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Acute enzootic muscular dystrophy of adult lambs at Basrah, Iraq

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Abstract

Acute enzootic muscular dystrophy of adult lambs due to vitamin E and/or selenium deficiency was suspected in local adult lambs of Basrah, Iraq. The study was conducted on 82 adults local breed lambs 8-11 months of age. Suspected animals show panting with increase abdominal respiration and mouth breathing, recumbence and unable to stand with acute death within 24-48 h. Fifteen clinically healthy lambs were considered as controls. The hematological changes indicated a significant decrease in RBC, Hb, and PCV reflected macrocytic hypochromic type of anemia. Indices of clotting factors show significant changes in diseased adult lambs. Results of the biochemical changes indicated a significant decrease of vitamin E, and the glutathione peroxidase, in diseased animals, whereas, a significant increase indicated in the values of AST, CK and troponin I. Results of the post-mortem examinations showed enlargement of the heart with a white-colored irregular patch. Furthermore, results of histopathological changes indicated an acute cellular degeneration of myocardial fibers associated with diffuse interstitial edematous fluid in the myocardial parenchyma and acute cellular degenerative myocardial fibers with a marked degree of degeneration in the myocardial parenchyma. It has been concluded that, acute enzootic muscular dystrophy has an adverse harmful clinical effect on adult diseased lambs which could always be terminated with death.

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Introduction

Acute enzootic muscular dystrophy is one of the important nutritional deficiency diseases affected animals caused by a deficiency of vitamin E and/or selenium. It has been documented that when the soils contain a trace amount of the selenium, Therefore, the grains and forages produced will be deficient in selenium, and on the same approach when the forages have less quality with bad stored it will be deficient in vitamin E, therefore when the animals eat those types of food without additional supplementation, that may be exposed to nutritional deficiency to this disease. The disease always affected lambs. calves, kids, and even foals (1-3).

Moreover, it has been shown that animal feed contains high polyunsaturated fatty acids such as fish meal, cod liver oil, soybean, linseed and corn oils reduce the absorption of vitamin E and selenium (4). Vitamin E and selenium are important micronutrients for good animal's health throughout the life cycle and the realization of a high level of production (1). Globally, there is a widespread occurrence of selenium and vitamin E diseases in animals, leading to substantial financial losses to animal producers. As the vitamin E and selenium contents of feed ingredients and their effective availabilities vary widely, a continuous supply of an active form of these micronutrients to farm animals is required (2).

It was documented that, the main function of vitamin E and Selenium in the body is that Selenium is a biochemical component of the enzyme glutathione peroxidase (GSH-PX). which protect tissues from oxidation. Where, they act as an antioxidant, which prevents the oxidative damage of tissues because they were responsible for the integrity of most of the tissue membranes (skeletal and cardiac muscles,

spleen, red blood cells, and adipose tissues) (3). Normally any cellular action especially of some specific cell membranes (those whom rich in unsaturated fatty acids) might produce normal west products such as hydrogen peroxide, therefore, the accumulation of these west products cause oxidation of tissues, thus, Vitamin E and selenium act as an antioxidant and prevent the oxidative damage of these cells or membranes (5).

Furthermore, selenium deficiency can affect the function of neutrophils (6). On the other hand, it thought that vitamin E can stimulate the immune mechanism (7). It has been shown that, at Basrah, Iraq, adult lambs show signs of acute enzootic muscular dystrophy with acute death which is considered as an infrequent disease registered in this area with little information provided, therefore the current work aimed to investigate the clinical biochemical and diagnostic studies of this disease.

Materials and methods

Animals and study areas

The current study was conducted on 82 adults local lamb breeds 8-11 months' age (males and females), at Basrah, Iraq. The study was started from March 2020 to June 2020. Animals are of different body weight and have a history of recent turnout for grazing at lush green grazing areas, however, animals have dry feeding at night time, moreover, the water sources were the usual field water sources.

Suspected adult lambs show signs of panting with increase abdominal respiration and mouth breathing with acute death within 24-48 h. Fifteen clinically healthy local adult lamb breeds were considered as a control group. Complete clinical and laboratory examinations have been applied to all suspected and control animals according to standard clinical techniques (3).

Samples and hematological analysis

Ten milliliters of blood were drawn from the jugular vein from each animal, 3 ml mixed with EDTA used for complete blood picture on full digital veterinary hematological cell counter (USA), another 3 ml of blood mixed with trisodium citrate for a complete evaluation of blood factor indices analysis using a special kit from (Biolabo, France). In addition, the clotting time was done according to Dayyal (8). The remaining blood is used for separation of serum used for biochemical analysis and kept under -20° until analysis.

Biochemical analysis

Biochemical analysis is done for evaluation of vitamin E (α-tocopherol) using commercial colorimetric assay kit from Elba science biotechnology by using spectrophotometer technique. Evaluation of serum glutathione peroxides was done using sandwich enzyme assay according to manufacturer's instructions from the Al-Shkairate establishment for medical supply, Jordon. Evaluation of troponin I assay ELISA kits from diagnostic automation, Cortez Diagnostics. Evaluation of aspartate aminotransferase (AST) and, creatinine kinase (CK) using commercial kits from Roche diagnostics.

Microscopic and histopathological analysis

A macroscopic and histopathological examination has been applied to all carcasses that died due to the disease (since twenty-nine of disease adult lambs was die after show the clinical signs and after sampling), as all the animal internal organs were examined visually and histologically (9).

However, the focus has been made on the heart because is considered as the most parts of the body affected by the disease. All examinations were done according to Maxie (9).

Statistical analysis

It was done by using the SPSS, for comparison between the diseased animals and controls. Mean and standard error of the mean was calculated, moreover, $P<0.05$ is the statistical value used between diseased adult lambs and the controls (10).

Results

Diseased adult lambs showed different clinical manifestations which included, loss of appetite 92.61%, panting with increase abdominal respiration and mouth breathing 89%, recumbence and unable to stand 85.32 %, and acute death within 24-48 hrs 53.31% (Table 1). Moreover, clinical examinations of diseased adult lambs revealed a significant increase in the respiratory and heart rate of diseased lambs compared with controls (Table 2). Furthermore, table 3 showed the hematological changes indicated in diseased adult lambs compared with clinically healthy lambs, which represents a clear significant decrease in RBC, Hb, and PCV values reflected a macrocytic hypochromic type of anemia of affected diseased lambs. On the other hand, indices of clotting factors of disease adult lambs and control group showed a significant decrease in the values of total platelet count and fibrinogen time, whereas, values of the platelet distribution width, the mean platelet volume, the clotting time, the prothrombin time, and the activated partial thromboplastin time are increased significantly in diseased adult's lambs than in controls (Table 4).

Results of the biochemical changes of diseased adult's lambs and controls indicated a significant decrease of vitamin E, and the glutathione peroxidase, in diseased animals, whereas, a significant increase has been indicated in the values of AST, CK and troponin I, in diseased adult's lambs compared with the control group (Table 5).

Table 1: Clinical signs of diseased adult lambs

Clinical signs	Number of diseased adult lambs n=82	%
Loss of appetite	76	92.61
Panting with increase abdominal respiration and mouth breathing	73	89.00
Recumbency and unable to stand	70	85.32
Acute death within 24-48 hrs	29	53.31

Table 2: Clinical examinations of diseased adult lambs and control group

Clinical examination Parameters	Control group (n= 15)	Diseased adult lambs (n =82)
Body temperature (C°)	38.3±0.63	38.6±0.55
Heart rate (minute)	88.0±4.21	168.0±8.57*
Respiratory rate (minute)	25.0±4.67	65.0±7.39*

*P<0.05 is the statistical value between diseased adult lambs and the controls.

Table 3: Hematological parameters of diseased adult lambs and controls

Parameters	Controls (n= 15)	Diseased adult lambs (n=82)
RBC ($x10^6$)	7.56±1.32	5.76±1.43*
Hb (mg/dl)	12.34±1.71	9.52±1.78*
PCV (%)	33.56±1.67	27.72±5.73*
MCV (fl)	44.39±1.43	48.12±2.75*
MCHC (g/dl)	36.76±1.67	34.34±1.77*
TLC ($x10^3$)	12.11±2.67	12.56±3.78.00
Neutrophils (absolute)	5733.00±304.16	5745.00±381.22
Lymphocytes (absolute)	5301.00±362.56	5298.00±213.11
Monocytes (absolute)	555.00±277.00	567.45±215.00
Eosinophils (absolute)	385.00±23.00	390.00±15.00
Basophils (absolute)	81.00±41.00	79.00±72.00

*P<0.05 is the statistical value between diseased adult lambs breeds and the controls.

Table 4: Clotting factor indices of diseased adult lambs and controls

Clotting factor indices parameters	Controls (n=15)	Diseased adults lambs (n=82)
The total platelet count (g/L)	476.54±14.57	321.11±70.66*
The platelet distribution width (%)	18.32±1.78	28.34±7.51*
The mean platelet volume (fL)	9.43±2.43	17.45±6.87*
The clotting time (minute)	3.44±1.34	4.12±1.87*
The prothrombin time (second)	16.22±1.56	20.25±4.34*
The activated partial thromboplastin time (second)	52.73±2.34	65.34±5.67*
Fibrinogen time (second)	21.56±2.65	13.22±7.91*

*P<0.05 is the statistical value between diseased adult lambs and the controls.

Table5: Biochemical changes of diseased adult lambs and controls

Biochemical parameters	Controls n=15	Diseased adult lambs n=82
α-tocopherol (mg/L)	22.00±1.34	9.32±2.78*
Glutathione Peroxidase (mU/mg Hb)	23.00±1.67	14.00±3.54*
AST (IU/L)	81.00±2.64	733.00±7.65*
CK (IU/L)	63.00±1.32	988.00±8.81*
Troponin I ng/ml	0.21±0.03	22.00±3.88*

*P<0.05 is the statistical value between diseased adult buffalo calve breeds and the controls.

As for the results of the postmortem and histopathological examinations of the dead carcasses the results were shown that enlargement of the heart was detected with a white-colored irregular patch. Moreover, different white patches of different sizes were also detected on the endocardial area of the heart (Figure 1).

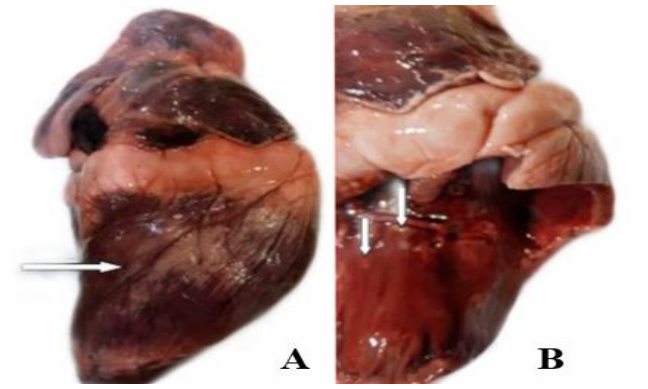

Figure 1: (a) Left side hypertrophy of the heart with multifocal whitish colored patches (arrow), (b) different multifocal whitish colored patches of different sizes were also detected on the cranial part of the endocardial area of the heart.

Results of histopathological changes were also indicated an acute cellular degeneration of myocardial fibers associated with diffuse interstitial edematous fluid in the myocardial parenchyma (Figure 2). Furthermore, acute cellular type of degenerative myocardial fibers with a marked degree of degradation in the myocardial parenchyma was also indicated (Figure 3). However, infiltration of inflammatory cells mainly macrophages that referred to multifocal coagulative necrosis of myocardial fibers associated with cellular debris in the myocardial parenchyma was also seen (Figure 4).

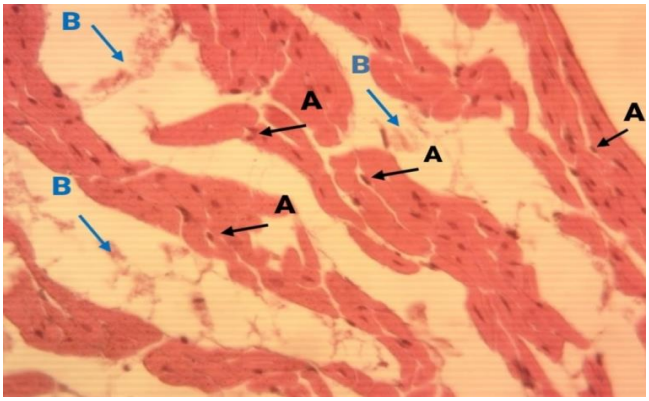

Figure 2: Histological section in the heart of diseased adults lamb shows acute cellular degeneration of myocardial fibers (A); associated with diffuse interstitial edematous fluid in the myocardial parenchyma (B). H&E stain, 10X.

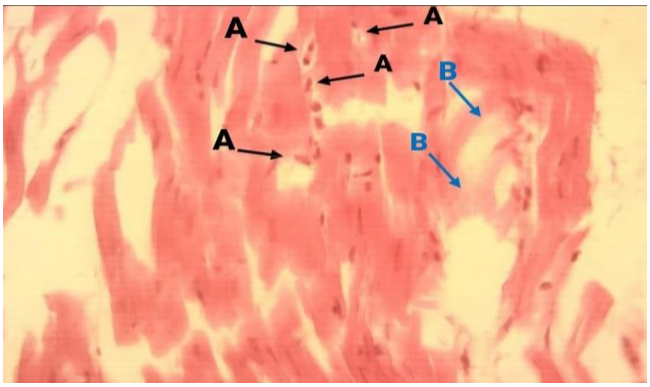

Figure 3: Histological section in the heart of diseased adult lamb shows the acute cellular type of degenerative myocardial fibers (A), as well as to the marked degree of degradation in the myocardial parenchyma (B). H&E stain, 10X.

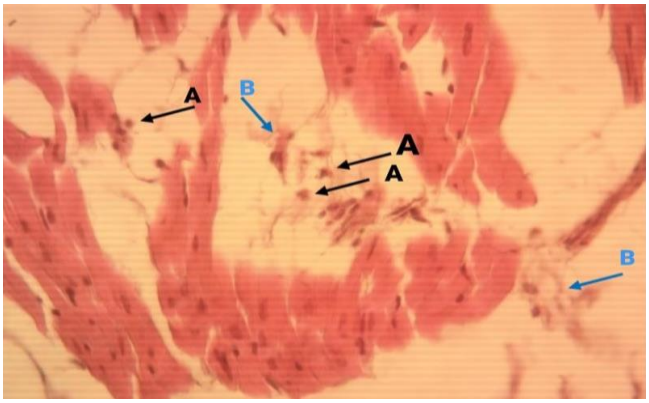

Figure 4: Histological section in the heart of diseased adult lamb shows infiltration of inflammatory cells mainly macrophages that referred to multifocal coagulative necrosis of myocardial fibers (A) associated with cellular debris in the myocardial parenchyma (B). H&E stain, 10X.

Discussion

Acute nutritional muscular dystrophy is an important and common disease associated with inadequate intake or utilization of vitamin E and Selenium, is a peracute to sub-acute myodegenerative disease of cardiac and skeletal muscle (3). The disease is commonly affecting ruminants and non-ruminant's animals, mostly those of younger ages and grow rapidly such as lambs, calves, kids, and foals, especially when their dams have long period feeding on vitamin E and /or Selenium deficient diet during the winter times (11).

It has been shown that, selenium and vitamin E are known as antioxidants, therefore, the deficiencies of these important nutrients could lead to harmful oxidative damage to tissue cells within the body. The muscle cells are the most vulnerable to damage in livestock species, as,

oxidative damage will cause degeneration of muscles, especially the skeletal and cardiac systems (1,12). If the cardiac muscles are affected the animal may show signs of respiratory distress. Whereas, when the skeletal muscles are affected it will result in weakness, stiffness, and recumbence (5). Moreover, it was documented that, sheep particularly can get sufficient quantities of vitamin E when grazing on natural herbs, as, the natural pastures were rich in vitamin E, on the contrary, it was found that cereal grains, root crops, silage, oilseeds, and even the dry hays could be tacky quality sources of vitamin E. Moreover, prolonged, stocked-up food could be ended in the decay of the vitamin E content (13). The deficiency of vitamin E could always affect animals those who feed bad and poor type of hay with fewer sources of diet contains vitamin E or those rich in polyunsaturated fatty acids. However, stress or unusual exercise due to prolong grazing time, could increase the myopathies (14).

In the present study adults, lambs exhibited different clinical manifestations which were also mentioned by others (1,5,7). Clinically two forms of the disease were identified, the cardiac and skeletal forms. The cardiac form occurs most commonly in neonates and typically has a rapid onset animal may be found severely debilitated or dead. However, difficulties of respiration will be a manifestation of damage to cardiac, diaphragmatic, and intercostal muscles (4). Moreover, in older animals, a locomotor disturbance and/or circulatory failure may accompany respiratory signs (5). In clinical practice, animals could display paresis, stiffness or inability to stand, rapid but weak pulse, and acute death, the Mortality may reach more than 70% (3).

It was shown that, sudden death preceded with paresis detected in deficient diseased animals with particular postmortem changes was always characteristic and diagnostic (5). In the skeletal form of the disease, the diseased animals show signs of stiffness, refuse to move due to painful muscles, however, young animals will be unable to rise with dysphagia, as well as that subclinical cases could result in subtle immune defects (15). Ill thrift and reproductive losses are the most common manifestation which could result from the deficiency of vitamin E and/or selenium, since, it could reflect poor growth rate and unthrifty young lambs, furthermore, both vitamin E and selenium could play specific roles in immune rezones of normal animals (12).

The mean function of vitamin E is a component of the antioxidant system that protects those cellular components rich in lipids (such as the mitochondrial membranes, cellular membranes, the plasma membranes, and also the endoplasmic reticular tissues) from lipid peroxidation by decreasing hydro-peroxide formation, moreover, selenium, which is a necessary component of glutathione peroxidase (GSH-PX), also acts to minimize the oxidative damages via devastating the peroxides in the body's more aqueous environment (3). Furthermore, feeds rich in vitamin E, but poor in selenium, may assist and predispose in the development of white muscle disease in younger animals since the non-membrane proteins are less conserved by the peroxidase enzyme, therefore, it still at the same of the oxidative damage activities (1). Contrariwise, when the food contains a low level of vitamin E and rich in selenium will be at the same risk some sub-cellular components that are not protected by the GSH-PX system (3).

In the current study, obvious hematological changes were indicated in diseased adult lambs compared with controls, since a significant decrease has been revealed in the values of RBC, Hb, and PCV which reflect macrocytic hypochromic anemia. same results were also indicated by Mohri *et al.* (4) and Ghanem *et al.* (5), It was documented that, hemolytic anemia is most likely happened as the fragility of erythrocytes is increased and premature lysis is undertaken as a function of vitamin E deficiency (2,4).

Alternation of clotting factor indices was indicated in the current work. Significant depression of platelets count as well as the fibrinogen time was seen in diseased adult lambs which reflected by the bleeding tendency indicated in the carcasses, moreover, a significant increase of the platelet distribution width, the mean platelet volume, the clotting time, the prothrombin time and the activated partial thromboplastin time was also indicated in diseased adult lambs compared with controls. same results were also mentioned by Smith *et al.* (1) and Hassan *et al.* (16) Who attributed the reasons for this to the relations between the vitamin E and/or selenium deficiency and the effectiveness of the indices of clotting factors, since, it was documented that vitamin E. is considered as the inhibitor factor for the platelets aggregation mechanism. Moreover, animals with vitamin E and/or selenium deficiency have an increased value of plasma thromboxane which relieved by vitamin E supplement (5), furthermore, it was postulated that vitamin E deficiency could encourage and enhance the formation of blood clots which assist in the creation of dissemination of intravascular coagulation mechanisms (3).

In this study, the levels of vitamin E and selenium were clearly decreased in diseased animals compared with the control group. Vitamin E is considered the main and important antioxidant factor in the body, nevertheless, not all its characterizations could be detected in this action and its main activities are confined to act in cell membranes to prohibit the spreading of free radical interactions and reactions, although it has been also indicated to have pro-oxidant activities (17,18). On the other hand, it was shown that the non-radical oxidation products could be created through the reaction between alpha-tocopheryl radical and the other free radicals, which are together with glucuronic acid and excreted through both bile and urine. In addition, vitamin E is transported in plasma lipoproteins, and after the absorption, via the intestine, it could be packaged into chylomicrons, which along the lymphatic pathway will be secreted into the systemic circulation (1). Furthermore, some workers have been also added that the accumulation

of the toxic peroxide activity could finally end with cell membrane damage followed by clear injury to the living tissue causing myopathies. This per-oxidative damage action is associated with the promotion of liberation of the lysosomal enzymes, as the activity of which can increase several times at the beginning of detection of the clinical signs (7). Deficiency of vitamin E and/ or selenium were less controlled of cardiac and skeletal muscles as well as the nerves, thorough a disturbance of muscle contractions allowing movement action, the heartbeats, rumen contractions, and lung functions, which are all could be affected by the deficiency. Moreover, the conversion rate of the food, the growth activities, and the reproduction are also could influenced (19). The serum biochemical analysis of the adult lambs affected with the deficiency of vitamin E and/or selenium is also associated with the changes in vitamin E and selenium status. As, the results indicate a significant increase in the values of AST, CK as well as troponin I.

It was shown that the most contributing factor in the laboratory diagnosis of muscular fibers degenerations is the creatinine kinase which is considered as the most particular and sensitive index of muscular damage. This enzyme is highly specific for both the skeletal well as cardiac muscles and is released directly into the bloodstream after the unusual exercise and muscular degenerations. Moreover, aspartate aminotransferase was also considered as an indicator for muscle degenerations, however, also liberated from other damaged liver cells. and, generally, the high levels of both enzymes in diseased animals have just been turned out and exposed to uncommon exercise (20-23). Furthermore, the cardiac degenerations could always lead to an increase in the level of serum troponin I. which also can indicate in different diseases affecting animals such occurs in inflammation of pericardial and endocardial tissues, In the foot and mouth disease of cloven-footed animals especially the cardiac form, on the other hand, there was a good relationship between the magnitude of the troponin levels and the severity of histological evidence of myocardial damage was also reported which in general indicated that serum troponin will always reflect the approved scale in the clinical and laboratory diagnosis of cardiac muscle injuries (24,25).

Carcasses die due to vitamin E and / or selenium deficiency showed different macroscopic and microscopic views which are also indicated by others (3). It was proved that post mortem lesions resulted from deficiency of vitamin E and/or selenium include the petechial hemorrhages and edema of the muscles. Furthermore, the most important hallmarks indicated in the carcasses are pale to white streaking of affected most skeletal and cardiac muscle, diaphragmatic area, and even the tongue (7).

Lesion caused by deficiency of vitamin E and /or selenium was also mentioned by Smith (1) and Constable *et al.* (3) in calves and lambs, who described the macroscopic appearance of the lesions found in the muscles is considered a constant illness, nonetheless, those muscular lesions could different extremely in different animals. Where, most lesions will contain localized areas of whitish or grayish discoloration which indicated the degeneration and necrosis of the muscle fibers. Moreover, it was known that in clinical cases associated with myocardial effects, a white color region of degenerations will indicate, which could found in various areas, especially under the endocardium rejoin of both left and right ventricles in lambs (5). However, these lesions might include the papillary muscles and the inter-ventricular septum and have a gritty character consistent with mineralization. Furthermore, on histopathological examinations, the pictures indicated a non-inflammatory cardiac muscle lesion which are seen in all animal species represented with hyaline degeneration is followed by coagulative necrosis and variable degrees of mineralization which are also indicated in the current study (1).

Conclusion

It has been found through the results of this study that the acute muscular dystrophy has obvious effects on adult lambs, causing harmful damage to their heart and skeletal muscles, as well as a clear negative impact on the animal's general health, which may lead to the death of these animals and the occurrence of great economic losses. Therefore, it is necessary to follow up the animal's diet to reduce the effects of this disease in farm animals

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Conflict of interest

In this scientific work, there is no conflict of interest.

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الحثل العضلي الحاد للحملان البالغة في البصرة، العراق

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الخلاصة

تم الاشتباه في حدوث الحثل العضلي الحاد للحملان البالغة بسبب نقص فيتامين هـ / أو نقص السيلينيوم في الحملان البالغة المحلية في البصرة، العراق. أجريت الدراسة على ٨٢ من الحملان البالغة تراوحت أعمارهم بين ٨-١١ شهرًا. أظهرت الحيوانات المشتبه بها زيادة التنفس البطني والتنفس الفموي، الاستلقاء مع عدم القدرة على الوقوف مع موت حاد خلال ٢٤-٤٨ ساعة. تم اعتبار خمسة عشر حملًا سليمًا سريريًا كمجموعة سيطرة. أشارت التغيرات الدموية إلى انخفاض معنوي في عدد كريات الدم الحمراء، وخضاب الدم، وسرعة ترسيب كريات الدم الحمر والتي انتتجت فقر الدم من النوع ذي الكريات كبيرة الحجم قليلة الصباغ. كما أظهرت نتائج الدراسة حدوث تغيرات معنوية واضحة وجلية في عوامل تخثر الدم في الحملان المريضة. أشارت نتائج الدراسة إلى حدوث انخفاض معنوي في مستوى فيتامين (هـ) وخميرة الكلوتاثايونين بيروكسيديز في الحيوانات المريضة، في حين لوحظت الزيادة المعنوية في قيم خميرة الاسبارتيت ناقلة الأمين والكرياتين كاينيز وخميرة التروبونين. أظهرت نتائج التشريح للحيوانات النافقة تضخم القلب مع وجود رقعة بيضاء غير منتظمة. فضلا عن ملاحظة تنكس خلوي حاد لألياف عضلة القلب المرتبطة بالسائل الوذمي الخلالي المنتشر في النسيج الحشوي لعضلة القلب وألياف عضلة القلب التنكسية الخلوية الحادة مع درجة ملحوظة من التنكس في النسيج الحشوي لعضلة القلب. استنتج أن الحثل العضلي الحاد له تأثير سريري ضار على الحملان البالغة المريضة والتي يمكن دائمًا قد تنتهي بالموت.