

## Electrocardiographic abnormalities and their association with some serum biomarkers in lambs with foot and mouth disease

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### Abstract

Little attention has been paid to the aspects of cardiac injury-related foot and mouth disease (FMD) in young animals. So, this research was conducted to evaluate the serum biomarkers and electrocardiographic (ECG) evidence of myocardial injury in the infected lambs with FMD. During an outbreak, 33 lambs with FMD and 11 healthy lambs were randomly enrolled. After blood sampling, ECGs were taken from 24 out of 33 infected lambs. Serum creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) activities were measured in both the patients and the control group. Interpretation of ECG showed abnormalities such as sinus tachycardia, premature ventricular complex (PVC) and ventricular tachycardia (VT) in 9, 5 and 6 sick lambs, respectively. The patients had significantly greater serum CK-MB activity ( $170 \pm 36$  IU/L) compared to the control group ( $51.4 \pm 4.7$ ), ( $P < 0.01$ ). The lambs with VT had the highest serum CK-MB ( $546.5 \pm 192$  IU/L) and LDH ( $2813 \pm 523$  IU/L) activities compared to the affected lambs with the other dysrhythmias ( $P < 0.01$ ). It seems that CK-MB has a reliable value for detecting cardiac injury in sheep. Due to the highest CK-MB level in the lambs with VT, it seems VT is associated with highest cardiac lesion compared to other arrhythmias in FMD lambs.

**Keywords:** ECG, Foot and mouth disease, lamb, VT.

### Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease of domesticated and wild ungulates characterized by vesicles in the mouth and on the feet. Very rarely, human beings may also become infected (Andrews *et al.* 2004). The virus exists as seven serotypes and cross-immunity is absent or incomplete among them (Constable *et al.* 2017).

In the recent years, several outbreaks of FMD have been taken place in newborn lambs with high fatality (due to myocarditis), in Iran (FAO, 2016; King & Henstock, 2016); thus, high fatality, especially in newborns has been

one of the major concerns of farmers. Despite the fact that FMD leads to the considerable losses in livestock due to myocarditis, especially in newborns, unfortunately, little attention has been paid by researchers to various cardiologic aspects of myocarditis-related FMD (Tunca *et al.* 2008; Aslani *et al.* 2013). Further, limited studies have been conducted on serum biomarkers and ECG abnormalities related to cardiac impairment in various pathological veterinary situations (Aslani *et al.* 2013; Fartashvand *et al.* 2013; Jafari Dehkordi *et al.* 2014; Nikvand *et al.* 2019).

The serum troponin I concentration provides an excellent and persistent cardiac biomarker in large animals (Constable *et al.* 2017). However, its value in sheep has not been well documented and recent studies have reported various serum troponin I levels e.g., 0.03 (De Souza *et al.*, 2019), below 0.4 (Constable *et al.* 2017) and less than 0.15 ng/ml (Basbugan *et al.* 2010) in healthy sheep. Therefore, in the current study serum creatine kinase-myocardial band (CK-MB), creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) activities were measured for evaluation of cardiac damage in the lambs with FMD, as has been shown by the authors (Tunca *et al.* 2008; Aslani *et al.* 2013; Aktas *et al.* 2015). El Beskawy *et al.* (2016) reported that affected lambs with FMD SAT2 strain had a rising serum CPK and LDH values than healthy ones. However, the ECG pattern of cardiac damage and its relationship with serum cardiac biomarkers in lambs with FMD is not well described. Therefore, this research aimed to evaluate the ECG alterations and serum biomarkers of myocardial injury due to FMD in naturally infected lambs in Khuzestan province, Iran.

### Materials and methods

This case-control study was performed on 33 lambs affected with FMD during an outbreak in January to June in Ahwaz city, Khuzestan province, southwest of Iran. According to the monthly reports on FMD status in the world by FAO, the foot and mouth disease virus type O, genotype O/ME-SA/Pan Asia was introduced as

a causative agent in this outbreak in Khuzestan province (FAO, 2016).

### Animals

During 3 visits to the rural areas of the suburbs of Ahvaz, from 5 sheep flocks affected with FMD, 33 lambs were randomly assigned for evaluation. All the lambs from the infected flocks showed slight salivation, depression, lameness, tachypnea and tachycardia. None of the affected adults and lambs had lesion in the mouth. Two months after the outbreak of the disease has subsided in the region, according to the farmers conversation, among the flocks that were formerly vaccinated for FMD and did not have a history of FMD and further were apparently healthy at the time of sampling, the eleven lambs were selected and served as the control groups. All the lambs were 2-wks to 2-months old.

### Blood sampling

After clinical examination, a blood sample (5 ml) was taken from the jugular vein into a free anticoagulant tube and sent to the laboratory within one hour. The samples were centrifuged at 2500 rpm for 10 min and the isolated sera were transmitted into micro-tubes and kept at -20°C for a short period of one week prior to serum analyses. After sampling of each affected flock, according to the professional ethics, the therapeutic and the preventive advice including anti-inflammatory and a broad spectrum antibiotic for the patients and further vaccination for the rest of flock was given to the farmers.

### *Electrocardiography*

Considering the tachypnea, tachycardia and undistinguishable irregular cardiac rhythms in auscultation of affected lambs, 10 minutes after blood sampling and resting, ECGs were taken by using the base-apex lead via a single-channel ECG machine (Fukuda Denshi Co. Japan) from 24 out of 33 affected lambs. The ECGs were recorded at least 1 to 3 min with the paper speed 25mm/sec and if needed 50 mm/sec. The device was calibrated at 10 mm equal to 1mV. Prior to using the alligator clips electrodes, their attachment sites (positive: skin over the left fifth intercostal space, negative: skin on the caudal third of the left jugular furrow and the earth electrode attached to metal object on the ground) were degreased by ethanol. The noel electrode was also attached away from both positive and negative electrodes on the left flank. In the following, the ECGs were interpreted in terms of any variations in heart rate and rhythm.

The criteria used to diagnose some dysrhythmias in electrocardiogram are as follows:

The criteria for ventricular tachycardia (VT): 1- Widened QRS complexes with regular R-R intervals (Garmel, 2008). 2- Excess of 120 complexes/min. 3- The T wave oriented in a direction opposite to the QRS complex. 4- The P waves lost within complexes (Constable et al., 2017).

The criteria for premature ventricular complex (PVC): 1- An early QRS complex without P wave. 2- Opposite polarity to the normal complexes. 3- Increased QRS duration

and a large T wave amplitude (Constable *et al.* 2017).

### *Serum biomarkers*

Serum activities of CPK, LDH and CK-MB were determined via a colorimetric assay method (biochemistry autoanalyzer, BT-1500, Biotechnica) using Parsazmun kits (Tehran, Iran) in all the patients and healthy animals.

### **Data analysis**

The Kolmogorov Smirnov test showed that the data of serum cardiac biomarkers had no normal distribution in the healthy lambs. So, to compare the mean of myocardial injury biomarkers among both groups the Mann-Whitney test was used. In contrast, the data in affected lambs were normal in distribution. Thus, One-way ANOVA and post-hoc Dunnett T3 tests were used to compare the means of the data among different dysrhythmia subgroups in the affected lambs. In addition, Pearson correlation coefficient was also used among the different serum biochemical markers. The level of significance was set at  $P < 0.05$ . All the analyses were performed using SPSS statistic software (SPSS Inc, SPSS statistics for windows, version 17.0. Chicago, USA).

### **Results**

The obtained ECGs from the affected lambs were interpreted so that, sinus tachycardia (ST), PVC, VT and normal sinus rhythm were observed in 9 (37.5%), 5 (20.8%), 6 (25%) and 4 heads (16.7%) of the patients lambs, respectively (Figure 1).

Serum biomarkers parameters of the affected and control lambs are listed in Table 1.

The affected lambs had significantly greater serum CK-MB activity ( $170 \pm 36$  IU/L) compared to the control group ( $51.4 \pm 4.7$ ); ( $P < 0.01$ ). Serum LDH and CPK activities were not significantly different in the affected lambs than the control group. The lambs with VT had highest serum CK-MB ( $546.5 \pm 192$  IU/L) and LDH ( $2813 \pm 523$  IU/L) activities compared to the affected lambs with the other dysrhythmias ( $P < 0.01$ ). Serum CK-MB activity in the affected lambs with PVC and ST were not significantly different from the control group (Table 2). The lambs with PVC had also the highest level of serum CPK activity ( $702.2 \pm 352$  IU/L) (Figure 2). The Pearson test showed a significant correlation ( $r = 0.836$ ;  $P < 0.01$ ) among serum CK-MB and LDH activities in the lambs with FMD (Table 3). All the affected lambs died within the days and had the tiger-heart sign (Figure 3) in postmortem examination.

**Table 1.** Serum biomarkers of affected and control lambs. Values are Means  $\pm$  SE.

Serum biomarkers	affected lambs (n=33)	Control lambs (n=10)	P-value
LDH (IU/L)	$1019 \pm 211$	$253 \pm 14$	0.153
CPK (IU/L)	$348 \pm 70.2$	$334 \pm 79$	0.574
CK-MB(IU/L)	$170 \pm 36$	$51.4 \pm 4.7$	0.003*

\*. Difference is significant at the 0.01 level (2-tailed). CK-MB, creatine kinase-myocardial band; CPK, creatine phosphokinase; LDH, lactate dehydrogenase.

**Table 2.** Comparison of serum biochemical parameters based on ECG findings in affected lambs. Values are Means  $\pm$  SE.

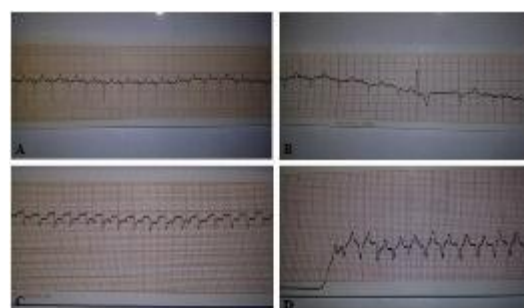
ECG	LDH (IU/L)	CPK (IU/L)	CK-MB (IU/L)
Normal ECG	$1030 \pm 749^a$	$58 \pm 23.5^a$	$282.5 \pm 220^a$
Sinus tachycardia	$669.2 \pm 247^a$	$232.4 \pm 62.5^b$	$151.2 \pm 72.5^a$
PVC	$635 \pm 235^a$	$702.2 \pm 352^b$	$84.6 \pm 20.8^a$
VT	$2813 \pm 523^b$	$243 \pm 55.8^b$	$546.5 \pm 192^b$

Unlike superscript letters (a, b) in each column denote significant difference among the data ( $P < 0.05$ ). PVC, premature ventricular complex; VT, ventricular tachycardia.

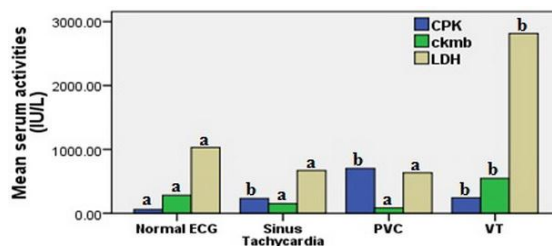
**Table 3.** Pearson correlations among the serum biochemical parameters in the affected lambs with FMD.

		LDH	CPK	CK-MB
LDH	Pearson Correlation	1	.370*	.836**
	Sig. (2-tailed)		.034	.000
CPK	Pearson Correlation	.370*	1	.238
	Sig. (2-tailed)	.034		.182
CK-MB	Pearson Correlation	.836**	.238	1
	Sig. (2-tailed)	.000	.182	

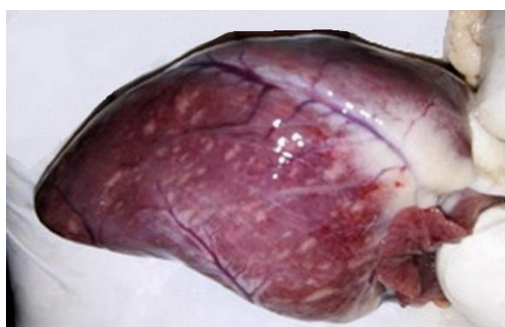
\*. Correlation is significant at the 0.05 level (2-tailed). \*\*. Correlation is significant at the 0.01 level (2-tailed). CK-MB, creatine kinase-myocardial band; CPK, creatine phosphokinase; LDH, lactate dehydrogenase.



**Figure 1.** A, Sinus tachycardis in a lamb with FMD (heart rate = 168 beats/min), Speed paper 25 mm/sec. B, Premature Ventricular Complex in a lamb with FMD. Note the increased QRS duration with opposite polarity to the normal complex and large T wave amplitude which follows by a pause. Speed paper 50 mm/sec. C, Ventricular Tachycardia with S-T depression in a lamb with FMD (ventricular rate = 270/min). Widened QRS complexes with regular R-R intervals (Garmel, 2008) without P waves and the T waves opposite to the QRS complexes (Constable et al., 2017). Speed paper 25 mm/sec. D, Ventricular Tachycardia in a lamb with FMD (ventricular rate = 270/min). Note the stable and regular QRS complexes without P waves and the T waves opposite to the QRS complexes. Speed paper 25 mm/sec.



**Figure 2.** Comparison of serum enzyme activities of LDH, CPK and CK-MB in patient lambs based on type of dysrhythmias. Unlike superscript letters (a, b) on the same colored bars show significant difference between groups ( $P < 0.05$ ). One-way ANOVA with supplementary Dunnett T3 tests for multiple comparing were used. **PVC**, premature ventricular complex; **VT**, ventricular tachycardia.



**Figure 3.** The heart of a dead lamb due to FMD with the multiple Tiger-heart lesions on the epicardium.

### Discussion

In this research, the mean serum CK-MB activity of the 32 affected lambs ( $170 \pm 36$  IU/L) was significantly higher than that in healthy lambs ( $51.4 \pm 4.7$ ). Limited sources have reported normal ranges of serum CK-MB activity in sheep, for example:  $41 \pm 4.7$  IU/L in less than 6-months of age lambs (Basbugan *et al.* 2010) and 50.7 IU/L in ewes (de Souza *et al.* 2019), which were in agreement with the present study. A few studies have shown a significant increase of serum CK-MB level in the sheep with myocardial damage due to FMD (Aslani *et al.* 2013; El Beskawy *et al.* 2016). In the human medicine, CK-MB has been known that has a high specificity for myocardial

impairment and it is also correlated with myocardial infarct size (Costa *et al.* 2008).

According to our results, insignificant elevation of serum LDH and CPK activities were identified in the affected lambs than the healthy ones. Based on a previous study on the lambs with FMD in Egypt, the serum activities of CPK and LDH were higher in affected than healthy lambs which was not in line with our results (El Beskawy *et al.* 2016). The reasons for this discrepancy may be attributed to the different strains of FMD virus or difference in the numbers of examined lambs in the El Beskawy *et al.* study (SAT 2 strain,  $n = 15$ ) against our study (O and A strain,  $n = 33$ ). FMD is dominantly associated with necrotic myocarditis in neonates (Constable *et al.* 2017), thus it is expected to increase serum CPK and LDH activities. The lack of significant changes in CPK and LDH levels in this study may be related to the age of the animal (less than 2-months old in the current research) compared with the study of El Beskawy *et al.* (2016) who studied on 2 to 6-months old lambs.

Therefore, it is possible that the patient lambs have died due to myocarditis before onset of skeletal muscle myositis.

The ECG findings in the affected lambs included 37.5% ST, 20.8% PVC, 25% VT and 16.7% normal rhythms. In a limited study on 5 lambs with FMD, some dysrhythmias such as atrial fibrillation, sinus tachycardia and ventricular fibrillation have been reported (Aslani *et al.* 2013), whose results are not in agreement with the present research.

Regarding the relationship between various types of dysrhythmias with some serum

skeletal muscle and cardiac biomarkers, Fakoor *et al.*'s (2017) study showed that myocarditis-related to subclinical selenium deficiency in Markhoz kid goats has been associated with increased serum CK-MB levels and dysrhythmias such as atrial and ventricular premature complexes. In the current study, based on type of dysrhythmias, the patient lambs were divided into several groups and their serum CK-MB levels were compared. The patient lambs with VT had a significant increase in serum CK-MB activity ( $546.5 \pm 192$  IU/L) compared to the control and the patient lambs with the other dysrhythmias. Also, higher serum CK-MB level in the lambs with VT than the patient lambs with PVC and sinus tachycardia seems to indicate that the VT is associated with more severe myocardial injury. A pathological elevated serum CK-MB level related to VT has been reported in cattle with FMD that supports the results of the current study (Nikvand *et al.* 2019). Further, the insignificant higher levels of CK-MB in the patient lambs with normal sinus rhythm compared to those with PVC and ST indicate that the serum CK-MB activity might increase before the ECG changes. Except for a previous study on cattle with FMD (Nikvand *et al.* 2019), no veterinary research was found to compare the amount of CK-MB and the other cardiac injury biomarkers based on different types of dysrhythmia. In this study, all the lambs with FMD died and had tiger-heart lesion in the necropsy. Given that the time intervals from blood sampling and ECG preparation to the death were not equal in the patient lambs, it is understood that the lambs which were sampled nearer to death, had more severe myocardial

damage, higher serum CK-MB and subsequently more malignant dysrhythmia (VT).

It seems that unlike other animals, CK-MB appears to be high in value for detecting cardiac injury in sheep. Regarding highest CK-MB level in the lambs with VT, it seems VT is associated with more severe cardiac lesion compared to the other arrhythmias in FMD lambs. Regarding no significant levels of the serum CPK and LDH levels in the patient lambs, it may be concluded that the FMD virus is not associated with the skeletal muscle myositis in the lambs under two months old.

#### **Conflict of Interest**

The authors declare that they have no conflict of interest.

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#### **Ethic approval**

This study was approved with attention to topics of research and animal welfare by local reviewers committee in faculty of veterinary medicine, Shahid Chamran University of Ahvaz, Iran (Approval No. 96/9/5, 1270).

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