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STUDIES ON EGG DROP SYNDROME-1976: 1- EXISTENCE OF ANTIBODIES IN EGYPTIAN COMMERCIAL CHICKEN LAYERS

BY

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INTRODUCTION

The entity of egg drop syndrome (EDS) was described for the first time in Netherlands in meat type parent and brown layer fowl by Van Eck et al., (1976) in which the disease displayed a number of remarkable characteristics included a sudden and severe drop in egg production (20-50 %) accompanied by the production of high percentage of soft shelled and shell-less eggs, aberrant shells had poor internal quality, short-lived slight diarrhoea and without obvious clinical signs or mortality. The condition seemed to be age related, near the onset or peak production (26- to 33 weeks of age) and egg production usually recovered completely or nearly completely within 6 to 10 weeks of disease onset, thus resulted in severe economical losses, 10-16 eggs losses per hen (McFerran, 1984).

Based on the results of serological examination by Van Eck et al., (1976) associated the condition with an adenoviruses infection. Soon after the first report of the syndrome, Baxendale (1978) isolated the virus which agglutinated chicken erythrocytes from buffy coat cells (BC 14 virus), while McFerran et al. (1977) also isolated a haemagglutinating virus

(virus 127) from pooled nasal and pharyngeal mucosa of affected birds. Subsequently, several EDS-76 isolates were reported in several countries as reviewed by Van Eck (1986).

The disease could be reproduced with both virus isolates BC 14 and 127 (Baxendale, 1978; Lutticken and Baxendale, 1980; and McCracken and McFerran, 1978). The virus isolates were indistinguishable (Baxendale, 1978; and Baxendale et al., 1978) and proved to be members of the Adenoviridae (Kraft et al., 1979; and Todd and McNulty, 1978).

The virus associated with EDS-76 is widespread in waterfowl and domestic ducks and geese. Three forms of EDS-76 have been recognized. The first, or classical, form infects breeding stock and is vertically transmitted. The virus remains latent until birds came into lay, when it is activated, causing classical EDS. The second, or endemic form arises from the classic form. Virus spreads into commercial egg layers and is often spreads by eggs through common packing stations. The third form is the sporadic form , which infects flock via drinking water contaminated by wild or domestic form. This form can give rise to the classical form(if-elite or grandparents were infected) or to the endemic form (McFerran, 1989).

In Egypt, virus-127 was isolated from duck farms, associated with detectable HI-antibodies (Hamouda, 1988).

The present work represents the first record for prevalence of HI-antibodies against EDS-76 in commercial chicken layers in Egypt associated with serious egg problems.

MATERIALS AND METHODS

Flocks examined:

Six-commercial chicken layers (five brown) and (one white) breeds not previously vaccinated against EDS-76 virus, situated in five governorates were tested serologically by HI-test for 127- virus infection in the period from March to December 1990 as a result of severe drop in egg production to sublevels of particular age accompanied by aberrant shells. The data of the examined flocks are presented in Table (1).

Sampling:

Blood samples submitted for testing were centrifuged for serum separation at 3000 rpm for 10 minutes , resulted supernatent serum was evacuated in sterile 1 ml plastic tubes and stored at -20 C till examined.

Hemagglutination (HA) and hemagglutination inhibition (HI) test: The tests were conducted by conventional microtiter method in Linbro SMCV 96 Conic microtitration plates, and the procedures were applied according Rhone Merieux (1985) procedure starting with 1:5 dilution. The HI-test was run against 4-HA units.

Reference Diagnostic Reagents:

IFFA-MERIEUX products specified for HI-test in the form of:

- Adenovirus-127 hemagglutinating undiluted antigen, inactivated by betapropiolactone (Lot 212-30).

- Freeze-dried negatvie control serum to dilute in 1 ml of sterile distilled water. SPF hen serum (Lot 63/05/04).

- Freeze-dried positive control serum to dilute in 1 ml of sterile distilled water. SPF hen serum, immunized with adenovirus-127 (Lot 206/09).

Interpretation of HI-results:

According the instruction of the used reference diagnsotci reagents of Rhone-Merieux(1985), the obtained HI-titer were interpreted as follow:

A titer > 40 was considered as positive.
A titer < 20 was considered as negative.

RESULTS

HA and HI-titer:

The obtained HA titer which produced 100% HA for the reference virus-127 inactivated antigen was 1:1280, while HI of the used reference positive antiserum was 1:640 (inhibited agglutination of 4-HA-units).

Serological Results: Sea Table (2).

DISCUSSION

In the period of March to December 1990, six-big commercial chicken layer flocks, in which brown (five out of six) and white (one out of six) breeds, five flocks were locally produced, and one flock was imported as one day old chick from Holland, situated in five governorates suffered severe drop in egg production around peak of production were examined serologically for EDS-76 virus infection.

Table (1): History of examined commercial layer flocks for EDS-76 antibodies

Flock No.	Governorate	Breed	Age at test (weeks)	House type	Source of day old chick	House capacity	Average egg fall %	Maximum egg fall %	Time for minimum fall (weeks)	Secured time for maximum production (weeks)	Maximum soft shelled and shell-less eggs %	Pale and white eggs	Signs	PM	REMARKS
1	SHARKIA	LB	25	B	L	18000	91-95	96	3	9	4.0	+	-	±	Wireless window, sparrow inside house.
2	GHARBIA	LB	35	B	L	27000	90-96	96	3	10	7.5	+	-	-	Reared breeder duck farm 1500 meter distance.
3	GIZA	HB	35	B+EL	L	32000	90-93	97	2	10	3.0	+	-	-	
4	MANUFIA	BK	30	B	I	23000	85-89	96	3	NR	9.0		-	-	
5	MANUFIA	HB	27	B	L	6000	91-90	91	3	27	2.5	+	-	-	
6	FAYOUM	LB	35	B	L	23000	90-96	90	2	NP	6.0	+	-	-	

LB : Lohman brown .
HB : Hilline brown .
WL : Novens white .
B : Battery.
EL : Deep litter .
B : for brown breeds .

L : Local .
I : Imported .
NR : Not secured .
NP : Not followed .
± : Normal PM lesions but few birds showed subcapsular haemorrhagic liver .

Table (2): Results of HI-antibodies -$\log_2$ against HAT-127 virus using 4-HA units.

Flock code	age at time of exam. (weeks)	tested sera No.	HI- titer-$\log_2$												GM	Reactors* %
			0	1	2	3	4	5	6	7	8	9	10	11		
1	26	30	2				2	16	10						4.9	93
2	36	32				4		4	8	6	2		6	2	6.9	87.5
3	37	28						24	4						5.1	100
4	45	30						1	1	18	10				7.0	100
5	29	91						3	3	6	7				6.9	100
6	37	61							4	8	4				7.0	100

GM : Geometric mean .

* : Birds with titer 1 : 40 (log 4) and over .

exam : examination .

The history of the examined flocks **(Table, 1)** showed that the incidence of the fall in egg production has occurred around an average peak production of 25-35 weeks age as initially reported (Van Eck, 1976) and the syndrome was mostly confined to the brown breeds (5-flocks) as also initially described (Van Eck,1976), in addition to white breed (1-flock) as also later reported (Higashihara et al., 1986), indicating that both breeds were susceptible to natural exposure to EDS-76 virus infection. The average of total egg production drop ranged from 20-41% and the observed time for the lowest point in egg-fall ranged 2-3 weeks afterward it began to increase and nearly to the corresponding predicted production at given ages within 9-10 weeks and this observation was as previously reported (Van Eck, 1986). In all the examined flocks neither obvious sick birds, apart from slight diarrhoea lasted about 2-3 days, nor increased mortality (less than 1% per month) could be observed, also no specific gross pathological lesions could be recorded in the daily dead birds, and this findings were also as previously reported (Van Eck, 1976), except flock No. 1, which showed subcapsular hemorrhagic liver in some dead birds within the first 2 weeks of egg fall.

The first signs of abnormality was the production (for at most 2 days) of eggs with shells that were pale and someones were completely white (in brown breeds) and thinner than normal and then a rapid changes to the production of soft shelled and shell-less eggs accompanied by decrease of size and weight of the produced eggs. The thin-shelled eggs often has a rough, sand paper like tixture or granular roughening of the shell at one end, all these findings were identical to the previous reports (McCraken and McFerran, 1978; Darbyshire and Peters, 1980 and Yamaguchi et al., 1981). Although some workers could not observe effect on albumen(McCraken

and McFerran, 1978; Darbyshire and Peters, 1980 and Yamaguchi et al., 1981) our findings revealed that the thin albumen of the shell-less and soft shelled eggs was always watery and not ropy as reported by other workers (Van Eck et al., 1976 and Meulemans et al., 1979).

Up to the present, outbreaks of EDS-76 attributable to 127- virus infection have been detected by the HI-test (McFerran, 1989) which is used for detecting specific antibodies in sera (McFerran et al., 1977), the results of HI-test performed for the six examined commercial layer flocks (**Table, 2**) revealed that the sera collected after the onset of the fall in egg production had a high frequency of antibody to adenovirus-127, on the basis that titers of 40 are significant positive, indicating exposure to adenovirus-127 infection, and the percentage of the positive reactors reached 100% when examined 2 weeks (flock 3,5 and 6) and 15 weeks (flock 4) post fall in egg production (**Table, 2**) as compared with flock 1 and 2 which were less than 100% reactors when examined 1 week post fall, and these were as the same findings of Van Eck (1986).

The origin of EDS-76 virus infections in the present work are not clear. Very probably, vertical transmission of virus which is well documented as a major method of spread, can not be excluded, particularly, our parent flocks are not vaccinated against EDS-127, hence, can confer the virus to their progney which is reactivated as birds came into production and the disease occur (McFerran et al., 1978; Baxendale et al., 1980 and Darbyshire and Peters, 1980), yet , this need confirmation by further examination for parent flocks regardless of the age, as any age birds is susceptible to infection (McFerran, 1984).

Another possibility for introduction of infection was observed in flock No. 1 where the windows of the house were wireless that allowed the sparrows and wild birds to be contacted with the fowls inside the house, thus, probably permitted deposition of their contaminated dropping in the feed or drinking water traugh of the fowl (Van Eck, 1986).

Third speculation, was observed in flock No. 2 which may originated the infection from neighbourhood breeder muscovy duck farm belonged to the same owner at a distance of approximately 1500 meter from the poultry house, and is supported by the view that the virus is widespread in duck population and of duck origin (Baxendale, 1978; Calneck, 1978; McFerran, 1977 and Schloer, 1980) and recently documented by prevalence of HI-antibodies and 127-virus isolation in Egyptian duck population (Hamouda, 1988).

Further probability for flock No. 4 which was imported as one day old chick from Holland and may be imported infected (exotic) or horizontally acquired the infection (McFerran et al. ,1977, 1979).

The last possibility reported to have among infected chickens, and it was speculated that introduction to chickens was "man-made" by injection of contaminated vaccine into breeder stocks, which then apparently disseminated the virus by vertical transmission(McFerran et al. 1977), so it would be prudent to test vaccines routinely for viruses such as 127-virus. Organization which import stock from area known to have the infection would be well advised to test their flocks serologically for infection.

Finally, we recommend the application of the available commercial oil-adjuvant inactivated vaccine which now appeared to control EDS-76 (Macpherson, 1980).

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SUMMARY

Six outbreaks of severe dropped egg production and production of pale eggs, soft shelled and shell-less eggs correlated with the appearance of hemagglutinating inhibiting antibodies to 127-virus are described. The outbreaks were selected from a larger number of flocks with similar problems in the field.

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