend in the medulla oblongata between the 2nd - 3rd week P/I. Necrotic changes of ganglionic cells were represented by chromatolysis, mostly of central type, shrinkage and rhexis of nuclei and even complete lysis of the nucleus accompanied with swollen or smaller neurons and oedema of some glia cells. Necrosis of purkinje neurons in the cerebellum accompanied with neuronophagia and even complete disappearance of the purkinje cells were observed (Fig. 4), 4-5 weeks P/I.

Birds inoculated either subcutaneously (S/C) or orally showed more or less similar cerebral and cerebellar changes which were less extensive particularly in the later. Similarly lesions observed were more obvious and more extensive in birds infected with the standard strain rather than with the local or field stain.

Experiment II:

Pathogenicity of the isolated AE virus strain to embryonated chicken eggs.

Muscular dystrophy and paralysis of legs and wings were the main findings observed both in dead embryos and in hatched chicken (Fig. 5 and 6). Mortalities and pattern of embryonic deaths are shown in table 2.

Histologically, the changes were advanced in those embryos inoculated with the reference virus strain, if compared with the local isolated virus which showed only mild to moderate histopathological alterations. They were mainly degenerative and necrotizing changes of the muscles of legs and wings; in which the muscles appeared eosinophilic, homogenous, having no or pyknotic nuclei (Fig. 7). Scattered areas of necrosis were observed in the brain, which were obvious and larger in those embryos inoculated with the standard virus strain (Fig. 8).

DISCUSSION

Three viral agents were isolated out of 15 chicken flocks 2-3 weeks old, that showed clinical signs of nervous disorders characterized by general depression, ataxia, tremors of head and neck and by abscence of post-mortem findings. Only one isolate has been subjected to physico-chemical and biological characterization. This isolate was identified as AE virus as indicated by the results. Seriological studies revealed a common reaction between the isolated strain and hyperimmune serum against the standard strain of AE virus using positive haemagglutination test (ANON, 1971). Furtherly, the cross neutralization test supported this seriological relationship between the isolated virus strain as shown in this study were similar to those reported by FELDMAN and WANG (1961); PAPPARELLA and SCHIAVO (1964) and BUTTERFIELD (1967). This confirms the annual report of the research committe on poultry viral diseases of economical importance in the farms of Arabic Republic of Egypt (1971), which recorded

the isolation of AE virus in Egypt. The criteria of VAN DER HEIDE (1975) and LUGINBUHL and HELMBOLDT (1984) for studying the pathogenicity of AE virus for embryos were adopted in this study. The isolated strain appeared pathogenic for 5-7 day-old embryonated chicken eggs producing muscular dystrophy and paralysis of the leg and wings as well as embryonic mortality from the 1st passage (70%) increased to 85% and 88.5% in the 2nd and 3rd passages respectively. The pathgenicity of the isolated strain as well as the standard strain for one-day-old chicken using different routes was studied. Both strains gave similar symptoms which appeared earlier in birds infected with the standard strain than in the isolated strain. Pathological alterations were severe and more frequent in the former one. Those birds infected through cerebral route were 1st to show symptoms. Moreover, virus reisolation from brain of sacrificed birds was only successful on the 7th day and the 10th day post infection with standard and isolated strains respectively.

Histologically, the brains of chickens infected either with the isolated or the standard strain showed similar changes in the brain tissue. These changes were much more obvious and extensive in the later strain. Necrotizing change of the ganglionic cells, neuronophagia, focal and diffuse gliosis, perivascular cuffs with lymphocytes and moderate to severe degenerative and necrotizing changes of the neurons were some of the common changes observed both in the cerebrum, cerebellum and brain stem of the infected birds in all groups; which indicates that the isolated virus is actually an AE virus. These changes were similarly observed in naturally infected cases from which the viral agent was successfully isolated. The results showed that the intracerebral route of inoculation is the most successful route (OLITSKY, 1939), whereas the subcutaneous and the oral routes were nearly similar both in the brain changes produced by either of them following infection and in the incubation period which is slightly longer in the oral route than in the subcutaneous one. Similar resutls were reported by CALNECK, et al. (1960) with a minimum incubation period of 11 days following oral transmission of infection. It can be concluded from results that infection with AE virus is widely spread among the examined chicken and this confirms the results reported by AHMED, et al. (1975), regarding the existance and widespread nature of AE virus infection among chicken in Egypt, which inturn makes the application of systemic vaccination programms of parent chicken flocks for the protection of newly hatched chicken urgently recommended.

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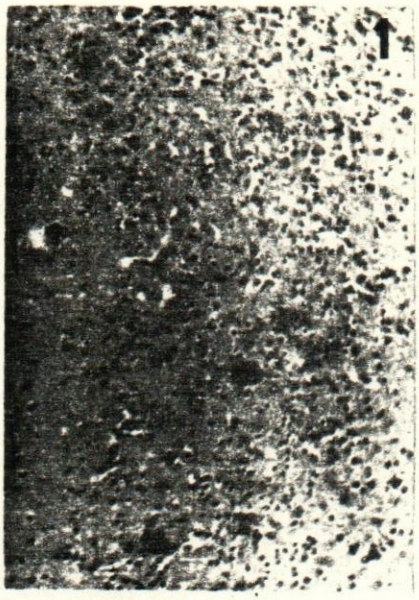
Table (1)
Results of pathogenicity of day-old chickens with both standard (Van Roekel) and field AE strains

Intervals (day)	Route of inoculation	Isolated strain		Standard strain	
		Reisolation	Histopath.	Reisolation	Histopath.
3	C	0/2	0/2	0/2	0/2
	S	0/2	0/2	0/2	0/2
	O	0/2	0/2	0/2	0/2
7	C	0/2	1/2	1/2	1/2
	S	0/2	0/2	0/2	1/2
	O	0/2	0/2	0/2	1/2
10	C	1/2	1/2	2/2	2/2
	S	1/2	1/2	1/2	1/2
	O	1/2	0/2	1/2	2/2
14	C	2/2	2/2	2/2	2/2
	S	2/2	1/2	2/2	2/2
	O	2/2	1/2	2/2	2/2
16	C	2/2	2/2	2/2	2/2
	S	2/2	2/2	2/2	2/2
	O	2/2	2/2	2/2	2/2
21	C	2/2	2/2	2/2	2/2
	S	2/2	2/2	1/2	2/2
	O	1/2	2/2	1/2	2/2
28	C	2/2	2/2	2/2	2/2
	S	1/2	1/2	1/2	1/2
	O	1/2	1/2	1/2	2/2
35	C	0/2	1/2	0/2	2/2
	S	0/2	0/2	0/2	0/2
	O	0/2	0/2	0/2	0/2

C : Intracerebral route
S : Subcutaneous route
O :Oral route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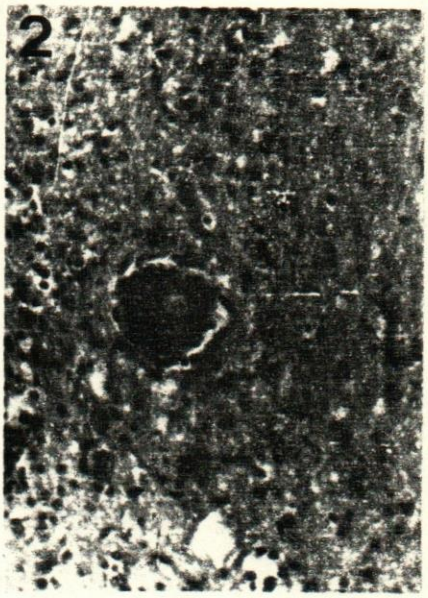
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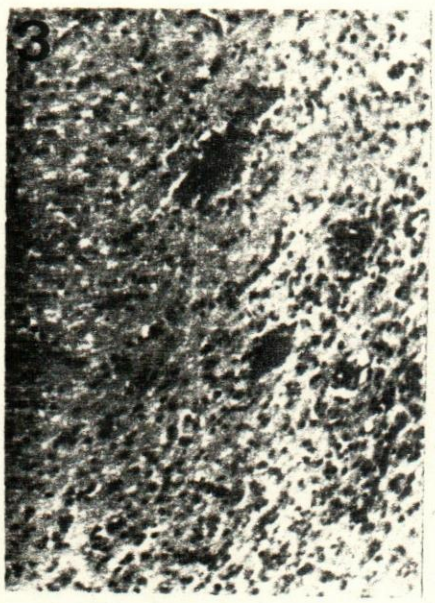
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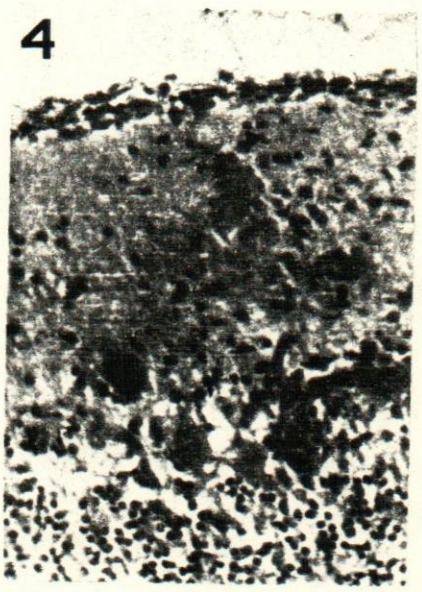
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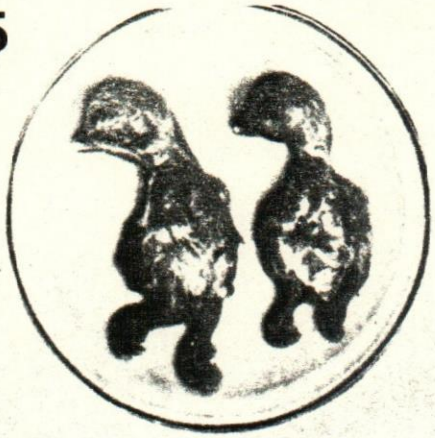

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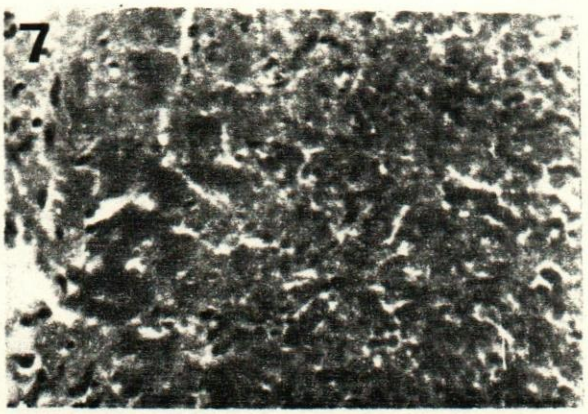

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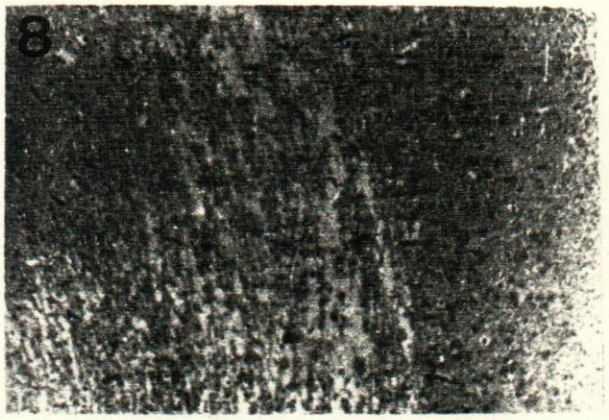

Table (2)

Mortality rate and pattern of deaths of embryonated chicken eggs, through the serial passages of the isolated AE virus strain.

Passage No.	No. of inoc. eggs	Death Pattern: Days post-inoculation												Ratio of dead/ inoc. eggs	Mortality rate %
		1	2	3	4	5	6	7	8	9	10	11	12		
1	10	-	-	-	-	-	-	-	-	-	1	2	4	7/10	70
2	7	-	-	-	-	-	-	-	-	1	1	2	2	6/7	85.5
3	18	-	-	-	-	-	-	-	3	2	3	4	4	16/18	88.5