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VIROLOGICAL AND PATHOLOGICAL STUDIES ON AVIAN ENCEPHALOMYELITIS AMONG YOUNG CHICKS AT EL- QASSIEM AREA, SAUDI ARABIA

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SUMMARY

A history of nervous disturbances and moralities (25%) among young chickens 10-21 day of age as well as a temporary drop in egg production and hatchability (10%) in parent flock occurred in a large poultry farm at EI-Qassiem area, kingdom of Saudi Arabia. Detection, isolation and identification of avian encephalomyelitis (AE) virus was done using embryonated chicken eggs, immunoflourscence (IF) and Immunodiffusion (ID) tests. Serological investigations were carried out on infected sera from both parent flocks and broiler chicks using Enzyme-linked immunosorbant assay (ELISA).

Histopathological studies were carried out on samples from naturally infected chick brains as well as brains from inoculated hatched chicks.

INTRODUCTION

AE is an infectious viral disease primarily affecting young chickens causing severe moralities and morbidities. So the disease is of great economic importance in poultry industry (Calnek et al., 1960) The incidence of clinical disease is very low unless the breeder flock were not vaccinated and become infected after egg production. Most strains of the virus were enterotropic where young chicks are readily infected via the oral route and shed the virus in their feces. However, some strains tend to be more neurotropic than others producing severe central nervous system lesions among young chickens (Calnek et al., 1995)

In the present study broiler chickens in a large poultry company were infected by AE virus, where no history of previous vaccination against AE of their parent flocks. The beginning of the

problem occurred in the form of neurological signs in young aged broiler chickens of 10 day old until 3 weeks old.

The aim of this study was to detect and isolate AE. Virus from young broiler infected farms and also to screen antibodies against the virus in sera from parents as well as from infected broiler chicks using ELISA, Besides, histopathological investigation was carried out to support and confirm diagnosis.

MATERIAL AND METHODS

A-Material: -

1- Embryonated chicken eggs 5-7 day and 9-11 day old were obtained from a susceptible flock at El-Qaassiem area kingdom of Saudi Arabia.
2- Brain impression smears for Immune Fluorescence (IF). and Acetone as a fixative.
3- Brain homogenates of infected chicks showing nervous signs for virus detection and isolation.
4- Tissue specimens from brain of naturally infected chickens as well as from inoculated hatched chicks submitted for histopathological studies.
5- AE positive antiserum used in Immunodiffusion test (ID) Spafas USA .
6- Chicken anti AE conjugated fluorescence isothiocynate used for IF technique (brought from poultry health centre Doorn institute-Holland).
7- Sera: either collected from infected layer flocks or from growing broiler chickens screened for ELISA test
8- Avian encephalomyelitis antibody ELISA test kits (KPI- Proflock Company, USA) used in testing of sera.
9- 8.9% saline of pH. 7.2 used as a virus diluent for ID test. Besides sodium merthiorate also used.
10- Hematoxylin and eosin (H & E) stain for histopathological investigation.

B-Methods:-

1- "5-7day" old chicken embryos from susceptible flocks were used for isolation of AE virus from brain homogenates specimens of infected chickens. The specimens were diluted 1/10 in sterile saline solution containing specific antibiotics. The procedure for virus isolation via yolk sac route of ECE was done according to Bülow Vv (1965).
2- 9-11 day old chick embryos were used for trials of NDV isolation from brain homogenate specimens of infected chickens. The specimens were diluted 1/10 in sterile saline solution containing specific antibiotics. The procedure for virus isolation via allantoic sac route of ECE was done according to Beard and Hanson (1984).
3-Impression smears: brain impression smears from infected chickens were examined for specific AE viral antigen by IF techniques accord-

ing to Vanderheide (1970).

4-Brain homogenates either collected from naturally infected chickens or from inoculated hatched chicks were examined for specific AE viral antigen by ID test according to Ikeda (1977).

5-Tested sera from infected parent flocks and from growing chicks either showing nervous signs or subclinical form of the disease were screened to AE antibodies using ELISA according to Garret et al. (1984).

6-Tissue specimens from brain of both naturally infected chicks as well as inoculated hatched chicks were fixed in 10% neutral buffered formalin solution. Paraffin sections of 5 micron thick were prepared, stained with Hematoxylin and Eosin for light microscopic examination (Clayden, 1971).

RESULTS AND DISCUSSION

AE Virus was detected in 15 impression smears and brain homogenates specimens of naturally infected chickens by IF technique and ID test according to vander heide 1970 and Ikeda's 1977 respectively.

On the other hand AE virus was isolated from the brain homogenates specimens of naturally infected chickens by inoculation of 5-7 day-old embryos obtained from susceptible flock according to Bülow, Vv 1965.

All of inoculated embryos were allowed to be hatched and observed for nervous signs of the disease during the first ten days of age.

The AE virus was identified by detecting of specific viral antigen in brains of inoculated hatched embryos by IF technique and ID test. These results were in agreement with those reported by Miyamae, T.1983 and Ikeda's 1977.

The brain homogenate specimens of naturally infected chicken were found to be negative for NDV isoaltion.

One hundred tested sera were screened for ELISA. 50 sample were collected from parent flocks and the others were collected from broiler infected chicken showing nervous signs (10 days-3 week old). All broiler sera were totally negative by ELISA. While the other parent flock sera were sero positive by ELISA test which gave and titer varying from 4603:8408 table.

According to AE antibody test kits produced by KPI, proflock ELISA titer greater than 5000 on the ELISA scale represent birds with high serum antibody levels. From the above mentioned results 19 sera gave ELISA titers lower than 5000, while the other 31sera gave ELISA titers more than 5000. These antibodies were not high and sufficient enough for virus neutralization during its transovarian transmission to embryos. These results were in agreement with those reported by

Table 1: Results of ELISA on 50 parent flock sera.

Sample No.	OD	S/P ratio	ELISA Titer	Sample No.	OD	S/P ratio	ELISA Titer	Sample No.	OD	S/P ratio	ELISA Titer	Sample No.	OD	S/P ratio	ELISA Titer
1	0.290	0.4583	4603	14	0.530	0.9583	7811	27	0.513	0.9229	7603	40	0.476	0.8458	7142
2	0.294	0.4666	4662	15	0.522	0.9416	7713	28	0.293	0.4645	4647	41	0.514	0.9250	7615
3	0.350	0.5833	5471	16	0.390	0.6666	6021	29	0.580	1.62	8408	42	0.512	0.9208	7633
4	0.414	0.7166	6342	17	0.382	0.6500	5913	30	0.506	0.9083	7517	43	0.527	0.9520	7774
5	0.512	0.9208	7633	18	0.318	0.5166	5015	31	0.414	0.7166	6342	44	0.460	0.8125	6939
6	0.296	0.4708	4692	19	0.300	0.4791	4751	32	0.294	0.4666	4662	45	0.579	1.060	8397
7	0.293	0.4645	4647	20	0.315	0.5104	4972	33	0.296	0.4708	4692	46	0.514	0.9250	7615
8	0.305	0.4895	4826	21	0.292	0.4625	4633	34	0.414	0.7166	6342	47	0.518	0.9333	7664
9	0.405	0.6979	6222	22	0.296	0.4708	4692	35	0.311	0.5028	4919	48	0.294	0.4666	4662
10	0.411	0.7104	6302	23	0.298	0.4750	4722	36	0.293	0.4645	4647	49	0.296	0.4708	4692
11	0.480	0.8541	7192	24	0.301	0.4812	4766	37	0.291	0.4604	4618	50	0.511	0.9187	7578
12	0.510	0.9166	7566	25	0.450	0.7916	6811	38	0.380	0.6458	5886	-Ve Cont. mean			
13	0.512	0.9208	7591	26	0.579	1.060	8397	39	0.411	0.7106	6303	+ Ve Cont. mean			

1- - Ve Cont. mean OD = 0.070
2- + Ve Cont. mean OD = 0.550
3- Corrected positive Cont. = 0.480

Garrett et al., (1984).

Histopathological studies:-

The gross picture in infected chicks was whitish areas in the muscularis of the ventriculus beside apparent enlargement of the eye ball marked opacity of the lens and total blindness in some cases. These observations are the same as those reported by Calnek et al. (1995).

The mid brain, brainstem and cerebellum as well as spinal cord of naturally infected chicks showed neuronal degeneration associated with neurophagia, edema and central chromatolysis or tygrolysis as pathognomonic to avian encephalomyelitis. (Fig. 1-4).

The prominent pathological alterations of the neurons occurred due to the primary neuronal affinity of the virus. In addition, focal to multifocal gliotic masses, particularly in the midbrain, brain stem and cerebellum, besides perivascular lymphocytic cuffing (Fig. 5).

Finally, congested cerebral and meningeal blood vessels, vasculitis, hemorrhage and subependymal lymphocytic infiltrations were also seen (Fig. 6). These results are in agreement with those reported by Jungherr, (1956) where he believed that all birds with clinical signs mostly had histopathological alterations represented by neuronal degeneration, gliosis and perivascular lymphocytic cuffing.

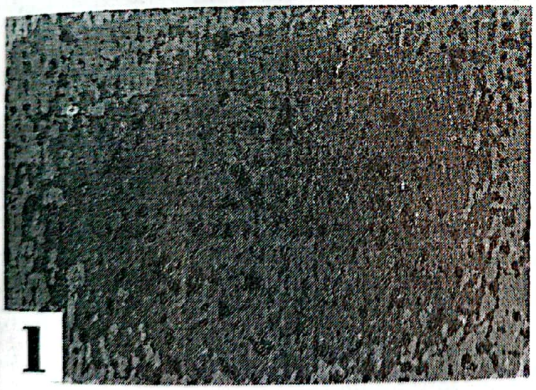

Fig. 1: Brain stem of naturally infected chicks showing neuronal degeneration (A) and gliosis (B).

Fig. 2: Brain of naturally infected chicks showing gliosis. (H & e x 1000).

Fig. 3: Mid brain of naturally infected chicks suffering extensive neuronal degeneration. (H & E, 400).

Fig. 4: Brain of naturally infected chicks showing central tygrolysis (A) and Edema (B). (H & E X, 1000).

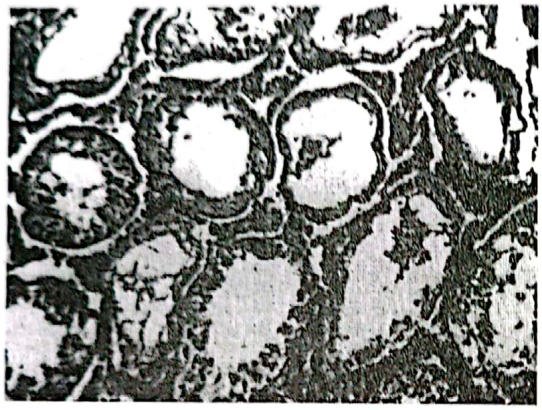

Fig. 5: Brain stem of naturally infected chicks showing perivascular lymphocytic infiltration and congestion. (H & E X, 400).

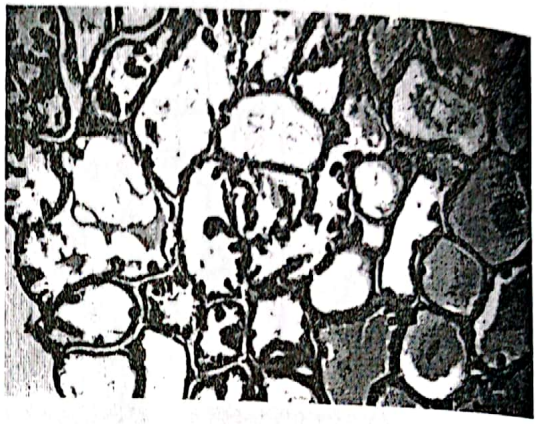

Fig. 6: Brain of naturally infected chicks showing vasculitis, hemorrhage, perivascular edema and subependymal lymphocytic cells infiltration. (H & E X, 400).

The brain and spinal cord of the newly hatched chicks inoculated via yolk sac has lesions in the form of mild similar lesions as in naturally infected birds, besides microgliosis of the cerebellar molecular layer. These observations are nearly similar to those found by Jugherr et al. (1956). During this work, mild lesions were observed. This finding could be claimed to that the field isolate is not adapted enough to chicken embryo tissue if compared to that of the egg adapted vaccinal strain. the latter was known to give marked lesions in brain of inoculated embryo.

In the present study, the obtained results besides AE virus isolation and negative Newcastle (NDV) isolation, central chromatolysis which is peculiar to AE virus, controversy to peripheral chromatolysis of ND which had been not shown, besides multifocal gliosis which is pathognomonic for AE than ND.

Conclusions

The brain was an excellent source of virus detection and isolation and the chicks exposed to AEV, developed antibodies that could be measured with ELISA test. ELISA test considered the method of choice for detection of AEV antibodies due to the purified viral antigen used, highly sensitive specific and is useful in both diagnosis and flock monitoring applications.

Histopathological evidence of gliosis, perivascular lymphocytic infiltration, neuronal degeneration in the CNS usually can be considered as a basis for a positive diagnosis

Control of AE achieved by vaccination of breeder flocks during the growing period to assure that they didn't become infected after maturity, thereby preventing dissemination of the virus by the egg-borne route. Also, maternal antibodies protect progeny against contact to AEV during the critical first 2-3 weeks.

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