Original Article

TJVR 2019; 3(2): 73-79

Turkish Journal of Veterinary Research

http://www.dergipark.gov.tr/tjvr

e-ISSN: 2602-3695

Clinicopathological Evaluation On Capture Myopathy Due To Chemical Immobilization In Spotted Deer

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Received: 30.07.2019 Accepted: 21.09.2019

ABSTRACT

Objectives: The study was focused to investigate the occurrence of a fatal capture myopathy (CM) after chemical immobilization during translocation in different places and to evaluate serum enzymes of stressed deer and pathologic alteration of vital organs of dead animals due to CM.

Materials and Methods: The experimental data was collected from Bangladesh National Zoo, Dhaka and the experiment was conducted at the Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh. Immediate after capture the animals were allowed to normalize body temperature by pouring sufficient water over the body. Peripheral blood was aspirated from jugular vein for serum biochemical analysis. Once the animals died, vital organs were collected and processed for histology.

Results and Discussion: Data from 2013 to 2018 revealed 178 animals captured through darting and among them 40 animals died due to post-capture myopathy reflecting death rate of 22.47%. We have closely studied on 16 animals captured on different occasions. Serum enzyme analysis were exhibited increased levels of ALT, AST, Bilirubin, Creatinine, BUN, LDH, CK, Troponin, Cholesterol, Triglyceride, HDL and LDL and were highly indicative of stress-linked muscle and organ damage. The macroscopic lesions consisted of muscular and cardiac degeneration, edema, hemorrhage and congestion in lung, adrenal gland and in kidney. Microscopically there were loss of striation and fragmentation of skeletal muscle, formation of contraction band necrosis in myocardial fiber, degenerative changes in renal tubule and formation of central intraluminal eosinophilic casts.

Conclusions: The pathological findings were indicative of capture myopathy in spotted deer. This report underlines that mortality from capture is a risk that must be considered during restocking programs.

Keywords: *Capture myopathy, Chemical immobilization, Spotted deer*

INTRODUCTION

Capture myopathy is a noninfectious disease of the muscle in which the muscle fibers do not function properly resulting muscle weakness, muscle damage, muscle cramps, stiffness and spasm can also be associated with myopathy. There are lot of species including ruminants and wild mammals susceptible to this disorder. Yet now the pathogenesis of capture myopathy is not well defined but it has been seemed that the high level of stress factor catecholamine affects the muscular system. Moreover, stressful conditions are also related with the onset of pathological condition

(Spraker, 1993). Persistent stress keeps this condition at a dangerous level.

Different forms of capture myopathy as capture shock, ataxic myoglobinuric, ruptured muscles and delayed per acute death have been reported (Spraker, 1993). Per acute of CM causing death sometimes animals may survive several days or even month, but will often die from heart failure. Ataxic myoglobinuric mild to moderate rhabdomyolysis are commonly reported form of CM that may occur after several hours of post capture. Pathologically moderate severe tubular necrosis with intratubular protein casts have been observed in kidneys.

Chemical immobilization is becoming prevalent in large mammal capture because of lower motility and injury rate than other techniques where low capture stress as animals are manipulated while sedated. Moreover, drug induced side effects depending on the specific drug used (Yadav et al., 2008; Ghurashi et al., 2009), the dread caused to animals during approaching, the stress caused by their displacement which is essential to perform manipulation procedure. For that sometimes manipulation procedures are essential (Northrup et al., 2014).

A lot of research, conservation and management programs focusing on wildlife require the capture and manipulation of animals. Certainly, the development of non or minimally invasive procedures allows obtaining information without the need to handle animals. Even, specific information of individuals can only be collected by capturing the animals. Live captures are also required in conservation biology for animal translocations. In this backdrop, the study has been focused to describe the occurrence of a fatal capture myopathy during the translocation of free-ranging specimens to a protected area and to evaluate serum enzymes of stressed deer and pathologic alteration of muscles and vital organs of dead animal due to CM.

MATERIALS and METHODS

Capturing of Animals

All the animal experimentation has been performed in accordance with the guideline approved by Animal Experiment and Ethics Committee (AEEC) of the Department of Surgery and all data about capturing followed by development of CM leading to death of animals were collected from the register book of veterinary hospital in Bangladesh National Zoo, Dhaka from 2013 to 2018. In the protected areas of the Bangladesh National Zoo, Dhaka, free-ranging deer, accustomed in large enclosures, were chemically immobilized. Capture operations were conducted in the morning, at temperatures of 25 °C.

Animals were captured by using a Dart Riffle (Teleinject, Germany), at a maximum distance of 20 to 25 meters. The time from darting until the animal recumbent (induction time) was between 6 and 10 minutes. The animals were immobilized with Xylazine HCl® 1.0- 1.1 mg/ kg body weight and Ketamine HCl® 2-5 mg/ kg body weight.

Serum Biochemical Examination

Blood samples from anesthetized deer were collected from jugular vein and rectal temperature, respiration and heart rate were recorded meticulously. For separating the serum blood samples without anticoagulant were centrifuged at 3000 rpm for 10 minutes. Serum samples were stored at -20 °C. The determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, creatinine, BUN, total serum cholesterol, total serum triglycerides, total serum HDL, total serum LDL levels were measured in serum samples using a biochemical analyzer device.

Gross and Histopathology

Once CM developed and animal died due to CM, postmortem examination was performed. Gross examination of vital organs and skeletal muscles was carried out meticulously and represent tissue sample were collected and processed for histology. Routine hematoxylene and eosin staining were done. The stained slides were observed and photographs of these slides were taken under photographic microscope (Micros®, Austria).

Statistical Analysis

Data were organized in the Microsoft excel spreadsheet and percentages of mortality due to capture myopathy in different years were calculated.

RESULTS

Capture Myopathy Statistics

Among the 178 captured deer from 2013 to 2018 in Bangladesh National Zoo, 40 deer were found dead due to capture myopathy. The highest number of mortality was found in the 2016 (44%) and the least mortality was recorded in 2018 (14%) followed by 2017 (17%). The mortality of male deer was higher than the female deer in each year.

Table 1. Post capture Clinical parameters.

Time after capture	Number of animal	Mean Temperature (°C)	Mean Respiration rate/min (RR/min)	Mean Heart rate/min (HR/min)
10 min.	3	40.2	38	103
20 min.	3	36.4	36	104
2 hrs	3	40.5	42.5	105
24 hrs (myopathic)	1	43.2	160	390
6 days (myopathic)	1	38.2	41	48

Table 2. Post capture status of Hepatic enzymes.

Parameters	Time post capture					Reference values
	10 min	20 min	2 hrs	24 hrs (myopathic)	6 days (myopathic)	
ALT (IU/L)	98.4	97.5	90.0	95.3	197.9	39 (5-209)
ALP (IU/L)	224	191	201.0	205.33	212	224 (7-1728)
Bilirubin (mg/dL)	0.4	1.3	0.2	0.6	0.3	1.46 (1.24-1.68)
AST (IU/L)	194.3	215.6	197.0	202.2	291.6	67 (17-204)

Table 3. Effects of capture followed by myopathy on marker of renal function and muscle damage.

Parameters	Time post capture					Reference values
	10 min	20 min	2 hrs	24 hrs (myopathic)	6 days (myopathic)	
Creatinine (mg/dL)	2.2	3.0	1.6	2.2	3.9	1.9 (.6-4.4)
Blood urea nitrogen (mg/dL)	55.2	69.0	53.5	59.2	63.1	25 (8-65)
CK (IU/L)	259.4	171.91	160.53	197.28	932.4	253 (38-1815)

Table 4. Post capture alterations of markers for cardiac damage/malfunction.

Parameters	Time post capture					Reference values
	10 min	20 min	2 hrs	24 hrs (myopathic)	6 days (myopathic)	
LDH(IU/L)	322	294	389	335	2668	552 (168-2274)
Troponin test (ng/ml)	0.02	0.02	0.03	0.06	0.08	0.01 (0.01-0.02)

Table 5. Electrolyte alterations after capturing and developing of myopathy.

Parameters	Time post capture					Reference values
	10 min	20 min	2 hrs	24 hrs (myopathic)	6 days (myopathic)	
Sodium (mmol/L)	172.33	177.5	183.2	176.2	159.5	143 (130-156)
Potassium (mmol/L)	7.3	11	8.2	8.83	15.6	4.8 (3-8)
Calcium (mg/dL)	6.52	6.13	6.08	5.21	5.2	9.5 (6.7-11.7)

Figure 1. Haemorrhagic areas between skin and fascia

Figure 2. Edematous lung with congestion and haemorrhage

Figure 3. Hemorrhage in the cortex of kidney

The mortality rate of deer was maximum from 2013 to 2016 when the combination of Xylazine and Ketamine was used contrast Ketamine 2018 (14%).

The results are summarized in Table 1-5.

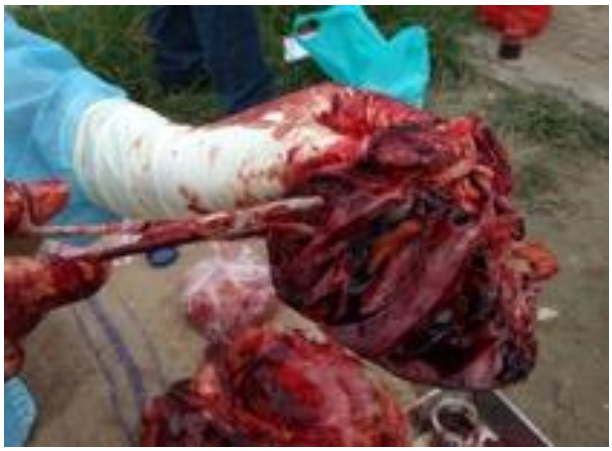

Figure 4. Haemorrhages and clotted blood in the heart

Figure 5. Edematous trachea

Gross Pathology

At postmortem examination, pale areas were observed in the skeletal musculature on both sides of the body (Figure 1). Pathological changes also found in lungs (edematous and congested) (Figure 2) and kidneys (hemorrhages) (Figure 3). Hemorrhages and clotted blood were also seen in heart (Figure 4). Huge amount of tracheal swab were present in the trachea (Figure 5).

Microscopic Lesions

Microscopical lesions were observed in lung, liver, kidneys, adrenal gland, spleen, heart and skeletal muscle are exhibited in Figure 6.

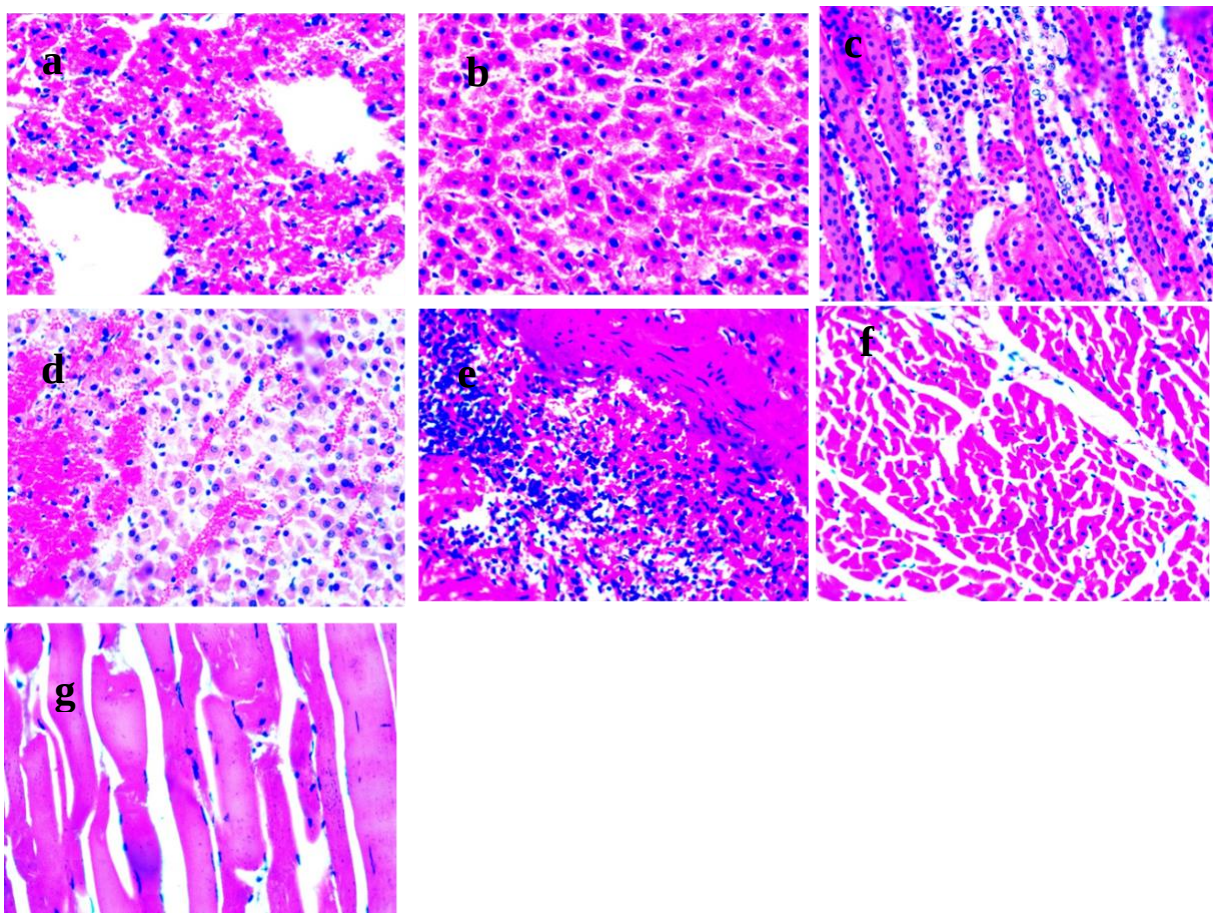

Figure 6: Microscopic lesions. (a) Lung with congestion, (b) Liver with necrotic cell, (c) Kidney associated with glomeruli congestion, (d) Adrenal gland with fat globules, (e) Spleen; severe congestion, (f) Heart; loss of striations and a mild granular degeneration, (g) Skeletal muscle; fibers distorted and loss of striation.

DISCUSSION

This study was conducted to investigate the occurrence of capture myopathy in spotted deer and it was based on the recorded data in the register book of Veterinary Hospital of Bangladesh National Zoo, Dhaka from the year 2013 to 2018. A total 178 animals were captured during the period registered where 40 animals of different ages and sexes were died due to capture myopathy. There is no such type of previous investigation in this line in zoo animals to compare and contrast our finding. In our study mortality was 14% to 44%. The mortality rates are concerned with some reasons that were capturing methods, anesthetic drug nature, capturing environment, sex and distance. Similar investigation reported that the causes of deer mortality are related to several factors that were more represented by forest fires, hunting, collision with vehicles and infectious disease than degenerative myopathy (Hattel et al., 2004). Another study found that death rates of captured deer vary widely from 2.1 to 48% based on different handling techniques (Cromwell et al., 1999). Gericke et al., (1978) reported that capture myopathy occurred as a result of acute stress and hyperthermia due to physical exertion and even resulted in acute death of Springbok. Increased temperature in animals may have been due to the initial psychological stress, physical exertion and interference of thermoregulatory centres by xylazine in the anaesthetic mixture during chemical immobilization. It has already been recognized that physical exertion and psychological stress could contribute to the incidence of hyperthermia during the immobilization of wild ruminants (Wolff, 2009). Xylazine in the anaesthetic combination may also have interfered with the thermoregulatory center resulting in hyperthermia. This is in agreement with the 67 observations of (Monteith et al., 2012) that thermoregulation was interfered by xylazine during chemical immobilisation.

The respiration rate decreased with the advancement of time from anaesthesia. It was also found that there was no remarkable difference in the respiratory rates upto 2hrs of capture. It was

observed by Doherty et al., (1986) that administration of xylazine resulted in respiratory depression leading to hypoxemia in sheep. The present findings suggest that attempts should be made to keep the dose of xylazine low in anaesthetic combinations to reduce complications associated with respiratory depression.

We have found extreme lower heart rate at 6th days postcapture when the animal died of due to CM, but it was highly elevated initially after capture. Susanne (2012) reported that the heart rate of deer increased in response to antropogenic external disturbances. Thus, the initial elevated heart rate due to the initial level of excitement. The lower heart rate of deer may have been due to severe damage of heart muscle to improper functioning of heart.

Muscle enzyme activity increases during capture and handling operations because of increased muscle cell permeability or muscle cell damage (Duncan and Prasse, 1986). These enzymes appear elevated in many stressed wild ungulates and in those suffering from capture myopathy (Vassart et al., 1992). Some authors have found that CK and AST levels are the most sensitive indicators of muscular disorders (Chapple et al., 1991). This indicated skeletal muscle damage during the procedure. Similar findings have been reported in Bighorn Sheep (Kock et al., 1987) and in Chital Deer (Chapple et al., 1991) during physical restraint. When muscle activity begins, blood flow increases but is intermittent. Blood flow decreases as muscle contracts because of the compression of vessels, and increases during relaxation - a process called the 'muscle pump' (Guyton and Hall, 1996). The muscle pump is active when the animal is running but it is inactive when it is immobilized by physical or chemical restraint or is standing in a crate. In most situations, the muscles of frightened animals that are not running are in a relatively high state of contraction, which hinders blood flow into the muscles. This leads to poor tissue perfusion. Transport stress in roe deer decreased heat dissipation, and hypoxia (Spraker, 1993). Catecholamines released in response to stress are potent vasoconstrictor agents that reduce the blood supply to muscles, thus causing hypoxia lesions.

Increased BUN and creatinine values were probably caused by prolonged exertion of deer. Severe, prolonged exercise in man causes an increase in BUN and creatinine of about 60% (Finco et al., 1997). Sealander et al., (1975) also found increased BUN values in deer physically restrained. If the cause of the elevation had been renal, urea and creatinine levels would have increased instead of decreasing in the 'after transport' sample.

Cardiac troponin enzyme is currently used in mammals to measure damage in cardiac myocytes. In this study, it was found that troponin enzyme activity was increased. This increasing tendency indicates that cardiac muscles were damaged due to capturing stress. Cardiac troponin is highly specific to myocardial tissues and is determined as the biomarker of choice to assess cardiac damage in humans (Scolletta et al., 2012). It is highly homologous in mammals it has been used in different specices like dogs, cats, ruminants even in wild animals like the white tailed deer (Boesch et al., 2015) to detect cardiac damage. Cardiac troponin concentration remain high with cardiac ischemia for about 7 to 10 days (Babuin et al., 2005). We also found that troponin level was remain high upto 6th days of capture.

In this study, the main gross lesions were present in the skeletal muscle, kidney and in heart. The lesions in these organs were mainly congestion with some nephrosis present in the kidneys. The muscle lesions appeared pale and necrotic at chronic cases and extensive and severe haemorrhages frequently being present at acute cases found. Microscopically, loss of striations which was found in skeletal muscle. The sarcoplasm of some fibers were calcified and where severe changes had occurred, the nuclei showed degeneration. Basson and Hofmeyr (1973) noted that most lesions were in the muscles of the limbs and back. Although the muscles were bilaterally affected the lesions were not symmetrically distributed fibrotic areas were present in the myocardium of apparently healthy animals that died approximately one month after capture. Due to muscular damage, there was release of myoglobin, which further act on kidney and ultimately lead to death. Basson and Hofmeyr (1973) described the adrenals as sometimes being congested and hemorrhagic and the cortex atrophied. Eosinophilic globules were sometimes present in the cortical zona glomerulosa and the outer zone of the medulla. The spleen and liver were congested and degenerated.

CONCLUSION

The findings of this study concluded that stress induced by capturing, transportation, and environmental condition is the precursor of capture myopathy. Most importantly, the condition affects vital organ function as evaluated by some enzyme

indicative of liver, kidney and heart. The pathological findings were indicative of capture myopathy in a spotted deer *Axis axis.* The study underlines that mortality from capture is a risk that must be considered during restocking programs.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

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