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Research Article

Sero-Evidence for Egg Drop Syndrome 76 in Poultry Flocks in Khartoum State

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Abstract

Due to the outbreak or an incident of egg drop syndrome 76 in Khartoum state, one thousand serum samples were collected from laying flocks of different breeds (Hisex-Bovans and local chicken) in Khartoum State, with a history of depression in egg production accompanied by rough shaped (small egg and abnormal shell). The investigation through detection of antibodies due to no previously vaccinated against egg drop syndrome 76 virus (EDS 76). Sera were tested by HI test and ELISA technique for EDS 76 antibodies. A high percentage (70%) of the serum samples were positive by the HI test for EDS 76; positive samples had a mean titer of up to 7 - 8 log2 indicating viral infection. Using the ELISA technique 85% of the serum samples were positive; the highest titer was (> 400) indicating viral infection.

Keywords: *Soft Shell Egg; Reduced Egg Production; Laying Hens; Khartoum State*

Introduction

Egg drop syndrome 76 is an economically serious syndrome of laying flocks due to a substantial collapse of eggs output at the predicted peak of production, which is a major reason for declining in egg production by up to 40 percent with rough shaped (small eggs, soft-shelled and shell-less eggs) by obviously healthy birds [2,3,13,14,20,21]. History of this syndrome firstly appeared in the Netherlands [25]. Later, several haemagglutinating adenoviruses were first isolated by McFerran., *et al*. [20] from affected hens in Northern Ireland, and the correlation between the syndrome and the isolate was demonstrated [7,11,20,23].

EDS virus is a double-stranded DNA virus of 33.2 kb and is classified as duck adenovirus serotype 1 (also known as EDSV), which belongs to the genus *Atadenovirus* [16,20,23]. Only one serotype has been recognized [22,24] and it differs from conventional adenovirus in that, the virus causing EDS76 agglutinate avian but not mammalian erythrocytes [8]. Duck is the natural host of EDSV [1], so the disease was introduced from the duck into chickens through a contaminated vaccine [2,5,15,23].

The epidemiology of the EDS-76 virus has been investigated by quantitative studies on virus spread under experimental conditions and by investigating outbreaks, they found that the EDS virus epidemiology is widespread as a result, first of vertical transmission that the oviducts have carried a large number of virus particles of some birds laying abnormal eggs, it would be suspected that the abnormal egg would also contain the virus and is more likely to be broken, allowing the spread of the virus [10,14,20] or through eggs, as the virus can spread from the dam to the offspring [26]. Second Lateral or horizontal transmission need contact with infected feces [10,14]. Transmission via insects is theoretically possible, but unproven (OIE report 2017). In this presentation, we study the investigation of the etiologic

agent which causes an outbreak in the Khartoum state leading with Virological and Serological methods for detection of EDS 76 antibodies in sera from fowl flocks by HI test and ELIZA technique.

Materials and Methods

A thousand infected chicken sera 29 to 52 weeks old, were collected from different areas in Khartoum State; Khartoum, Khartoum North and Omdurman. Whole blood was collected from the jugular vein of the infected birds in sterile test tubes and incubated at room temperature overnight, then these sera were separated and kept at -20˚C till used. The sera were examined for EDS 76 antibodies by HI test and ELISA technique.

HI test

A known EDS 76 HI antigen (V-127 Rhone milieux) and a known antiserum (V-127 Rhone milieux) were used in the HI test. The test was performed as described according to [13,20]. Titers were expressed as the reciprocal of the highest dilution of serum causing inhibition to 4 units of a virus. Volumes of 0.025 ml of test serum, 0.025% ml of 4 haemagglutinating units of virus antigen, and 0.025 ml of 0.8% of chicken RBC were used.

ELISA technique

CIVTEST Avi (Laboratories HIPRA, S.A Spain), an indirect ELISA for detection of specific antibodies against EDS virus in avian serum, was used in this study. The test was performed as described by the manufacturer using (IDEXX FLOCKCHEK*U.S.A.EDS76). The titers 138, 238, and > 400 are considered low, medium, and high range, respectively.

Results

Haemagglutination inhibition (HI) test

When the 1000 blood serum samples were examined by HI test, 700 samples (70%) were positive. The highest positive HI titers (7 - 8 log2) were detected in serum samples collected from Khartoum North. The samples that showed positive HI reaction were 450 from the Khartoum North area (90%) and 250 from Khartoum (71.43%) while no antibodies were detected in all serum samples collected from Omdurman (Table 1).

Area	Age (week)	Total flock number	Number of Positive samples (%)
Khartoum North	29-52	500	450(90%)
Khartoum	29-52	350	250 (71.43%)
Omdurman	29-52	150	000 (0%)
Total		1000	700 (70%)

Table 1: *Haemagglutination inhibition positive sera collected from localities areas in Khartoum State.*

ELISA

The same 1000 samples of blood serum that have examined by the HI test were again examined by the ELISA test, we found that 85% of the samples examined were positive samples. 300 positive samples from the Khartoum area and 500 positive samples from the Khartoum North area, 50 positive samples were detected among serum samples collected from the Omdurman area (Table 2). The highest titers (> 400) were detected in 200 samples from Khartoum (Elgeraif) and in 300 samples from Khartoum North (Shambat).

Area	No. of positive samples/ No. examined	ELISA titer range		
		Low (%)	Medium (%)	High (%)
Khartoum	300/350	50 (16.67%)	50 (16.67%)	200 (66.67%)
Khartoum North	500/500	100 (20%)	100 (20%)	300 (60%)
Omdurman	50/150	50 (0%)	0 (0%)	0 (0%)
Total	850/1000	200	150	500

Table 2: *ELISA antibody titer against EDS 76 virus in chicken's sera were collected from three different areas in Khartoum State.*

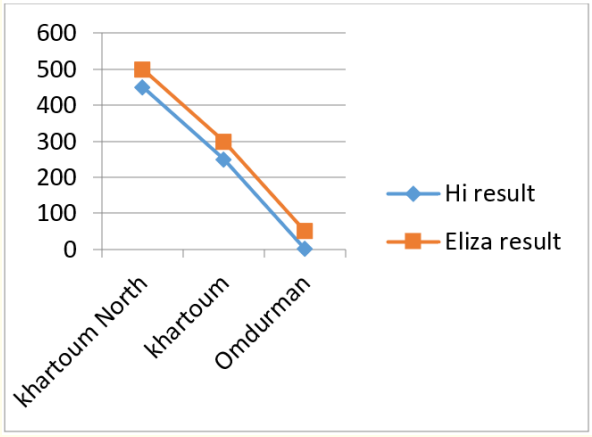

Figure: *This figure has showed the Elisa technique and HI test results and Khartoum north positive result is higher than Khartoum.*

Discussion

Egg drop syndrome is a cosmopolitan disease in chickens and quails. The initial outbreaks that appeared in Sudan in laying hens were probably caused by a contaminated Marek's disease vaccine, which grown in duck embryo fibroblasts and these suggestions agree with [2,5,15].

In this recent study a cascade drop of eggs production at the predicted peak, which was accompanied by egg abnormalities (decreased shell thickness and size), was observed in different areas at Khartoum State, mineral deposition on the shell surface of some eggs was noticed. These observations agree with the first report in Sudan, findings of Ballal and Kheir [4], who recorded signs of egg abnormalities consisting of lack of shell pigment, decreased shell thickness leading to cracks, decrease in size, and soft-shelled or shell-less egg. Changes or failure to achieve predicted production levels were mentioned and an EDS 76 virus (adenovirus) agglutinates fowl erythrocytes has been isolated from these infected chickens [6,11,13,18].

Examination of the sera which have collected from the three different areas in Khartoum State using HI test and ELISA technique revealed detectable EDS 76 virus antibodies, which indicated that the sampled birds were affected by EDS virus. Our results, therefore, support a previous report by Ballal and Kheir [4] on the occurrence of the disease in Sudan.

High titers up to 8 log2 and high titer up to 400 ELISA titer were recorded in Khartoum North (Shambat) and Khartoum (Elgeraif). The results of this survey showed that EDS infection is more prevalent in Khartoum North compared to the Khartoum area (shown in the above figure). This may be attributed to the fact that Khartoum North farms are located close together and are crowdy.

Also, the movement of workers between farms may play an important role in the spread of the disease in Khartoum North. Reused trays may also play a role in the transmission of the disease [9,10].

In the Omdurman area, no antibodies against EDS 76 were detectable by the HI test and a low result was detectable by the Elisa technique. Our result shows that the sensitivity and specificity of the Elisa technique are high compared with the HI test [17]. The result of Omdurman is low because the farms are marginal and far away from each other and they were not crowdy, as crowdedness enhances the horizontal transmission of the disease within the same poultry house [9,10].

Conclusion

Virological and serological investigation on causes of cascade egg production and production of rough shaped (soft-shelled and shell-less eggs) by the laying hens revealed that the responsible causative agent is the EDS virus. This is the second report of an outbreak Of EDS in Sudan.

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