

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Electrocardiogram and serum cardiac biomarkers changes in FMD in cattle

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Received: 19.10.2018	•	Accepted/Published Online: 04.02.2019	•	Final Version: 04.04.2019	
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Abstract: This study was designed to evaluate serum and electrocardiographic (ECG) changes in cattle with foot-and-mouth disease (FMD). Thirty-two cows with clinical signs of FMD and 13 healthy cows were randomly enrolled. After blood sampling, ECG was performed on 23 of the 32 ill cows. Serum circulatory troponin I (cTnI), CPK, LDH, and CK-MB activities were determined in the patient and control groups. Arrhythmias including ventricular tachycardia (VT), sinus tachycardia (ST), atrial fibrillation (AF), and premature ventricular complex (PVC) were seen in 4, 4, 2, and 1 of the patients, respectively. The patients had higher cTnI levels than the control group (P = 0.05). A significant rise in cTnI was observed in patient cows with VT (0.86 ± 0.33 ; P < 0.01) and AF (0.22 ± 0.01 ng/mL; P < 0.05) in comparison with the control group (0.14 ± 0.04 ng/mL). The most significant finding of this study was the death of all of the cows with VT. It appears that the cause of death in the cows with VT was cardiac impairment, which was marked by serum cTnI elevation. It can be concluded that cows with VT have a grave prognosis. In some FMD cases with an increase in cTnI, AF was also observed, but with a fairly good prognosis.

Key words: Cattle, cTnI, electrocardiography, ventricular tachycardia

1. Introduction

Seven immunologically distinct serotypes of viruses (O, A, C, Asia 1, South African Territories (SAT) 1, SAT 2, and SAT 3) that belong to the foot-and-mouth disease virus (genus Aphthovirus, family Picornaviridae) cause footand-mouth disease (FMD) (1). In recent years, several outbreaks of FMD have taken place in Iran. In this regard, a remarkable level of mortality, especially in calves over 6 months of age and occasionally adults, was one of the major concerns of farmers. While some attention has been paid to the different aspects of myocarditis due to FMD in lambs (2), insufficient published information has been documented regarding FMD-related myocarditis in calves and especially adult cattle (3). Aktas et al. (4) showed that among calves affected with FMD, only those less than 2 months old died due to FMD-related myocarditis. In various studies, serum activities of LDH, AST, CPK, and CK-MB have been used as biomarkers to detect myocardial injury in farm animals, but they did not show a good predictive value (4,5). Serum concentration of cTnI provides an excellent and persistent cardiac biomarker in large animals (1). Troponins play a role as regulatory proteins in the contraction of skeletal and cardiac muscle tissue (6). The troponin complex includes 3 distinct forms: troponins I, T, and C. Troponin C has limited validity

for diagnosis of heart disease (6). Because of the greater myocardial selectivity and qualitative point-of-care devices that are widely available for the on-farm detection of myocardial injury, cTnI is preferred to type T (1).

Apart from FMD, there are also a few studies on ECG changes and the identification of various types of myocardial damage-related dysrhythmias in different veterinary pathological situations (7,8). Considering the limited research in this regard, except for one study in which ECG was performed on 5 lambs with FMD (2), and also due to an inadequate understanding of FMDrelated myocardial injury in cattle, the present research was designed to evaluate the serum biomarkers and ECG evidence of cardiac impairment in cattle with FMD.

2. Materials and methods

2.1. Ethical approval

This study was performed conforming to the requirements of the ethical committee of the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran (Approval No. EE/97.24.3.49696/scu.ac.ir).

2.2. Animals

This case-control study was carried out on 13 healthy cows without FMD (control group) and 32 indigenous cattle

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with FMD that had been infected during an outbreak of the disease from January to June 2016 in Khuzestan Province, Iran.

During several visits to the clinic of the veterinary teaching hospital of Shahid Chamran University of Ahvaz and to rural areas of the suburbs of Ahvaz, 32 indigenous cattle with FMD from 7 herds were selected in a simple random sampling model. All of the patient cows had vesicular lesions on the muzzle, buccal, and nasal mucous membranes with saliva drooling; they were also febrile and lame, with vesicles in the clefts and on the coronets. Signs of tachypnea, severe tachycardia (120-230 beats/min), and undistinguishable irregular cardiac rhythms were noted in auscultation in the majority of the patients. According to the monthly reports on the global FMD situation by the Food and Agriculture Organization and reports from the Iran Veterinary Organization, the FMD virus type O (genotype O/ME-SA/Pan Asia) was confirmed via antigen detection ELISA and VP1 sequencing as a causative agent in the affected cattle from this outbreak in Khuzestan Province.

The patient cows ranged in age from 6 months to 8 years. The age distribution in the 23 cows that underwent electrocardiography is presented in Table 1. Two months after the outbreak had subsided in the region, 13 clinically healthy cattle with normal rhythms and rates of auscultation and without a history of FMD in their herds were selected as the control group.

2.3. Blood sampling

A blood sample of 10 mL was collected from the jugular vein of each animal and transferred to the laboratory within 1 h. The samples were centrifuged at 2500 rpm for 10 min and the isolated sera were transmitted into microtubes and frozen at -20 °C for 10 to 15 days before testing.

2.4. ECG preparation

In this step, 10 min after blood sampling and rest for the patients in the clinic or at the farm, an electrocardiogram was prepared using the bipolar base-apex lead via a singlechannel ECG machine (Fukuda Denshi Co., Tokyo, Japan) on 23 of the 32 patients (some owners refused consent). During the ECG procedure, the patients were standing and their movement was restricted. The ECGs were recorded for at least 1 to 3 min with a paper speed of 25 mm/s or, if needed, 50 mm/s. The device was calibrated at 10 mm equal to 1 mV. Prior to using the alligator clip electrodes, their attachment sites (positive: skin over the left fifth intercostal space; negative: skin on the lower third of the left jugular furrow; neutral: on the flank; earth electrode: a metal object attached to the ground) were degreased using ethanol. The ECGs were interpreted in terms of dysrhythmias and any alterations in heart rate and rhythm.

The criteria used to diagnose some dysrhythmias in the electrocardiograms were as follows:

- Diagnostic criteria for VT: 1) stable and regular QRS complexes; 2) excess of 120 complexes/min; 3) P waves lost within complexes.

- Diagnostic criteria for AF: 1) irregular in the duration of R-R intervals and deflection of QRS complexes; 2) P waves lost within complexes; 3) narrow QRS complexes.

- Diagnostic criteria for PVC: 1) early QRS complex without P wave; 2) opposite polarity to the normal complexes; 3) increased QRS duration and large T wave amplitude that ended in a pause (1).

- Heart rate of 60–80 bpm was considered normal for the cows; a higher value was assumed to be sinus tachycardia (1).

After a sampling from each herd, therapeutic and preventive advice was given according to professional ethics. In routine clinical practice, all of the patients similarly received 10% oxytetracycline, flunixin meglumine, and fluid therapy. Antiarrhythmic therapy with 2% lidocaine (Pharmaceutical Co., Rasht, Iran) was only prescribed for the patients with sustained VT. In line with the aims of the study, in the subsequent follow-ups, the fates of the studied animals were also recorded via telephone conversation with owners.

2.5. Serum myocardial biomarkers

Serum cTnI level was determined using an ELISA kit (Monobind Inc., Lake Forest, CA, USA) with a detection range of 0–75 ng/mL. The activities of CPK, LDH, and CK-MB were assessed via the colorimetric assay method and biochemistry kits (Parsazemon Co., Tehran, Iran). All samples were batch-analyzed.

2.6. Statistics

In order to compare the mean of serum analyses between the patient and control groups, the Mann–Whitney test was used. One-way ANOVA with a Dunnett T3 post hoc test was also performed to compare the data of patients with different dysrhythmias. To determine the relationship between age and mortality with the type of dysrhythmias, Fisher's exact test was used. Additionally, Pearson correlations were calculated between different variables. The level of significance was set at P < 0.05. The data were analyzed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

In this study, 23 of the 32 animals with FMD underwent electrocardiography. The ECGs were interpreted such that ST, sustained VT, AF, PVC, and normal sinus rhythm were recorded in 4 (17.4%), 4 (17.4%), 2 (8.7%), 1 (4.3%), and 12 (52.17%) of the patients, respectively (Figure 1). ECG findings and mortality rate according to age distribution in the 23 patients are presented in Table 1. VT occurred at a significantly higher rate in the 6–12 months of age group compared to the oldest patients (P < 0.05). In one of the patient cows, which simultaneously had AF and PVC on

ECG	6–12 months	1-2 years old	>2 years old	Death frequency
Norm ECG	2	4	6	0
ST	1	2	1	0
PVC	0	0	1	0
AF	0	0	2	0
VT	3	0	1	4

Table 1. ECG findings and death frequency according to age distribution in 23 of 32 patient cattle.

The data indicate the number of patient cattle. AF, Atrial fibrillation; Norm ECG, normal electrocardiography; PVC, premature ventricular complex; ST, sinus tachycardia; VT, ventricular tachycardia.



Figure 1. A) Atrial fibrillation with premature ventricular complex (eleventh complex) in an adult cow with FMD (ventricular rate = 180 beats/min). Note the irregular patterns of R-R intervals and narrow QRS complexes without P waves. Paper speed: 25 mm/s. B) Ventricular tachycardia with S-T elevation in an 8-month-old calf with FMD (ventricular rate = 210 beats/min). Note the stable and regular QRS complexes without P waves and the T waves opposite the QRS complexes. Paper speed: 25 mm/s. C) Ventricular tachycardia in a cow with FMD (ventricular rate = 240 beats/min). Note the stable and regular QRS complexes and the T waves opposite the QRS complexes. Paper speed: 25 mm/s. C) Ventricular tachycardia in a cow with FMD (ventricular rate = 240 beats/min). Note the stable and regular QRS complexes without P waves and the T waves opposite the QRS complexes. Paper speed: 25 mm/s. D) Ventricular tachycardia in a cow with FMD (ventricular rate = 240 beats/min). This is a continuation of Figure C, which was prepared at a speed of 50 mm/s.

ECG (Figure 1A), AF disappeared within 2 months after recovery, but PVC remained. S-T (S-T segment) elevation was also observed in one of the patients with sustained VT (Figure 1B). ECGs of the cattle with VT were performed with stable and regular QRS complexes without P waves at paper speeds of 25 (Figure 1C) and 50 (Figure 1D) mm/s. All cows of the control group had normal cardiac rhythms and rates in auscultation.

Serum cTnI and other pertinent biomarkers in the patient and healthy cattle groups are listed in Table 2.

The mean serum cTnI of the 32 patients and 13 healthy cows was 0.21 ± 0.08 (range: 0.04–1.80) and 0.14 ± 0.04 ng/mL (range: 0.1–0.16), respectively (P < 0.05). Unlike CPK, serum CK-MB and LDH activities were significantly higher in the patients than in the control group (Table 2).

Serum cTnI level and the enzyme activities of CK-MB, CPK, and LDH in the patient cows with VT, AF, ST, and PVC are presented in Table 3. A significant increase in cTnI was observed in the patient cows with VT (0.86 \pm 0.33 ng/mL) and AF (0.22 \pm 0.01 ng/mL) compared to the controls $(0.14 \pm 0.04 \text{ ng/mL})$ (P < 0.05) (Table 3; Figures 2 and 3). The patients with PVC (0.14 ng/mL) and ST (0.12 \pm 0.01 ng/mL) did not have higher levels of cTnI than the control cattle. Except for the patients with VT, the patient group had no significant difference with concern to CK-MB activities compared to the control group. The Pearson correlation coefficient showed positive and significant correlations between serum cTnI with serum activities of CPK (P = 0.487, sig. = 0.005) and CK-MB (P = 0.494, sig. = 0.004) enzymes. The means (\pm SE) of heart rate in the patient cows with ST, AF, and VT were 132.5 \pm 12.5, 180 \pm 28, and 182 \pm 46 beats/min, respectively. No significant correlation was observed between heart rate and cTnI level

in the patients (P > 0.05). Antiarrhythmic therapy with lidocaine (IV bolus at 0.5 mg/kg BW every 5 min for 3 treatments) was done for only 2 of 4 patients with VT. The therapy, however, could not return the patients to normal sinus rhythm. All of the cows with VT died, while none of the cows with the other dysrhythmias died.

4. Discussion

In the recent outbreaks of FMD in Iran, the authors observed that a high fatality rate occurred in mature cattle and calves older than 6 months of age. Given the severe irregularity in heart rate and rhythm on clinical auscultation and unexpected deaths in the cattle and calves, we decided to investigate the possible role of myocardial impairment in the deaths of these animals. To evaluate the patients' cardiac damage, serum biomarkers of cardiac injury, including cTnI, LDH, CPK, and CK-MB, and their relationship with ECG changes were considered. In this study, the dysrhythmias of ST, sustained VT, AF, and PVC, as well as normal rhythm, were recorded in 4 (17.4%), 4 (17.4%), 2 (8.7%), 1 (4.3%), and 12 cattle (52.17%) out of 23 patient cows, respectively. To confirm the diagnosis of VT, it should be noted that is difficult to

Table 2. Serum biomarkers (mean \pm SE) of patient and control cows.

Animal groups	cTnI (ng/mL)	LDH (IU/L)	CPK (IU/L)	CK-MB (IU/L)
Patient cows (32)	0.21 ± 0.08	514.7 ± 48.6	262.3 ± 56.4	53.6 ± 4.1
Control cows (13)	0.14 ± 0.04	347.9 ± 37.6	157.8 ± 37.1	36.4 ± 7.7
Sig. (Mann–Whitney)	0.05*	0.017*	0.67	0.006*

*Difference is significant at the >0.05 level (2-tailed). CK-MB, Creatine kinasemyocardial band; CPK, creatine phosphokinase; cTnI, circulatory troponin I; LDH, lactate dehydrogenase.

Table 3. Comparison of serum parameters (mean \pm SE) based on ECG findings in 23 of 32 patient cattle.

ECG	Troponin (ng/mL)	LDH (IU/L)	CPK (IU/L)	CK-MB (IU/L)
Norm ECG $(n = 12)$	$0.09\pm0.006^{\mathrm{a}}$	431 ± 34^{a}	155 ± 63^{a}	41.7 ± 3.1^{a}
ST (n = 4)	0.12 ± 0.01^{a}	486 ± 83^{a}	374 ± 196^{b}	49.7 ± 9^{a}
PVC (n = 1)	0.14*	222*	39*	30*
AF (n = 2)	$0.22\pm0.01^{\mathrm{b}}$	625 ± 323^{a}	626 ± 455^{b}	67.5 ± 55.8^{a}
VT (n = 4)	$0.86 \pm 0.33^{\circ}$	$949 \pm 183^{\mathrm{b}}$	733 ± 122 ^b	$89.7\pm5.4^{\rm b}$

*The numbers refer to 1 cow and were not included in the variance analysis. Different superscript letters in each column (a, b, c) denote significant differences between groups (P < 0.05). AF, Atrial fibrillation; Norm ECG, normal electrocardiography; PVC, premature ventricular complex; ST, sinus tachycardia; VT, ventricular tachycardia.



Figure 2. Comparison of cTnI concentrations in cattle with FMD based on the type of dysrhythmias. Different letters (a, b, c) on the bars show a significant difference between groups, b (P < 0.01), c (P < 0.05). One-way ANOVA with complementary Dunnett T3 tests was used for multiple comparing. AF, Atrial fibrillation; Normal ECG, normal electrocardiography; PVC, premature ventricular complex; ST, sinus tachycardia; VT, ventricular tachycardia.



Figure 3. Comparison of serum enzyme activities of LDH, CPK, and CK-MB in patient cows based on the type of dysrhythmias. Different letters (a, b) on same-colored bars show a significant difference between groups (P < 0.05). One-way ANOVA with complementary Dunnett T3 tests was used for multiple comparing. AF, Atrial fibrillation; Normal ECG, normal electrocardiography; PVC, premature ventricular complex; ST, sinus tachycardia; VT, ventricular tachycardia.

differentiate VT from supraventricular tachycardia. For 2 reasons, it was concluded that the observed arrhythmias in 4 patients were VT. First, supraventricular arrhythmias are not fundamentally associated with bad outcomes, while ventricular arrhythmias are (1). Second, supraventricular tachycardia is accompanied by inverse P waves and sometimes may be buried in the QRS complex and not seen (9), whereas in the 4 patients with sustained VT, the

P waves were not seen even with increased paper speed (from 25 to 50 mm/s).

A significant increase in cTnI was observed in the cows with VT and AF compared to the control group. The AF and VT arrhythmias are not physiologic in cattle (1) and are thought to occur in the context of myocardial damage.

In a study on 5 lambs with FMD, dysrhythmias such as ventricular premature beats, sinus tachycardia, and ventricular fibrillation, together with increased serum CPK and LDH activities, and a higher level of cTnI, were observed (2). There are few studies on ECG changes and the identification of various types of myocarditisrelated dysrhythmias in different pathological veterinary situations (7,8,10). In a previous study on low-production cattle, a meaningful correlation of higher serum cTnI and CK-MB with sinus bradycardia, ST, AF, and PVC was observed, which suggests myocardial damage; this result is consistent with our findings (8). Despite the high levels of cTnI and CK-MB in cattle with theileriosis, no pathologic dysrhythmias were detected in them (7). The results of that study do not agree with those of the current study. This disparity can be attributed to the lesser severity of cardiomyopathy in theileriosis versus that of FMD in cattle. In the present study, the patients with PVC and ST had no greater levels of cTnI and CK-MB than the control group. It has been accepted that ST is a physiological arrhythmia in cattle (1) and would not be expected to be associated with high serum cTnI levels.

Normal serum cTnI value in farm animals has not been well described and various sources have reported varying amounts of it in healthy cattle, e.g., <0.02 (11), <0.04 (1), <0.08 (12), and 0.18 (13) ng/mL. Variations are expected in measuring cTnI concentrations when samples are evaluated with different immunoassays (14). The cTnI level has recently been used for determination of myocardial cell damage in different pathologic conditions such as FMD in lambs (15), traumatic reticuloperitonitis (16), endotoxemia (17), monensin toxicosis (12), theileriosis (7), bovine respiratory disease (18) in calves and cattle, and downer cow syndrome (19). In our study, the serum cTnI concentration of healthy control cow was 0.14 ± 0.04 ng/mL, which is close to and consistent with the findings of Başbuğan et al. (13), but does not agree with findings from other studies (1,12,14). In this way, we concluded that 9 (28.1%) of the 32 patient cows with a cTnI concentration higher than 0.14 ng/mL may have been impaired by myocardial damage resulting from FMD. The ECG readings of these 9 patients revealed that 4, 2, and 1 of them had VT, AF, and normal ECG, respectively. The 2 remaining cattle did not undergo electrocardiography. Mean (±SE) serum cTnI concentrations of 4 patients with sustained VT (0.86 \pm 0.33) and 2 patients with AF

 $(0.22 \pm 0.01 \text{ ng/mL})$ showed that both dysrhythmias may be indicative of cardiac impairment in cattle with FMD. Serum levels of cTnI were determined in 4 patients with sustained VT to be 0.25, 0.51, 1.8, and 0.87 ng/mL, which are significantly higher than the normal values of the other studies (12–14). Considering the higher serum cTnI level in patients with AF compared to the control group, it seems that this dysrhythmia is in the context of FMD-related myocardial injury but is not accompanied by a bad outcome in patients. Outcome or survival may be related to the serum cTnI level as well as the type of dysrhythmia. Given the lower cTnI level in the patients with AF compared to the patients with VT, it seems that the cows with AF had milder cardiac injury in comparison with cows with VT.

All of the patients with VT died, while none of those with other dysrhythmias were lost. Based on the significant increase in serum CK-MB activity of patients with VT compared to the patients with the other dysrhythmias, it can be concluded that CK-MB is a good indicator of myocardial cell damage in ill cows with VT. The results of an experimentally induced endotoxemia study on calves showed a low sensitivity of CK-MB for detecting myocardial cell damage that did not concur with our results (17). The reason for this inconsistency may be the lower degree of myocardial injury in endotoxemia versus FMD. In this regard, some efforts have been made to treat some arrhythmias in horses (20) and cows (21). Ruminants with AF are not usually treated with specific

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antiarrhythmic agents; the heart will usually revert to sinus rhythm following the correction of the underlying disorder. However, IV quinidine has successfully been used in the treatment of 7 of 9 cows with AF (1). Lidocaine as a first-choice therapy can be used for treating VT (22). In our study, lidocaine was not able to reverse sustained VT to normal sinus rhythm. Magnesium sulfate has also been suggested to treat VT during milk fever therapy (23). However, investigating the efficacy of magnesium or quinidine sulfate as antiarrhythmic drugs for treating VT in cattle with FMD is recommended.

In conclusion, death due to FMD in cattle seems to be due to cardiac impairment. Regarding the higher levels of serum cTnI and CK-MB in ill cattle with sustained VT, this arrhythmia seems to be associated with more severe myocardial injury and has a serious prognosis. Although AF may be seen in FMD-related myocarditis, it was not accompanied by a bad outcome in the patients. As a guideline for future studies, it is recommended that the efficacy of magnesium or quinidine sulfate as antiarrhythmic agents for treating VT in cattle with FMD be investigated within the endemic area.

Acknowledgments

The authors would like to thank the staff at the Large Animal Internal Ward for helping them accomplish this research. This work was funded by a grant from Shahid Chamran University of Ahvaz Research Council (derived from Research Project No. 1270).

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