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A CASE STUDY OF SUSPECTED INFECTIOUS BURSAL DISEASE FIELD INFECTION IN LAYERS IN SHARG ELNEEL- KHARTOUM STATE, SUDAN

SARRA ELNAEIM [1]; HIND OSMAN E. [2]; MOHAMMED ABDALSALAM [2]; SARA BASHER TAHA[2]; MOHAMED T. IBRAHIM [3]; JEDDAH, IBRAHEEM ELHAG [4] AND HAYFA MOHAMMED ISMAIL[5]

[1] Faculty of Post Graduate Studies, Sudan University of Science and Technology.
[2] College of Veterinary Medicine, Sudan University of Science and Technology.
[3] Faculty of Animal Production Technology and Wild Life, Sudan University of Science and Technology.
[4] Central Veterinary Research Laboratory, Sudan.
[5] Department of Preventive Medicine and Public Health, Faculty of Veterinary Medicine, University of Khartoum.

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ABSTRACT

Infectious bursal disease (IBD) is a severe, highly contagious immunosuppressive disease. This study aimed to investigate a suspected Infectious bursal disease field infection with apparent morbidity and elevated mortality in a floor-reared Hy-line layer flock in Khartoum State, Sudan. The investigated flock showed depression, dullness, ruffled feathers, diarrhea, pasted vent, and decreased feed and water intake. The affected birds were underweight (800gm to 870gm). The mortality rate was found to be 14.7%. A post-mortem examination revealed enlarged, edematous bursae with various degrees of petechial hemorrhage in the serosal and mucosal surfaces. Hyperemia, inflammation, yellowish exudate, and atrophy were also seen in some of the affected bursae. Extremely distended ureters filled with a whitish material were detected. Enlarged kidneys with degenerative changes and obvious necrotic foci were detected grossly. Agar Gel Immuno-diffusion (AGID) tests and inoculation of the virus in chick embryos were found to be negative. Indirect IBD conventional ELISA test for VP3 for serum of 20 birds revealed 100% positive cases. Creatinine, uric acid, and urea in the serum of ٢2 infected birds from the diseased flock and 19 noninfected birds from the original flock showed a recognizable elevation in the three parameters that reached about 5 times, 6 times, and 2 times respectively. The presence of bursae with obvious lesions in a layer flock of 21 weeks of age was considered the main criterion for the diagnosis. The flock was diagnosed with IBD. ELISA results support the diagnosis. Infection with IBD at this age (21 weeks) could be attributed to bad management in general and bad nutrition in specific which led to being underweight and delayed the regression and disappearance of the bursa of Fabrecious.

Key words: *IBD, Layers, Sudan*

Corresponding author: Hind Osman E.
E-mail address: hmmhmm12345@yahoo.com / hindosman@sustech.edu
Present address: College of Veterinary Medicine, Sudan University of Science and Technology.

INTRODUCTION

Infectious bursal disease (IBD) is caused by a virus that belongs to the genus Avibirnavirus and the family Birnaviridae (Leong *et al*., 2000). It is a highly contagious viral infection that affects young chickens (Hafez *et al*., 2003) that have lymphoid tissue as its primary target with a special preference for the bursa of Fabricius (cloacal bursa). It was first recognized as a distinct disease entity by (Cosgrove, 1962) in 1962 and was referred to as avian nephrosis due to the extreme kidney damage found in birds (Saif *et al*., 2008) It was first identified in 1974 in Belgium (Meulemans *et al*., 1974). Because the first outbreak occurred in the Gumboro, Delaware area, "Gumboro disease" became a synonym for this disease (Saif *et al*., 2008). The disease may appear as subclinical form in chicks aged 0-3 weeks with immunosuppression or clinical form in older birds (Sellaoui *et al*., 2012). Chicken is the only host known to develop clinical disease with distinct lesions following exposure to IBDV (Raj *et al.*, 2009). The poultry industry has been concerned about infectious bursal disease (IBD). It is most commonly found in high-density poultry-producing areas around the world (Berg, 2000) but it is present in 95% of OIE Member Countries (Eterradossi, 1995).

In 1981, the first outbreak in Sudan was observed in El-Obied, North Kordofan State (Shuaib *et al*., 1982). An outbreak of IBD was reported in Kassala in 1988 (Gaffer *et al*., 1988) and they found that there was a seroepidemiological relationship between the virus strain of Kassala and the strain of El-Obied. The disease has been reported in many parts of Sudan, and it has become a major problem for the poultry industry (Hajer and Ismail, 1988) and is considered to be the most devastating poultry disease in the country. This study aims to investigate a suspected field infectious bursal disease infection in a layer flock at age 21 weeks in Khartoum State, Sudan.

METHODOLOGY

An investigation was carried out for a complaint of elevated mortality in a Hy-line breed layer flock of 7000 birds at about 21 weeks of age. The flock was reared for 16 weeks in battery cages after which they were housed on the floor. A descriptive observational study was conducted to investigate a suspected IBD field infection. The weight of the diseased birds was 800-870 grams. They were housed in a semi-closed system in a recommended capacity for floor rearing. The farm is located between latitudes 15°39'34.5"N 32°36'28.4" E in the Sharg Alneel. The flock was previously vaccinated with the Vaxxitek IBD vaccine on day one and with a conventional vaccine on day twenty. This flock had been purchased at the age of 16 weeks.; they constituted 50% of an original flock. The second part of that original flock- the healthy flock- was kept on another farm in another geographical area (Alkadaro, Khartoum North)

Blood samples were collected in 5 ml syringes from randomly selected birds from the affected and the healthy flocks for serological profiling. Part of the collected blood was transferred to heparin-containing tubes for plasma collection. The rest of the blood was set at room temperature to clot and the serum was then collected in Eppendorf tubes for the ELISA test.

Dead birds were recorded daily throughout the outbreak. A post-mortem examination was performed for recently died and ten morbid chickens. Pathological changes were recorded and photographed. For coccidiosis, intestinal scrapings were used for the fecal flotation test. Microscopic examination was used to detect the presence of Eimeria oocysts. Samples from affected bursa were taken and kept at -20ºC and affected kidneys were immediately preserved in 10% formal saline.

An indirect ELISA diagnostic kit (VP3) is used to detect antibodies directed against infectious bursal disease (IBD) during the outbreak. It is a quantitative test used to detect IBD-specific antibodies in chicken sera. Kit components were preserved and used as described by the manufacturer (IDvet, France). For the interpretation of the ELISA test and calculation of the antibody titer, ID^{soft} software was used.

For histological examination, affected tissues were preserved and processed as described by Bancroft and Stevens (1990).

Frozen bursae were homogenized and suspension was centrifuged for 10 minutes at 1000 rpm. Until utilized, supernatant fluids were stored at -20° C in sterile bottles. Lyophilized IBDV Hyperimmune serum was reconstituted in 1ml distilled water and used. For the test, purified agar with 6 outer and one inner hole was used in the petri dish. 20 µl of bursal homogenates and 20 µl of sodium deoxycholate were mixed using a pipette, and then 20 µl of the mixture was put in each of the six surrounding wells and 20 µl of the hyperimmune serum was located in the center well. The gel was then incubated for 24 to 48 hours at room temperature in a humidified chamber. The test was read against an illuminated chamber, clear precipitin lines were said to be positive results.

Blood biochemistry

Kit components for creatinine, urea, and uric acid were preserved and used as described by the manufacturer (Biomed Diagnostics). Collected serum samples were tested within 24 hours. The sample and standard were read against a reagent blank using a spectrophotometer (BioSystem: Spain).

RESULTS

The investigated flock showed depression, dullness, ruffled feathers, diarrhea, pasted vent, decreased feed, and water intake, and decreased weight (800 gm to 870 gm). The mortality rate was found to be 14.7%.

Post-mortem examination of the affected birds revealed enlarged, edematous bursae with various degrees of petechial hemorrhage in the serosal and mucosal surfaces (Figure 1), hyperemia (Figure 2), inflammation with yellowish exudate (Figure 3), and atrophy. (Figure 4). The kidneys were enlarged with degenerative changes and obvious gross necrotic foci. The ureters were extremely enlarged and filled with a whitish material (Figure 5). Microscopically, degenerative and necrotic changes in kidney tissues were detected (Figure 6, Figure 7, Figure 8).

Agar Gel Immunodiffusion (AGID) test for IBD antigen and test for coccidia were found to be negative. Table 1. shows the results of an indirect conventional IBD ELISA test for the affected 21 weeks laying flock.

The data of creatinine, uric acid, and urea were taken for the affected flock and the healthy flock. Table 2. shows the mean of creatinine, uric acid, and urea LS in each group. Figure 9 and Figure 10 show the serum creatinine, uric acid, and urea levels in the hens of the healthy and the affected flock respectively. Creatinine, uric acid, and urea showed a recognizable elevation from the normal that reached about 5 times, 6 times, and 2 times respectively in the three parameters measured.

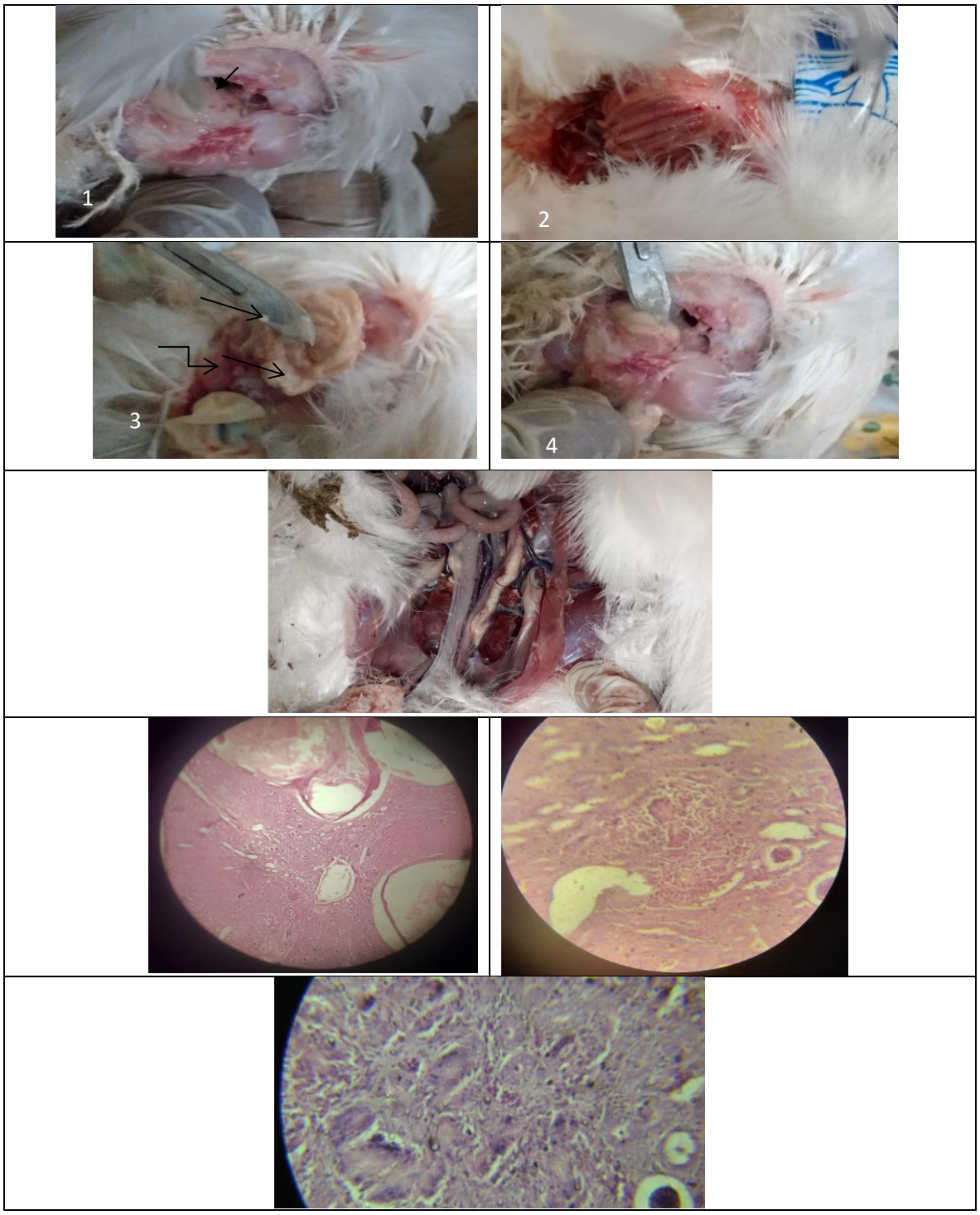

Fig. 1: Petechial hemorrhage on the serosal surface of the bursa of Fabrecious of 21 weeks laying flock tentatively diagnosed with IBD.
Fig. 2: Enlarged and hyperaemic bursa of Fabrecious of 21 weeks laying flock tentatively diagnosed with IBD.
Fig. 3: Inflammatory exudate inside bursa of Fabrecious of 21 weeks laying flock tentatively diagnosed with IBD.
Fig. 4: Atrophied bursa of Fabrecious of 21 weeks laying flock tentatively diagnosed with IBD.
Fig. 5: Enlarged kidney and distended ureters of a 21-week laying flock tentatively diagnosed with IBD.
Fig. 6: Kidney, degenerative, and necrotic changes. H&E: 10X.
Fig. 7: Kidney, degenerative, and necrotic changes. H&E: 20X.
Fig. 8. Kidney, degenerative and necrotic changes. H&E: 40X.

Table 1: ELISA test for IBD titer in about 21 weeks laying flock tentatively diagnosed with IBD.

Age	Geometric Means of antibody titer	Cv %	Minimum titer	Maximum titer
145 days old	3523	26	2511	5676

Table 2: Mean of creatinine, uric acid, and urea levels in serum of a healthy flock and an affected flock tentatively diagnosed with IBD at 21 weeks of age.

	Mean of serum creatinine level	Mean of serum uric acid level	Mean of serum urea level
Group 1	5.23± 2.9 [a]	19.0±10.5 [a]	13.04±4.5 [a,b]
Group 2	0.64± 0.1 [b]	3.68 ± 1.9 [b]	7.34 ± 1.7 [b, a]
F	28.910	16.330	4.435
Sig.	0.000	0.000	0.042

a,c means within the same columns followed by different superscripts significant ($p < 0.05$) different.

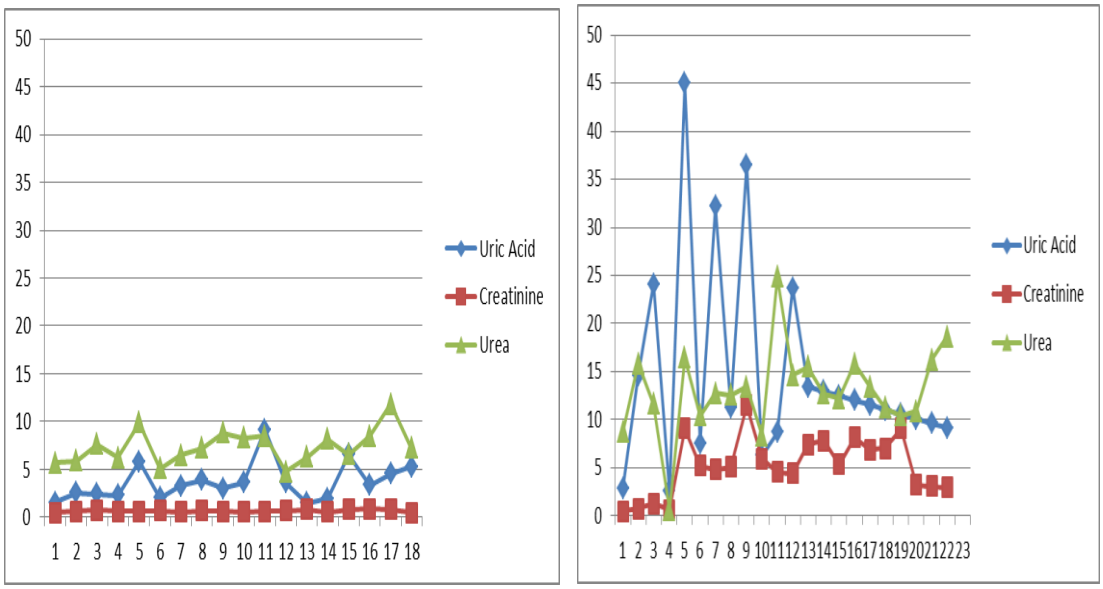

Fig. 9: Uric acid. Creatinine and urea of a 21-week healthy laying flock (left). Fig. 10. Uric acid. Creatinine and urea of a 21-week laying flock affected with IBD (right).

DISCUSSION

Infectious bursal disease (IBD) is a viral disease of the immune system (Teshome *et al*., 2015). The disease is a highly contagious chicken infection that causes severe economic losses to poultry around the world (Hon *et al*., 2008). The objectives of this study were to investigate the cause of mortality in a layer flock in Khartoum State, Sudan, and to compare the renal function in the affected flock with a healthy one.

Depending on the age of the bird, IBD may appear in a clinical form or as a subclinical form (Sellaoui *et al*., 2012), in the current study clinical signs suggestive of the disease were observed and the post-mortem results confirmed the IBD taking into consideration the presence of detectable bursa with pathological changes that are characteristic

lesions of IBD. During an IBDV infection, the BF is the primary diagnostic organ in which gross pathological changes occur (Regenmortel, 2003).

In our study, the presence of the bursa was considered as an increase in weight and size without referring to the body weight because it should be completely absent at that age. That change together with the pathological edema and yellowish transudate is consistent with the results of Lukert, and Saif. (2003) reported an increase in bursa to body weight ratio- most likely due to bursal edema- and a yellowish coloration of the bursa of Fabrecious serosal surface that is caused by the accumulation of serous transudate caused by marked inflammation of the bursal mucosa.

Since The first two outbreaks in 1982 in El-Obied (North Kordofan State) Sudan (Shuaib *et al*., 1982) and 1988 in Kassala (Gaffer *et al*., 1988) research has been going on to complete the seroepidemiological relationship between the virus strains in different areas and the effect of the disease in poultry as it becomes a major problem for poultry industry in Sudan since then (Hajer and Ismail, 1988). The virus's resistance to various disinfectants and virucidals may contribute to the virus's rapid spread (Jackwood and Sommer-Wagner 2010). In Sudan, the absence of poultry extension and failure of vaccination due to the wrong predicted date of vaccination aggravate the situation. In the current case, another factor was added and appeared as a major risk factor: the underweight of the birds. This decrease in the weight at the age of expected onset of laying is most probably due to malnutrition which is not uncommon in rearing bullets for sale at four months.

In our study, the onset of mortality was at age 21 weeks. This age is higher than that recorded in previous studies of IBD infection in layer hens. In Finland, Tammiranta *et al.* (2018) reported signs in a younger age- 6.5-15 weeks The signs reported were similar to those in our study including increased mortality, apathy, decreased food intake, and diarrhea. In the current study, the birds were administered a dose of vector vaccine on day one in addition to a second dose of conventional vaccines on day twenty (as recorded). The conventional vaccine was given after an ELISA test for MDA at the age of 10 days (Appendix 1) which is far time for the test that is recommended to be done on days 1 to 5. At day 20, the vaccine is said to cover 20% of the flock. It is expected that the vaccine has no effect on the 80% that had an MDA higher than 250 and there would be a virus neutralization. The titer due to the conventional vaccine should be detected in 20% of the flock only and the titer of the rest 80% should be due to the vector vaccine which is detectable by the VP2 ELISA kit. The expected result of the titer using a conventional ELISA kit was not detected before in birds above 3 months of age because this test is not routine for almost all farm managers in Khartoum State. The Kit that should be used to detect the vector vaccine should be directed to the VP2 vaccines and it usually gives detectable titer after 7 weeks of vaccination. It is expected to give a higher titer if measured several weeks later but it is usually difficult to estimate using the VP3 conventional kit that gives a false low titer. The resulting titer in this study is high compared to that detected at shorter intervals post-vaccination with conventional vaccines (Appendix 2). This suggests an increase in the titer due to field infection rather than vaccination. In Tammiranta *et al.,* (2018) study, the birds' vaccination status was not included in the referrals; ELISA tests revealed 100% positive samples in the two studies with the difference of vaccination in our study.

The emergence of antigenic variants and very virulent strains of the virus complicates the field situation, contributing to increased bird mortality. Recent and future developments in molecular epidemiology, as well as the development of a new generation of vaccines, could significantly aid in the prevention of this disease shortly (Dey *et al*., 2019).

A new generation of vaccines based on recombinant forms of the VP2 protein is currently being developed to overcome the limitations of inactivated and live-attenuated vaccines against IBDV in terms of efficacy and safety (Müller *et al*., 2012). The recombinant vaccines are formulated with a replication-competent herpesvirus of turkey (HVT) whose genome has been grafted with the nucleotide sequence encoding the IBDV VP2 nucleotide sequence (Darteil *et al*., 1995). The vaccination program used for the surveyed flock did not offer a satisfactory protective level to prevent clinical field infection although both conventional and recombinant vaccines were used; the same conclusion was reached in the IBDV vaccinated flocks surveyed after clinical infection in Khartoum State (Babiker *et al*., 2008). One of the major obstacles facing poultry health in Sudan is the absence and inappropriate dating and interpretation of the ELISA tests.

Depression, dullness, ruffled feathers, diarrhea, pasted vent, and decreased feed and water intake were observed in the investigated flock. These signs are similar to those reported by Mohammed *et al*. (2019) in Sokoto State where 14-week-old IBD-vaccinated hens died suddenly. The clinical signs in those hens were depression, ruffled feathers, drowsiness, huddling, and milky-watery feces. In the current study, the signs also agreed with what was found by Babiker *et al*. (2008) Who reported soiled vent feathers, diarrhea, anorexia, depression and ruffled feathers as clinical signs.

The mortality rate in the current study was low (14.7%) compared with that reported in the previous studies in Khartoum where it was 9%-49% (Babiker *et al*., 2008), and in Sokoto in which it was 40% (Mohammed *et al*., 2019).

To the best of our knowledge, this is the first report of a field IBD infection at age 21 weeks in a laying flock in Sudan. Worldwide, it was reported before in Bangladesh (Rashid, *et al*., 2013).

The study of the serum biochemistry of two flocks- infected and healthy- revealed significant differences in the three blood biochemical parameters tested which indicates a change in the serum biochemistry in the affected birds. The infection adversely affected the renal function of the affected flock and a significant difference in the mean serum level of creatinine uric acid and urea compared to a healthy flock at the same age. Fluctuation of the three parameters was obvious in the affected flock.

In conclusion, pathological changes were manifested in twenty-one weeks of age laying flock including an obvious bursa of Fabrecious with edema, hemorrhage, hyperemia, inflammatory exudation, hypertrophy, and atrophy was detected. Kidneys were enlarged and contained degenerative and necrotic changes. The ureters were severely affected and distended with urates. The infection adversely affected the renal function of the affected flock and a significant difference in the mean serum level of creatinine uric acid and urea compared to a healthy flock at the same age. Fluctuation of the three parameters was obvious in the affected flock. The flock was diagnosed with IBD infection.

Experimental studies concerning the effect of the different types of IBD vaccines on the immunity level of layers. Increase the awareness of the owners and farm managers on the importance of ELISA tests to accurately predict the date of vaccination.

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Shuaib 1982 it is not 1988.

دراسة حالة لإصابة الحقلية محتملة بمرض القمبورو في شرق النيل-السودان

سارة النعيم , هند الريح عثمان , محمد عبد السلام , سارة بشير طه, محمد تاج الدين , جدة إبراهيم الحاج , هيفاء محمد اسماعيل

مرض الجراب المعدي (القمبورو) هو مرض حاد شديد العدوى مثبط للمناعة، إن الهدف من هذه هو التحقق من الإصابة الحقلية بمرض القمبورو المحتملة في وجود إمراضية ظاهرية وارتفاع في نسبة النفوق في قطيع بياض بولاية الخرطوم ، السودان. أظهر القطيع الذي تم فحصه إكتئاب وتبلد وتغير في الريش وإسهال والتصاقات فتحة الاخراج وانخفاض في كمية العلف والماء المتناولة بواسطة الطيور. هنالك نقصان في اوزان الطيور المصابة (٨٠٠ جم الى ٨٧٠ جم). تم تقدير معدل الوفيات ب ١٤,٧٪. أظهر فحص ما بعد الوفاة عن وجود جرابات متضخمة و متورمة مع وجود درجات مختلفة من النزف النقطي في الأسطح المصلية والمخاطية. كما لوحظ وجود فرط في الإدماء والتهاب وإفرازات صفراء وضمور في بعض الجرابات المصابة. كما لوحظ تضخم الحالب الممتلئ بمادة بيضاء اللون مع تغيرات تنكسية في الكلى وبؤر نخرية واضحة كشف عنها بشكل عياني ومجهري في الطيور التي تم فحصها. أظهر الإنتشار في هلام الجل نتيجة سلبية لعدد ١٠ عينات اي عدم وجود الفيروس النشط كما ان نتيجة تزريع الفيروس في جنين الفرخ كان سلبياً. كما أظهر فحص الانزيم المناعي المرتبط التقليدي غير المباشر لعدد ٢٠ عينة نتيجة موجبة بنسبة ١٠٠ %. أظهر الكرياتينين وحمض البوليك واليوريا في مصل ٢٢ طائرًا مصابًا من القطيع المصاب و١٩ طائرًا غير مصاب من القطيع الأصلي ارتفاعًا ملحوظًا في المعايير الثلاثة لدي القطيع المصاب والتي بلغت حوالي ٥ مرات و٦ مرات ومرتين على التوالي. في قطيع البياض البالغ من العمر ٢١ أسبوعًا، تم اعتبار وجود الجراب والآفات التي تؤثر عليه والتغيرات الظاهر للعين من المعايير الرئيسية للتشخيص. يمكننا أن نعزى الإصابة بمرض القمبورو إلى سوء الإدارة بشكل عام وسوء التغذية بشكل خاص مما أدي إلى نقصان في الوزن وتأخير اضمحلال واختفاء جراب فابريشوس.