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OUTBREAKS OF EGG-DROP SYNDROME-1976 IN TAIWAN AND ISOLATION OF THE ETIOLOGICAL AGENT

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A condition similar to egg-drop syndrome-1976 (EDS-76) occurred in 8 layer and broiler breeding flocks in 4 counties in Taiwan from April to Oct. 1982, and it was diagnosed as EDS-76 by virological investigations. Egg production fell suddenly when the hen were 24-41 weeks of age, and the depression lasted 4-12 weeks. Production reduced 6 to 25%. Depressed egg production was accompanied by the laying of shell-less, soft-shelled, and thin-shelled eggs associated with loss of egg-shell pigment.

Seven isolates of hemagglutinating adenovirus were isolated from cloacal swabs and faeces of infected hens. One isolate, cloned and named TN strain, had the same antigenicity in serologic test and same biological and physicochemical properties as the JPA-1 strain of EDS-76 virus.

This is the first report on the outbreaks of EDS-76 in Taiwan and the isolation of the etiological virus.

In 1976, a syndrom causing depressed egg production associated with the laying of soft-shelled and shell-less eggs was first described in the Netherlands by van Eck et al.[3] who suggested the possible involvement of fowl adenovirus in the syndrome. Later, McFerran et al. isolated several hemagglutinating adenoviruses from affected hens in Northern Ireland[9] and demonstrated a correlation between the syndrome and the isolate.[7,8] Outbreaks of egg-drop syndrome-1976 (EDS-76) have been reported in esveral European and Asian countries since 1976.[2,3,9,10,11,12,14]

The authors observed 5 cases similar to EDS-76 during the period April to Oct. 1982 in Taiwan.

This report deals with isolation and characterization of virus from outbreaks of EDS-76.

MATERIALS AND METHODS

Samples

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Cloacal swabs, trachea, lung, liver, spleen, kidney, uterus, and rectum were obtained from infected hens for virus isolation. A 10% tissue suspension was made in Eagle's, minimum essential medium (MEM) by sterile mortar and pestle. The suspension was centrifuged at 700Xg for 10 minutes, and antibiotics (penicilin and dihydrostreptomycin) were added to the supernatant fluid with respective final concentration of 200 U and 200 μg per ml. The supernatant fluid was held at room temperature for 30 minutes before inoculation into the cell cultures.

Cell cultures

Embryo-fibroblasts and embryo-liver cells of chicken, duck, and goose were prepared in Eagles's MEM on Petri dishes respectively as described previously.[13]

Viruses

The TN strain (one of the 7 isolates) was cloned-purified by well-isolated individual plaque from infected DEL cell culture after isolation in GEL cell culture. The virus clone was passaged 3 times in DEL cell culture and used in this experiment. Control virus were the JPA-1 strain of EDS-76 virus.[13,14]

Fluorescent-antibody (FA) technique

The fluorescent-antibody technique was performed as described by Imada et al..[4] Fluorescent antibody against the JPA-1 strain EDS-76 virus was kindly supplied by Dr. Kawamura, H., National Institute of Animal Health, Japan.

Electron microscopy

Purified viral materials were negatively stained with 2% potassium phosphotungstate and observed with a Hitachi H600 electron microscope at 75kV.

Hemagglutination (HA) test

The HA test was carried out by the conventional microtitre method as described previously.[13]

Hemagglutination-inhibition (HI) test

The HI test was carried out by the conventional microtitre method using 0.025ml volumes. Antigens of the TN strain and JPA-1 strain of EDS-76 virus consisted of formalin-inactivated cell-culture

fluid from infected DEL cell cultrues. Four hemagglutinating units of antigen were reacted against 2-fold serial dilutions of test sera with inital dilution of 1:2. Serum dilution of 1:2 without antigen was served as control.After 30 min. of reaction at room temperature, 1 drop of 1% chicken erythrocytes was added to each well,mixed thoroughly,then allowed to stand at room temperature for 40 min. The reciprocol of the highest serum dilution showing complete inhibition of HA pattern was considered as the titre.

Clinical observations

Signs similar to EDS-76 were observed in 4 layer farms and one broiler breeding farm. Summary of the clinical observations is given at Table 1.

In farm I, located in Tainan county, falls in egg production were recognized inapril of 1982. Three layer flocks in the farm were subsequently affected. Production fell suddenly when the flocks were 28-41 weeks of age and not recovered until 5 to 10 weeks later. The reduction rates in egg production ranged from 6 to 25% when compared with the predicted production curves for the same breed of chicken.

In farm II, located in Hsinchu county, similar condition occurred in April of 1982. Abnormalities of egg shells were similar to those observed in farm I, including to the laying of shell-less, soft-shelled, and thin-shelled eggs in addition to loss of eggshell pigment.(Fig.1)

Farm III and farm IV were the layer farms located in Kaoshiung county. Farm V was the broiler breeding farm located in Taichung county. The rates of reduction in egg production in 2 flocks of farm V and the decrease of total egg weight in a flock of farm IV was indicated in Fig. 2-4

Pathological findings

In some cases, soft atrophic ovarian folllicle (Fig.5), remarkable edematous swelling in both mucosa and wall of uterus (Fig.6) and a yellow chalky exudate was found among the uterine mucosal fold. No changes was noted in any other viseral organ of the infected hens.

Virus isolation

In farm I, 2 hemagglutinating agents were isolated from faeces; In farm II, 1 hemagglutinating agent was isolated from cloacal swab. No hemagglutinating agent was isolated from farm III. In farm IV and farm V, 2 hemagglutinating agents were isolated from cloacal swabs, respectively. All hemagglutinating isolates induced intranuclear inclusion bodies in cell cultures, and characteristic fluourscent antigen were noted in the nucleus (Fig.7) when stain with fluorescent antibody against JPA-1 strain. One of the isolates from cloacal swabs was cloned by the plaque-cloning method on a monolayer of DEL cells; it was designated as TN strain.

Electron-micriscopic examination of the virus

An aliquot of duck-embryo propagated virus, TN strain, was used for EM examination by negative staining. The virus particles showed the typical adenovirus morphology with the clear rounded capsomere

Table 1. Effect of EDS-76 Outbreaks on Egg Production in Chicken Farms

Farm	Location of farm	Flock*	Reduction rate of egg production			Maximum reduction (%)	Increase of cull eggs	Decrease in average egg-weight
			Period (1982)	Age (wks)	Duration (wks)			
I	Tainan	A	April	39	5	6	5 times	decline from
		B	April	41	10	7	4 times	
		C	April	28	7	25	20 times	51g to 30g
II	Hsinchu	D	April	23	12	20	Yes	not tested
III	Kaoshiung	E	Sep.	24	6	10	Yes	decline from
IV		F	Sep.	27	15	15	Yes	52g to 44g
V	Taichung	G	Sep.	36	4	21	5 times	Yes
		H	Oct.	37	6	20	5 times	

*Flock A-F were layer flocks, flock G-H were broiler breeder flocks.

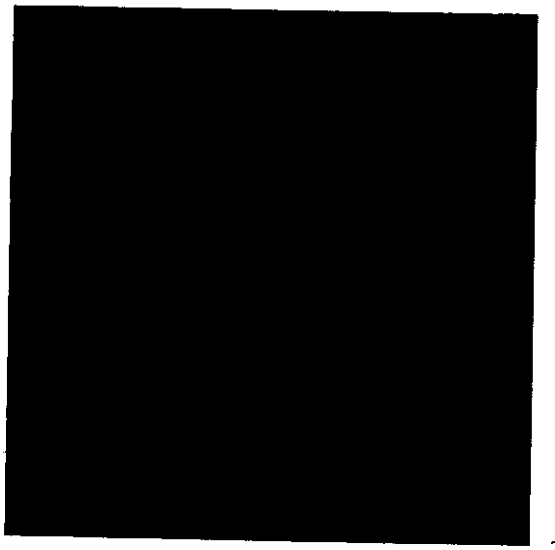


Fig. 1. Normal brown egg (above 4) and abnormal eggs, such as loss of egg-shell pigment, cracked, and soft-shelled laid by layer of farm H.

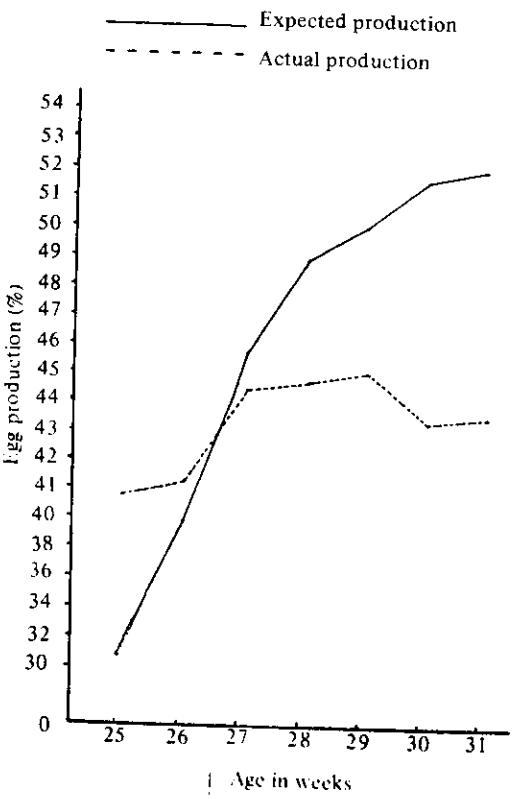
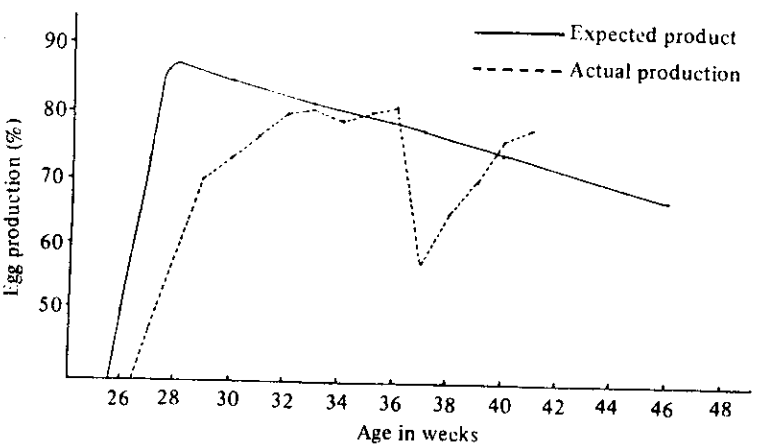


Fig. 2. The expected and actual egg production curves for F flock suffering from EDS-76.

Fig. 3. The expected and actual egg production curves for G flock suffering from EDS-76.



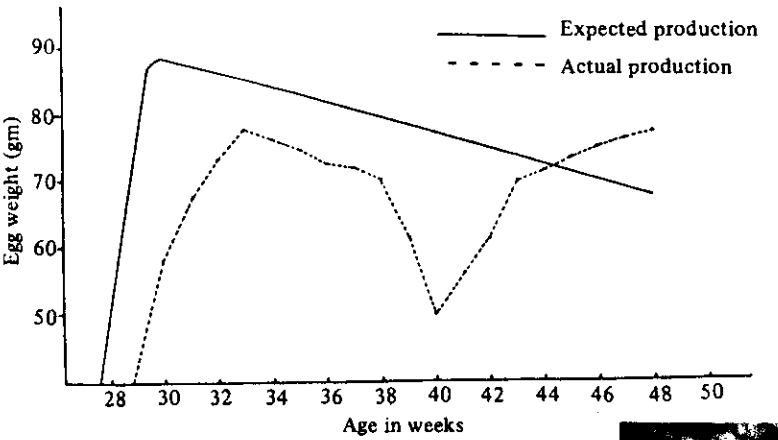


Fig. 4. The expected and actual egg production curves for H flock suffering from EDS-76.

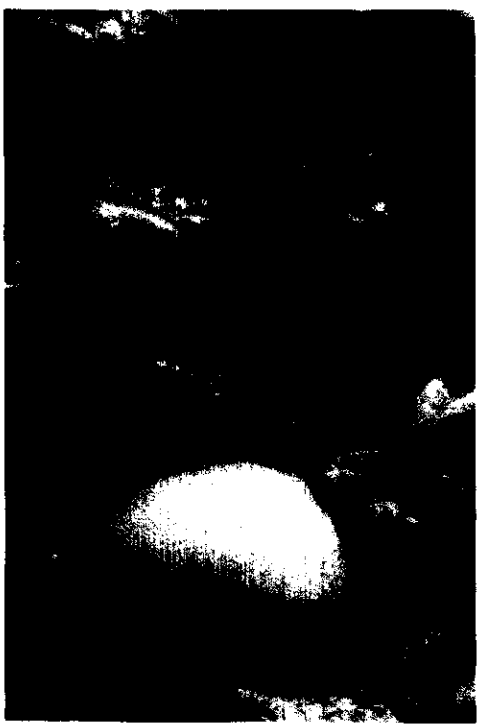


Fig. 5. Soft atrophic ovarian follicle of an hen suffering from EDS-76.

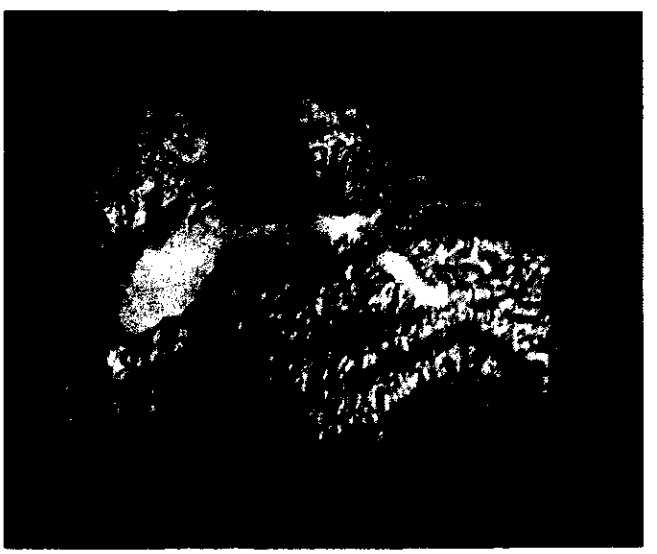


Fig. 6. Remarkable edematous swelling in both mucosa and wall of uterus of an hen suffering from EDS-76.

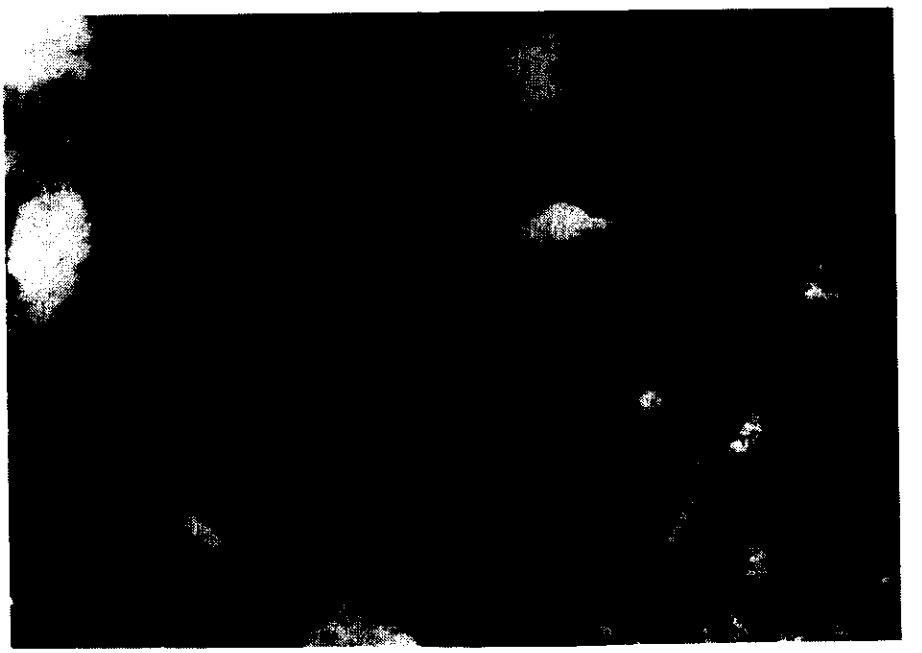


Fig. 7. Specific granular intranuclei fluorescent antigens in the duck embryo liver cell infected with TN strain observed 24 hours postinoculation. (X 200).

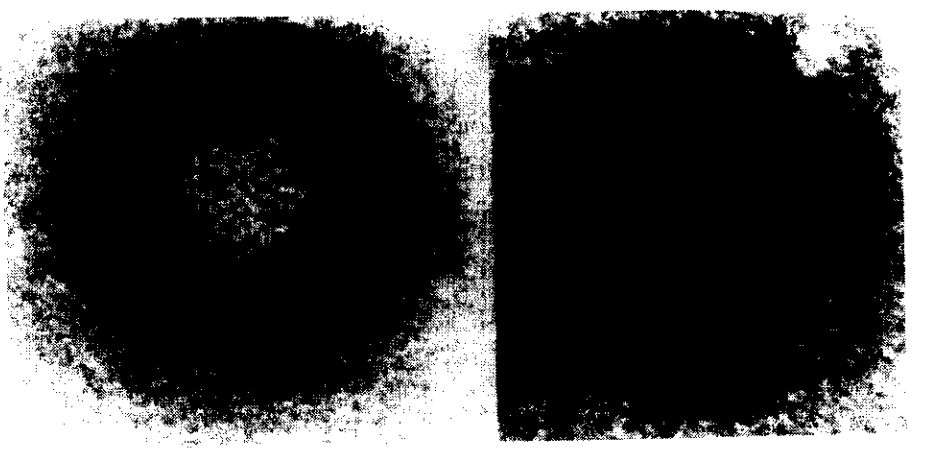


Fig. 8. Electron micrographs of purified virus particles of the TN strain. Stain is potassium phosphotungstate. (X 300,000)

stucture. Fiber structure were observed at the penton base of the virus particle. The diameter of the virus particles ranged from 70 to 80 nm.(Fig.8)

Hemagglutination

The virus can agglutinate the RBC of chicken, duck, goose, pigeon, peacock, and sparrow, but can't agglutinate the RBC of sheep, goat, horse, cattle, rabbit, pig, and human.

Antigenicity

No antigenic difference betwiin TN and JPA-1 strains was demonstrated by means of HI test.

DISCUSSION

The work describes a deliberate attempt to isolate a haemagglutinating adenovirus from flocks showing an egg drop syndrom that was clinically similar to EDS-76. In Taiwan, EDS-76 antibodies were not recognized in chicken flocks until 1980.[6] This is the first report on the presence of a EDS-76 virus in Taiwan.

The seven isolated viruses were identical to the JPA-1 strain EDS-76 virus, shown by the following facts (1) all hemagglutinating viruses produced the same CPE and intranuclear inclusions in the infected cells as the JPA-1 strain; (2) the viruses possessed the same fouorescent antigens as JPA-1 strain in the nuclei of the infected cells; and (3) the virus of the TN strain, which was selected as prototype strain from 7 isolate had the same biological and serological cheracteristics as those of the JPA-1 strain.

Many cells, such as CEF, DEF, TEF (turkey embryo fibroblast), CEL, DEL, TEL, CEK, DEK, TEK, CK, DK, TK, have chosen for virus isolation.[5]

In our experience, it is suggested that GEL cells were also a sensitive substrate for EDS-76 virus isolation. The observation of an in vitro preference for goose and duck over chicked cells EDS-76 virus,[13] coupled with the demonstration of HI antibodies against the agent in goose and duck flocks in Taiwan[6] and Hungary,[1] suggested that waterfowl might be the natural host for the agent.

McFerran [9] reported that the oldest flock showing a fall in production was 36 weeks of age and the majority had falls in production between 29-31 weeks. Yamaguchi [14] also described the outbreaks of EDS-76 in the hens at the age of 30 to 55 weeks old. In Taiwan, the authors observed the occurence of the EDS-76 in hens of 23 or 41 weeks of age. Hence there are a quite large range of the agegroup of hens which are susceptible to the disease.

McFerran [9] reported that most EDS-76 infected flocks did recover to meet their predicted production levels and in some cases some degree of compensation was found. In our cases, compensation of the egg production was noted in Flock H.

The faeces, oviduct, WBC, pharynx and nasal mucosa are preferred for isolation of EDS-76 virus. [9] In our cases, all the isolated virus came from faeces or rectum contents, and none was isolated from other organs. This might have some connection with the stage of the disease when the samples were collected.

In Taiwan, dropped egg production and egg shell formation disorders in laying ducks has been recognised recently. High percentage of the antibody to the virus have been detected in sera if these duck flocks. Whether the drop in egg production of laying ducks was caused by EDS-76 virus infection is under investigated.

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台灣鷄產蛋下降症(EDS-76)之發生及病毒性狀之研究

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1982年4～10月，在台南、新竹、高雄及台中縣5個養雞場，發現白色蛋雞4群，褐色蛋雞2群，肉種雞2群，突然發生產蛋率下降（6～25%），產下異形蛋（褪色、軟卵、薄殼、粗殼蛋），發生雞齡爲24－41週，從患雞之糞便以鵝胚肝細胞或雞胚肝細胞分離呈細胞病變之7株病毒，分離毒對雞、鴨、鵝、鴿、孔雀及麻雀的紅血球有血球凝集性。又接種於鴨胚肝細胞以螢光標示抗體染色在核內可檢出螢光抗原，電子顯微鏡上觀察病毒呈圓形，正二十面體樣，其直徑有70～80 nm之腺病毒，分離毒血清學性狀與JPA-1株EDS-76病毒相同。

此爲台灣首次EDS-76發生及病毒分離成功之報告。

台灣省家畜衛生試驗所