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Clinical and Histopathological Findings Associated with Ataxic Myoglobinuric Syndrome in Persian Onager (Equus Hemionus Onager)

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Abstract

The current study is a unique case of captive capture myopathy in an eight-year-old Persian onager. Depression, tremor, muscular stiffness, Tachycardia, mucosal hyperemia, and red-brown urine were all symptoms of the onager when died 5 hours after being captured. The biochemical parameters revealed increases in the levels of creatine kinase, lactate dehydrogenase, potassium, and cortisol. Necropsy revealed prominent contraction band necrosis that involved heart Purkinje fibers. In the kidneys, tubular epithelial cells exhibited hypereosinophilic cytoplasm and loss of nuclei, and frequently contained fine, yellow-brown granular intracytoplasmic pigment. In the liver, edema and peripheral fibrosis, and moderate congestion expanded sinusoids. The lung parenchyma showed focal alveolar emphysema with atelectasis. In the spleen, lymphoid atrophy along with lymphoid necrosis, in the small intestine, atrophy of intestinal villi, the obvious loss of superficial epithelium, the number of the mucosal glands were decreased and there was a small number of chronic inflammatory cells. In conclusion, this report presents Persian onager capture myopathy, emphasizing the need of knowing the causes of death in wild animals to successfully conserve them in captivity.

Keywords: Capture stress, Histopathological findings, Myopathy, Persian onager

Introduction

The Persian Onager is one of six Asiatic onager species now found in Iran, and it is listed as being at an extremely high risk of extinction by the International Union for Conservation of Nature and Natural Resources (IUCN). Physiological stress is an unavoidable aspect of wild animal survival and transmission. Globally, myopathy's weakness as a malignant result of stress during captivity operations accounts for some wildlife-related deaths. In addition to having a significant impact on safeguards, these deaths also have direct and indirect financial consequences and huge losses. Such deaths frequently reveal how animals' safety and well-being were jeopardized and harmed during transfer procedures. The essential point is that this has serious implications for the survival of endangered species. The threat of extinction grows as the survival process and the unprincipled movement persists [1]. Jarrett et al [2] reported the first case of myopathy in a hartebeest (Beatragus hunter) necropsy examination in 1964. Myopathy is a non-infectious muscle decrease in which muscle fibres do not work properly, resulting in muscular weakening, cramps, muscle damage, stiffness, and spasms. This

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condition is similar to a human clinical lesion known as stress cardiomyopathy (SCMP) [3]. Although the exact pathological course of the lesion is unknown and frequently misdiagnosed, it is now recognized internationally as a unique reversible cardiac illness triggered by a stressful incident [4]. Capture myopathy (CM) is more commonly known in ungulates and birds, although it probably affects all captured wildlife. In addition, it is found in coyotes, primates, badgers, fishes, and amphibians [5]. The pathogenesis of CM is not well understood at the present, however, elevated levels of the stress hormone catecholamine appear to affect the musculoskeletal system. Furthermore, the start of the pathological state is linked to stressful situations. Constant stress keeps this situation at a dangerous level. Different forms of CM have been reported as capture shock, ataxic myoglobinuria, muscle rupture, and delay in any acute death [6]. Severe central muscular damage is the primary cause of CM (rhabdomyolysis). When damaged muscle fibers' basement membrane and sarcolemma are damaged, cytoplasmic components including myoglobin and creatine kinase (CK) are released into the circulation [7]. An increased blood lactate concentration, lead to lowers pH and produces acidosis. These changes in metabolism may help explain why body temperature rises in the early stages of capture myopathy [8]. The Heart Failure Association defines stress cardiomyopathy diagnostic criteria, which include echocardiography, electrocardiography, cardiac catheterization, and biochemical analysis. Anxiety, tachypnea, shivering, bent neck (torticollis), red-brown urination, and fever are the most common clinical symptoms in animals with myopathy. In more severe conditions, the animals may develop lame or stiff limbs, lose their appetite, get constipated, as well as seem weak or sluggish. When an animal displays these signs, its chances of surviving are decreasing [9]. The present study aimed to investigate the clinical and histopathological findings of the ataxic myoglobinuric syndrome in Persian Onagers, which was a rare case of capture myopathy that occurred as a result of unavoidable events when handling captured animals at the zoo.

Methods

The present study was bout an eight-year-old Persian onager in captivity. Before being relocated, the onager was quarantined in a separate box for one night. However, due to high mobility and physical activity, the right hind leg was stuck in the gap between the wall and the entrance door of the quarantine box. After three hours, the caregivers spotted the problem and intervened to save the onager. The animal's physiological indicators were monitored after it was released from the imprisoned condition. Debilitation, lassitude, depression, tremor, ataxia, muscular rigidity, hard striding, and tachycardia (up to 25 beats per minute) were among the signs. The other signs were including open-mouth breathing, quick shallow breathing, laminitis, recumbency, mucosal hyperemia, anus temperature of 40.3ºC, and red-brown. Hematological and biochemical analyses were performed on blood samples taken from the jugular vein. For Persian onager, the parameters were compared to reference ranges (Equus hemionus onager) [10]. Except for cortisol, which was assessed by Arfuso et al [11]. Creatine kinase (CK > 562 U/L), lactate dehydrogenase (LDH = 398 U/L), potassium (K+ =

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5.9 mmol/L), and cortisol (750 ng/ml) levels in the blood serum all increased. The biochemical and hematological results were within normal limits (Table 1).

Table 1. **Comparison of hematological and biochemical results with normal values in Persian** Onager (Equus hemionus onager)

Parameters	Results	Reference range
Erythrocytes (1012/L)	9.8	9.4-14.4
Hemoglobin (g/dL)	126	125.4-196.9
Hematocrit (%)	0.44	0.4-0.5
MCV (fL)	36.7	36.3-44.6
MCH (pg)	11.9	11.8-15.4
MCHC (g/dL)	315.8	315.6-352.0
Leukocytes (109/L)	8.1	6.2-8.3
Neutrophils (%)	88.2	48.7-88.5
Neutrophil band (%)	3.7	0.0-3.9
Lymphocytes (%)	47.1	7.5-47.4
Eosinophils (%)	1.9	0.0-2.0
Monocytes (%)	1	0.0-3.0
Basophils (%)	0	0.0-1.0
Platelet count (109/L)	1500	128.5-1611.1
Na (mmol/L)	128.3	126.1-140.2
K (mmol/L)	5.9 H	2.8-3.9
Cl (mmol/L)	100	89.5-102.0
pH	7.35	7.35-7.47
pCO2 (kPa)	7.3	5.5-7.5
pO2 (kPa)	26.3	4.7-26.6
O2 SAT (%)	92.1	65.9-99.5
ctCO2 (mmol/L)	33.8	29.9-34.1
HCO3act (mmol/L)	30.9	28.9-32.4
HCO3std (mmol/L)	29	26.5-30.4
BEecf (mmol/L)	51	4.4-53.9
BEb (mmol/L)	5.8	3.0-6.1
Anion Gap	12.3	4.1-13.3
Total protein (g/L)	63.5	57.3-71.5
Albumin (g/L)	36.1	28.9-40.9
Globulin (g/L)	27.2	22.5-42.0
Albumin/globulin	1.4	0.7-1.7
Triglycerides (mmol/L)	0.3	0.2-0.5
Total cholesterol (mmol/L)	3.0	2.4-4.5
Gamma-glutamyl transferase (U/L)	21.5	14.3-33.6

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Alanine aminotransferase (U/L)	6.3	2.0-8.3
Aspartate aminotransferase (U/L)	3500H	100-600
Creatinine Kinase (CK)(U/L)	2036 H	10-350
U/L)) Lactate Dehydrogenase (LDH)	2800 H	250 - 2070
Cortisol (ng/mL)	750 H	20-90
Alanine aminotransferase (U/L)	3.52	3.40-7.49
Gamma-glutamyl transferase (U/L)	25.70	25.94-19.59
Albumin (g/L)	31.93	31.80-38.13
Albumin/globulin	1.15	1.14-1.46
Triglycerides (mmol/L)	0.28	0.26-0.37
Myoglobin(nmol/L)	18.2H	1.28 to 3.67

H: High

A mixture of intensive fluid therapy (dextrose), Metagin (metamizole sodium), nandrolone laurate, selenium, and vitamins E, and B12 were used in the treatment program. Medical treatment was ineffective, and the animal died five hours later. Multiple foci of petechiae and ecchymosis hemorrhage multi focally on the gastrointestinal tract, signs of interstitial pneumonia and pulmonary hemorrhage due to struggling at death and hyperemia, petechial hemorrhage in the pericardium and heart muscle, pale foci of leg and heart muscles, inflammation and darkness of kidneys, and red-brown urine in the bladder were found on necropsy.

Myocytes and Purkinje fibers both had contraction band necrosis in cardiac histopathological lesions. Degenerative cardiomyocytes had contracted fibers stained with a dark red cytoplasm that was pyknotic, thick, and hyalinized. Degenerated cardiomyocytes showed varied sarcoplasmic and perinuclear vacuolization. In deteriorated cardiomyocytes, there was a sarcoplasmic depletion of myoglobin. In degenerating cardiomyocytes, non-injured fibers displayed uniform intracytoplasmic immune labeling for myoglobin and intracytoplasmic fibrinogen staining (Fig. 1,2,3).

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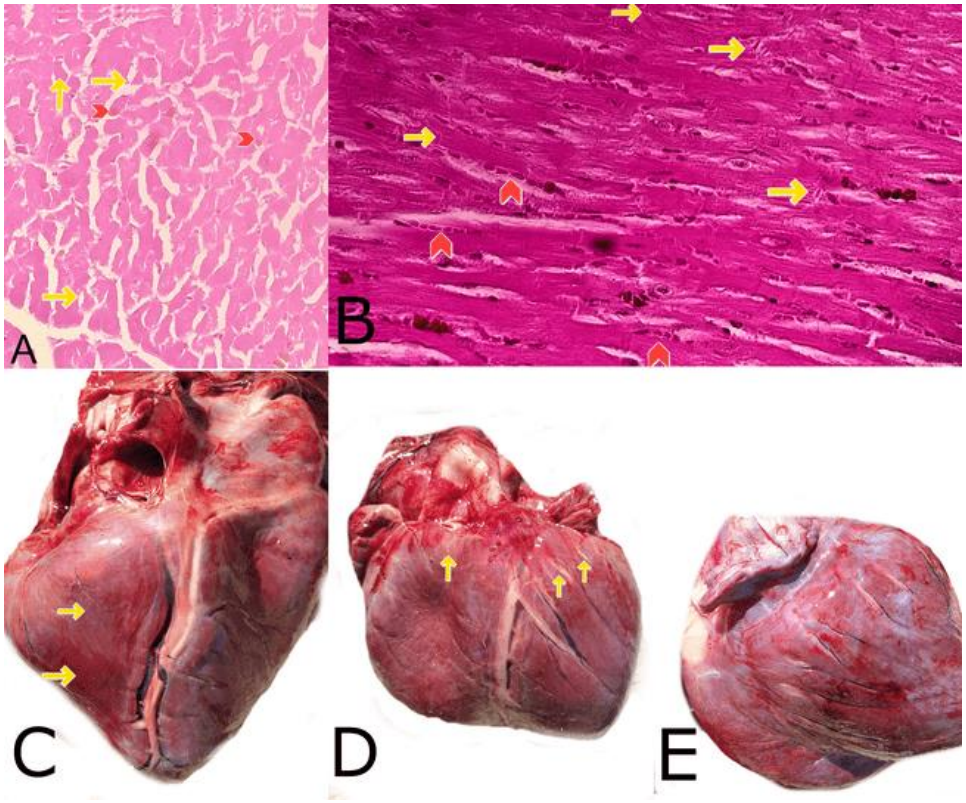

Fig 1. Histopathological lesions of the heart in a Persian onager (*Equus hemionus onager*). A- Myocardium stained with hematoxylin and eosin. Hypercontracted fibers surrounded by endomysial edema rounded and deeply stained fibers represent hypercontracted segments of myofibres, the initial stage of necrosis (Yellow arrows). Globular accumulation of myoglobin in the interstitial space (Red arrows head), B- Multiple fibers have bands transverse to the longitudinal axis of myofibres typical of contraction band necrosis (Yellow arrows). The granular material and globules under the basal lamina and extracellularly in necrotic myofibres (Red arrows head), C- multiple whitish areas in the papillary muscles and the sub endocardial myocardium of the right ventricle (Yellow arrows), D- multifocal to coalescing haemorrhages in the Cardiac muscle (Yellow arrows).

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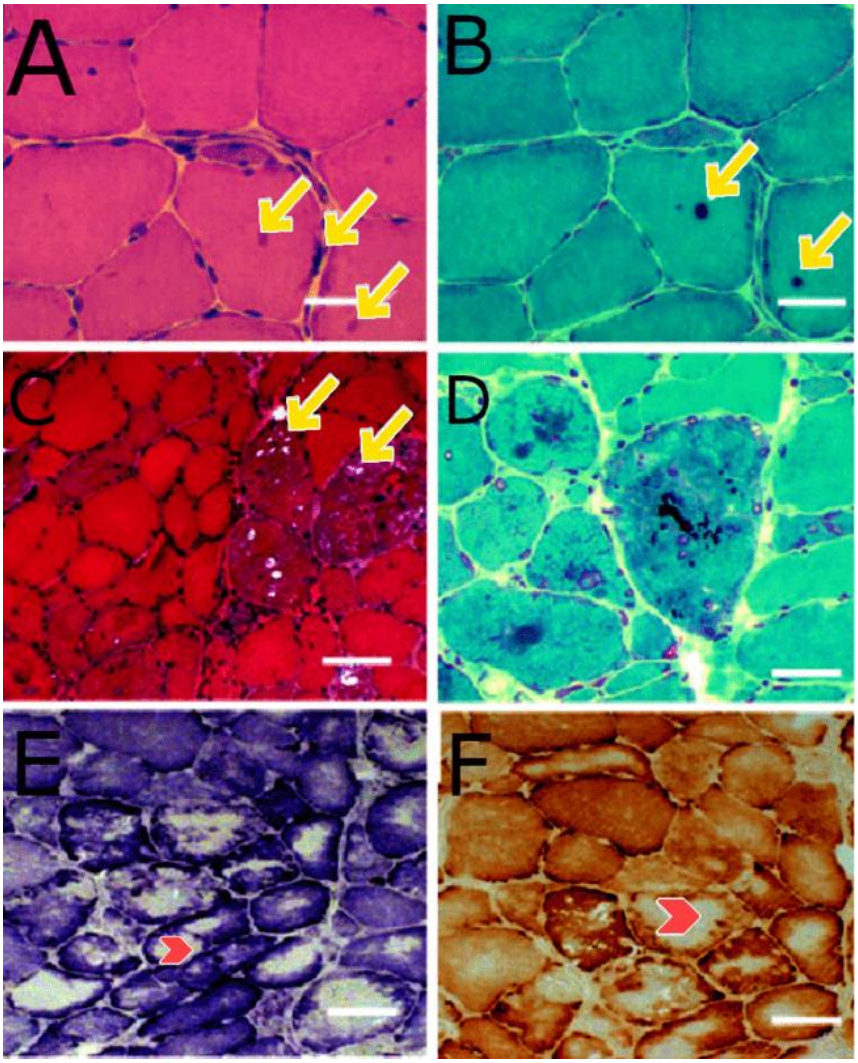

Fig 2. Histopathological findings in Ataxic Myoglobinuric Syndrome in muscles of a Persian onager with Capture Myopathy. Hematoxylin & Eosin (H&E) (A) and Gomori trichrome (G-Tri) (B) staining in Capture myopathy. Arrows indicate the presence of isolated sarcoplasmic and subsarcolemmal protein aggregates (bars = 35 m m). H&E (C) and G-Tri (D) staining in Capture myopathy in a Persian onager (Equus hemionus onager, respectively. Note the vacuolar changes (arrows) in myotilinopathy (bar = 50 m m) and the polymorphic protein aggregates in CM (bar = 35 m m). Succinic dehydrogenase (E) and cytochrome-C oxidase (F) staining in CM in Persian onager (Equus hemionus onager). Note the presence of rubbed-out fibers (Red Arrow) and multiple core-like lesions (bars = 60 m m).

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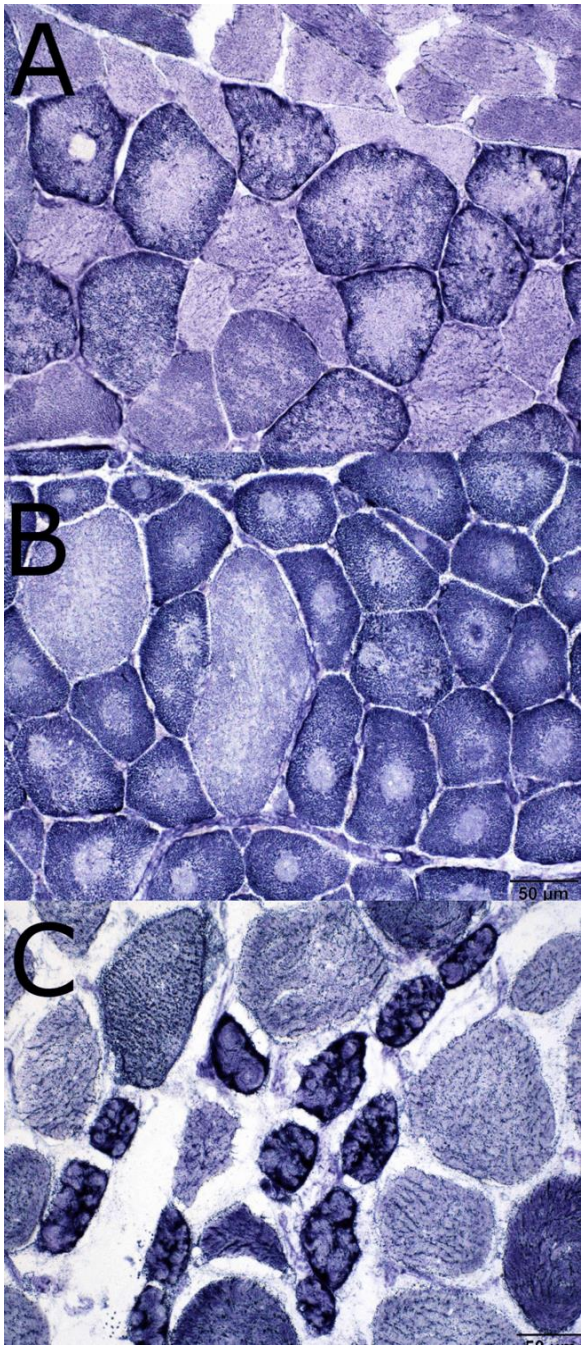

Fig3. Capture Myopathy, NADH-TR staining. Note the architectural changes in the muscle, e.g., central cores, whorled, lobulated and moth eaten fibers. Speckled pattern within myofibers Intensity is proportional to number of mitochondria and NADH activity Type I (dark, dense purple appearance oxidative fibers) and Type II (light, scattered purple speckles nonoxidative fibers)

In the kidneys, some tubular epithelial cells had hypereosinophilic cytoplasm and lost nuclei (Renal cortical necrosis), and small, yellow-brown granular intracytoplasmic pigment was frequently seen. Tubular cell degeneration and congestion were the most noticeable

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abnormalities in the renal parenchyma. Intratubular homogeneous eosinophilic material (proteinaceous casts) was commonly found across the cortex. Increased bowman's space with or without serous exudate was among the other modifications (Fig. 4).

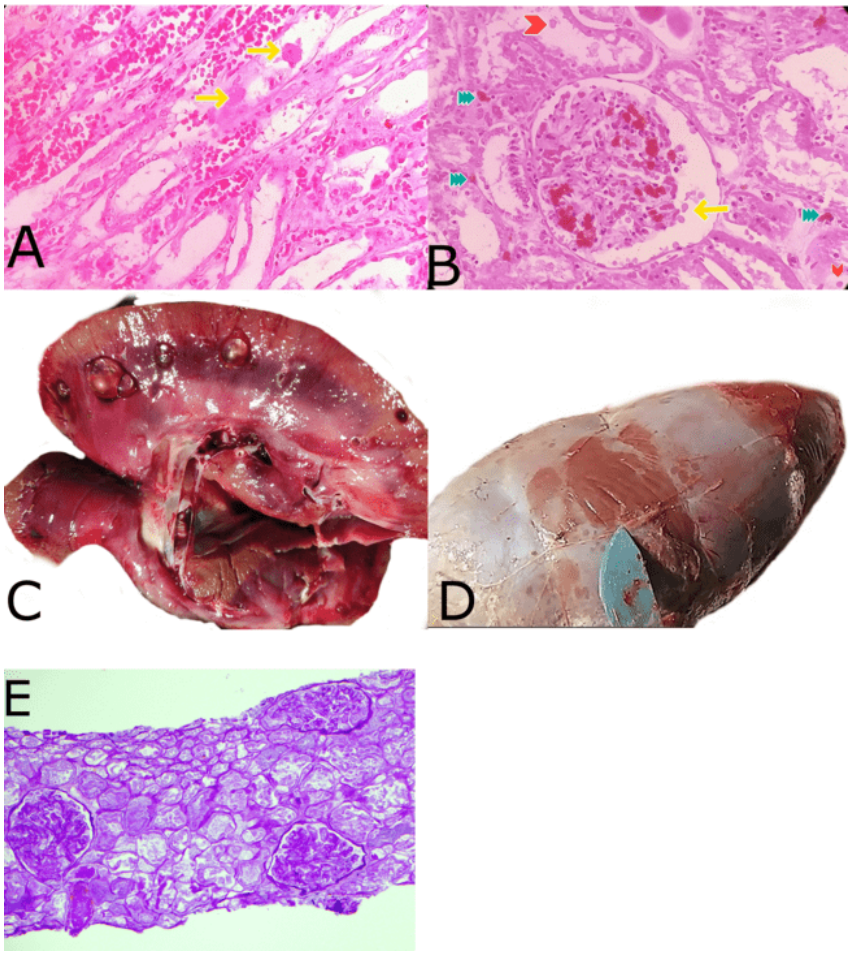

Fig 4. Histopathological lesions of the kidney in a Persian onager (*Equus hemionus onager*). A- Affected tubules have swollen epithelium necrosis with pyknotic nuclei in tubular cells and proteinaceous granular casts in the lumen (Yellow arrows), B- Bowman's space (yellow arrow) and tubules (Red arrows head) are distended with eosinophilic proteinaceous material. proximal tubular cells containing an intracytoplasmic pigment (probably myoglobin, see Blue arrows) Haematoxylin and eosin. (400X), C- Both the kidneys were found to have developed hydronephrosis with severe congestion within the cranial pole, D- Right kidney: enlarged kidney with focal congestion.E- Cortical necrosis with frank tubular necrosis with ghost-like outlines of cells without discernible nuclei, characteristic of coagulative necrosis (periodic acid-Schiff stain)

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Edema, peripheral fibrosis, and mild congestion with enlarged sinusoids were observed in the liver (Fig. 5).

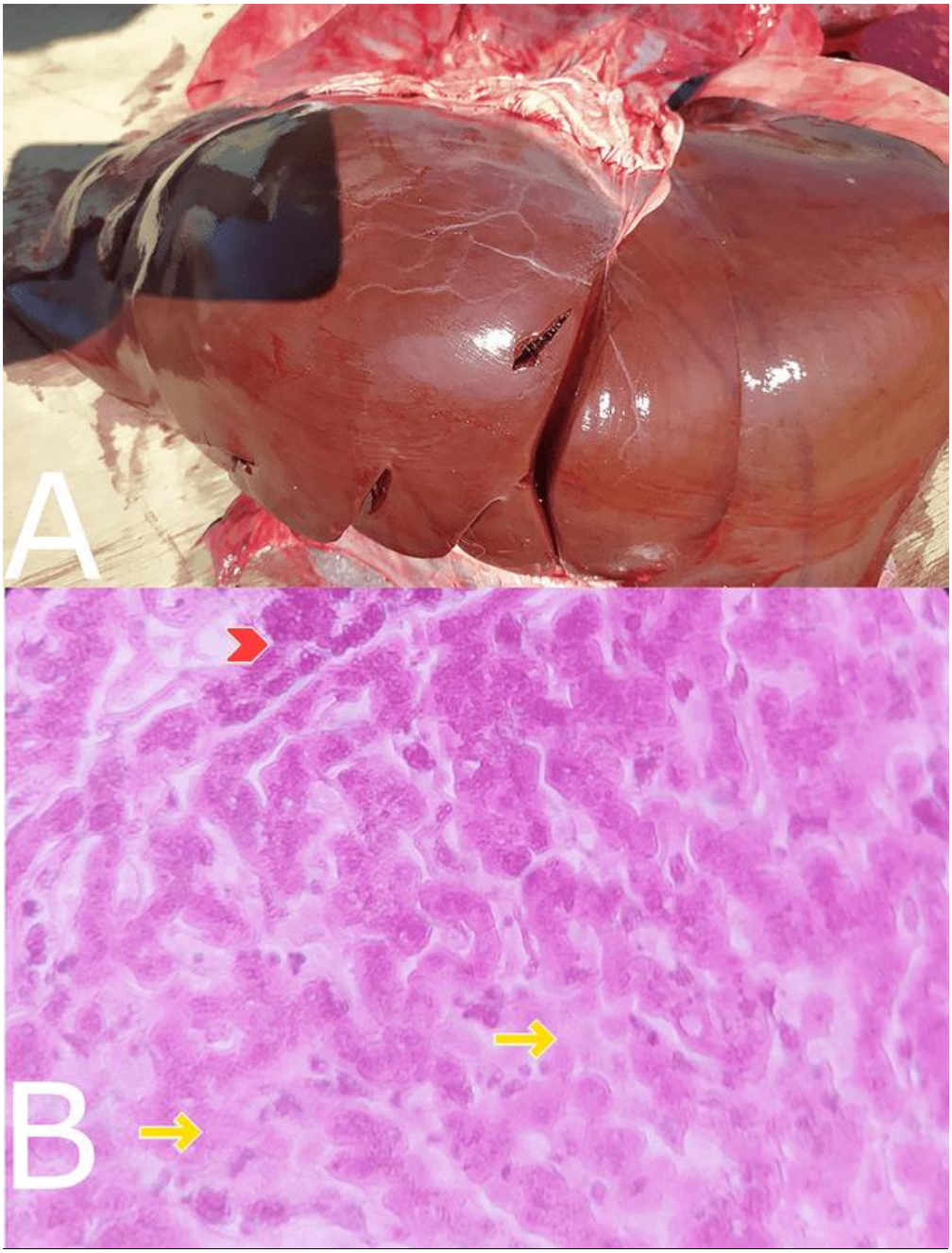

Fig 5. Histopathological lesions of the liver in a Persian onager (*Equus hemionus onager*). A- No obvious macroscopic changes were observed in the liver. B- erythrocytes expanded the sinusoids (Red arrow), degenerated and necrotic cells (Yellow arrow).

Localized alveolar emphysema with atelectasis was the most common histological finding in the lung parenchyma. Alveolar walls were thickened in some locations and the obvious edema and congestion were seen in the lungs (Fig.6).

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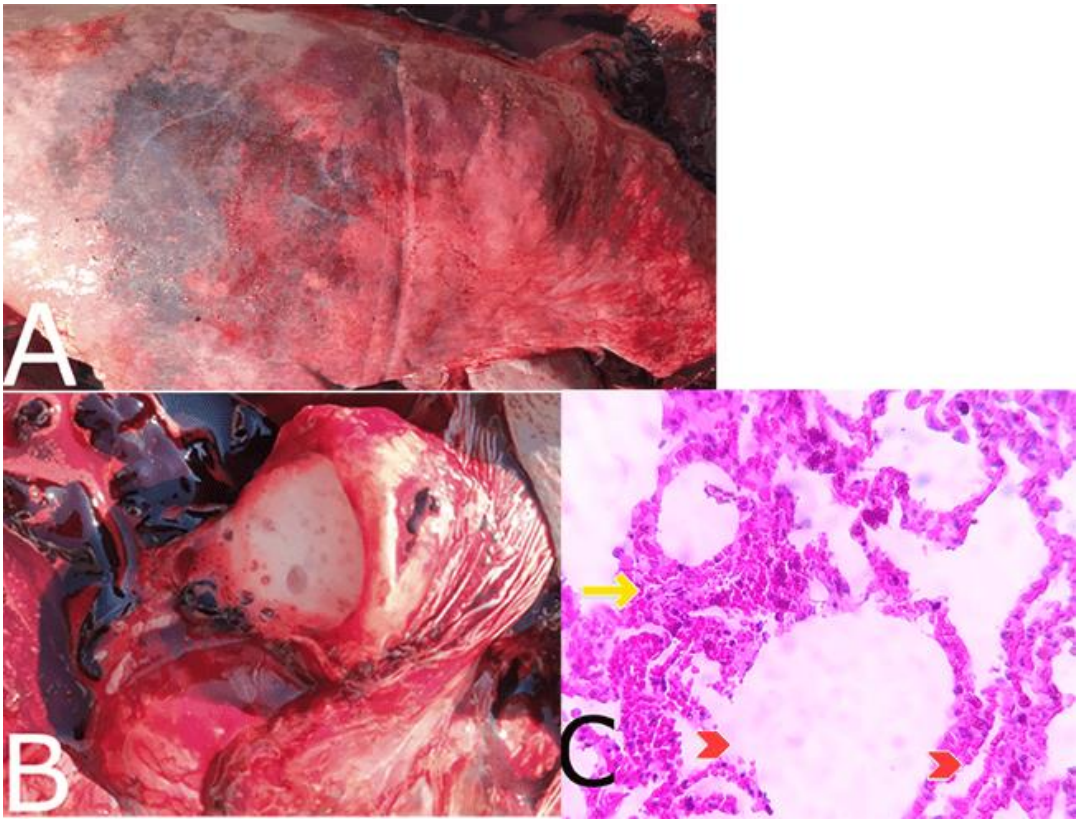

Fig 6. Histopathological lesions of the lung in a Persian onager (*Equus hemionus onager*). A- Edematous lung with congestion and hemorrhage. B- The trachea was edematous and full of foamy discharge, C- Alveolar emphysema with atelectasis (Red arrows), Thickening of the alveolar wall (Yellow arrow).

Cells in the spleen with severe congestion included an intracytoplasmic pigment (possibly myoglobin), as well as lymphoid atrophy and necrosis (Fig.7).

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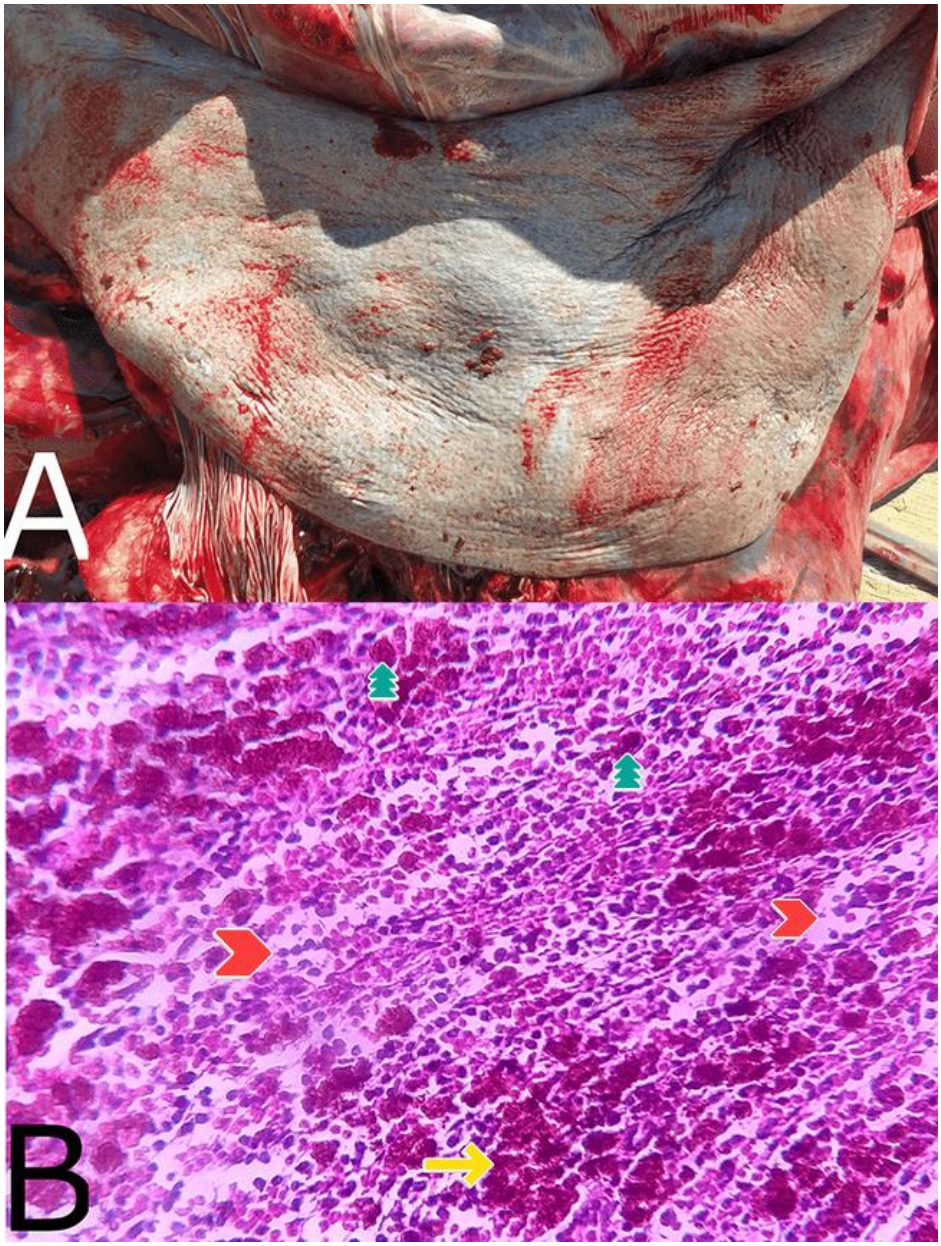

Fig 7. Histopathological lesions of the spleen in a Persian onager (*Equus hemionus onager*). A- Enlargement and splenic pallor indicating lymphocyte depletion, B- Lymphoid atrophy and lymphoid necrosis (Red arrows), intracytoplasmic pigment (probably myoglobin (Blue head arrows)) severe congestion (Yellow arrow).

The intestinal villi were atrophic, with the apparent loss of superficial epithelium, a reduction in the number of mucosal glands, and a limited number of chronic inflammatory cells. In the lumen and between the glands, there was a reduction in the number of mucous glands and an accumulation of chronic inflammatory cells and erythrocytes. The mucosa was atrophic in certain regions, with no glands or villi (Fig.8).

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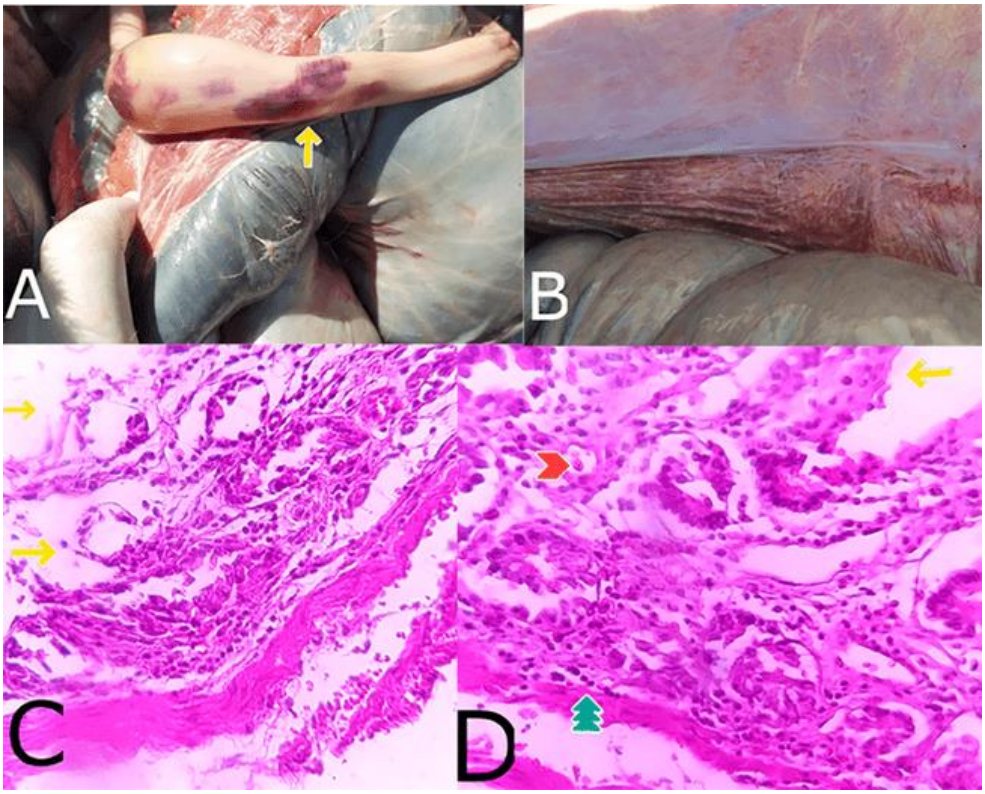

Fig 8. Histopathological lesions of the small intestine in a Persian onager (*Equus hemionus onager*). A- Macroscopic view of the small intestine, the presence of extensive multifocal hemorrhage on the serous wall (Yellow arrow) Hemomelasma ilei(subserosal haemorrhagic lesions in the ileum), B- Extensive petechiae hemorrhages on the inner surface of the serous wall of the ventricular area, C- Villi atrophy and loss of superficial epithelium (Yellow arrows), D- Infiltration of chronic inflammatory cells into the space between the villi walls (Blue arrow) Presence of red blood cells in different amounts (Red arrow).

Discussions

Capturing and translocating wildlife animals has become a major and essential tool in the conservation and sustainable management of captive and free wildlife populations, and both of these aspects have played a critical part in the survival of many species. Capturing and translocating wildlife species has been frequent over the last fifty years as the agricultural economy has expanded in places traditionally known as wildlife reservoirs [12]. The protection and commercial importance of particular animal species has expanded considerably as a result of this industry and the implementation of these procedures [13]. In this regard, due to the high value of wildlife animals and the rising trend of trading them throughout the world, the loss of even a single species might have huge conservation or financial consequences. Furthermore, CM has affected wildlife more than any other disease cause in the previous fifty years [14]. As a result, the little economic impact of the death of wildlife animals due to CM emphasizes the need to learn more about the origins, treatments, and prevention of this lethal disease. The key stresses that induce the "fight or flight" response in

these animals are now thought to be the human presence, restraint, and heightened dread of motorized vehicle sounds during capture. However, the rapidly expanding experience gained during the capture and relocation of living animals, which is currently being updated and enhanced, has quickly become a significant management tool, lowering the incidence of CM to less than 2%. Unfortunately, despite the advancement of these efficient methods, CM still occurs, owing to a lack of understanding of the underlying pathophysiological mechanisms that cause this disorder, as well as how stress and effort can cause muscle injury [15]. In conclusion, finding a definitive therapeutic option and minimizing the lesions produced by CM can provide professionals in this field with a clear and definite perspective direction to work in.

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