Kocatepe Veterinary Journal

Kocatepe Vet J (2019) 12(4):396-399

DOI: 10.30607/kvj.599089

RESEARCH ARTICLE

Determination of D-Dimer Levels in Calves with Cryptosporidiosis

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ABSTRACT

The aim of the present study was emphasized to estimation of D-dimer levels among calves with cryptosporodiosis. The study was conducted on 1-3 weeks old, 11 Holstein calves with *Cryptosporidiosis* (study group) and on 1-3 weeks old ten healthy Holstein calves (control group). Diagnosis of Cryptosporidiosis made by rapid test kits. Blood samples were taken from each animal, by puncture of the jugular vein and were collected into plain tubes without anticoagulant. D-dimer concentrations were detected by fluorescent immunoassay techniques in both study and control group calves. As a result, D-dimer values were significantly increased in infected calves when compared with the control group animals.

Keywords: Calf, Cryptosporidiosis, D-Dimer

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Kriptosporodiazisli Buzağılarda D-Dimer Seviyelerinin Belirlenmesi

ÖZ

Bu araştırmada kriptosporodiyazisli buzağılarda kan D-dimer seviyelerinin belirlenmesi amaçlanmış olup, çalışmanın materyalini 1-3 haftalık, hızlı test kitleri ile Kriptosporidiyazis belirlenen 11 Holstein buzağı (çalışma grubu) ve 1-3 haftalık on sağlıklı Holstein buzağı (kontrol grubu) oluşturmuştur. Her buzağının, *v. jugularis*'lerinden alınan kan örnekleri antikoagülansız tüplere toplanmış, D-dimer konsantrasyonları, hem çalışma hem de kontrol grubu buzağılarda floresan immunoassey tekniği ile belirlenmiştir. Sonuç olarak, D-dimer seviyeleri, kontrol grubu hayvanlara kıyasla enfekte buzağılarda önemli ölçüde yüksek saptanmıştır.

Anahtar Kelimeler: Buzağı, Kriptosporidiazis, D-Dimer

To cite this article: Cıngı C.Ç. Kabu M. Erdoğan H. Haydardedeoğlu A.E. Ural K. Determination of D-Dimer Levels in Calves with Cryptosporidiosis. Kocatepe Vet J. (2019) 12(4):396-399.

Submission: 31.07.2019 **Accepted**: 02.10.2019 **Published Online**: 09.10.2019
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INTRODUCTION

Cryptosporidium parvum infection is very common in young ruminants and can also be detected in many mammals. The infection occurs frequently in calves and can be detected at the earliest five days. 70% of cases occur in calves within 1-3 weeks. The main symptom is diarrhea and occurs at the age of 5-15 days. Transmission occurs directly by contact between calves. Stool excreted sporozoites and infected oocytes are the source of infection. The agent may also be spread indirectly through fomite or human transfer. *Cryptosporidium spp.* it may be infectious when sporulated in the host cells and passed into the feces. The feces are yellow in color and watery and contain mucous. (Kahn 2010).

D-dimer, a well known break down or degradation product of cross-linked fibrin, might be elevated due to clot formation/ fibrinolysis (Freyburger et al. 1998, Goldstein et al. 2001). Elevated circulatory D-dimer concentrations in relationship with DIC, thromboembolic disease, coagulative disorders thromboembolic disease and other relevant diseases (Goldstein et al. 2001, Nelson and Andreasen 2003, Griffin et al. 2003, Stokol 2003, Dewhurst et al. 2008). An elevated D-dimer value in plasma is a marker of a prothrombotic state, and its measurement might be helpful for prediction or prevention of thrombotic diseases (Marcucci et al. 2000). Therefore, in an attempt to investigate whether calves with a parasitic disease, as was the case in this study, could even exist probable prothrombotic condition, plasma D-dimer vales were analyzed in a subset of calves with naturally occuring cryptosporodiosis, compared to those of a healthy control calves. Only few (absolutely lacking) data is available on the occurrence of precoagulative status in calves with cryptosporodiosis Therefore, the objective of the present work was to study D-dimer levels among calves with cryptosporodiosis.

MATERIALS and METHOD

The study was conducted on 1-3 weeks old, 11 Holstein calves with Cryptosporidiosis (study group) and on 1-3 weeks old ten healthy Holstein calves (control group). Diagnosis of *Cryptosporidium* made by rapid test kits from Bovid-5 Ag Test Kit, (BionoteInc.,Korea) Blood samples were taken from each animal, by puncture of the jugular vein and were collected into plain tubes without anticoagulant. D-dimer concentrations were detected by use of the Point-of-Care fluorescent immunoassay. The present author's clinic utilizes the Finecare FIA meter (FIAm) (Fluorescence Immunoassay Rapid Quantitative Test, Guangzhou Wondfo Biotech Co., Ltd., Atasan Ata teknik Ltd. Sti, Turkey) which is automated. The use of FIAm D-dimer analyzer has been validated for the first time in veterinary practice in our clinic in August 2014. In an attempt to determine whether the results obtained for D-dimer using the latter analyzer differed significantly and to quantify the limits of agreement between the results of immuno turbidometric assay and this analyzer, four of the samples were randomly selected and then were sent out for a private commercial laboratory for measurement and comparatively determined on same samples, which gave same results.

The FIAm requires 10 µl of undiluted serum and utilisesan Fluorescence Immunoassay methodology. A human anti-D-dimer monoclonal antibody was impregnated into the well on a supplied test cartridge by the manufacturer. Calves sera sample is then added; along with buffer solution, then were shaked for 30 seconds. Within this mixture 75 µl was taken, then were put on to the reading stribes, forwarded to the analyzer. The appropriate time for reading was 180 seconds [involving incubation]. FIAm D-dimer analyzer has a detectable range between 0.1-10 mg/L. The method was adapted within the manufacturer directions. Elevated D-dimer levels was set as (>0.1 g/ml).

Ethics committee approval was not enrolled in the present study, but it has been denoted that there is no need for approval of the ethics committee in non-experimental clinical veterinary practices index of Article 2 (b) of the Regulation on Working Procedures and Principles of Animal Experiments published in the Official Newspaper dated 15.02.2014 with no 28914 as was expressed. In the present study sera samples were withdrawn from sick animals, in an attempt to control their health status since it was understood that there was informed consent form in the study, ethics committee approval was not required for this study

Statistical analysis

D-dimer data were shown as mean and standard deviations (mean ± SD). Data were checked for homogeneity and Mann-Whitney U test was performed to analyze the significance of alterations between Control and Infected groups. SPSS (22.0, IBM) program was used in statistical tests and $p<0.05$ was considered significant.

RESULTS

D-dimer values were significantly increased in infected calves compared with the control animals ($p < 0.05$) (Table 1).

Table 1. D-Dimer Levels In Control and Study Groups.

Data	Groups		p value
	Control n=10	Infected n=11	
D-dimer (mg/L)	0.09±0 (0.09)	2.07±2.22 (0.09-6.2)	**0.001**

DISCUSSION and CONCLUSION

Increased D-dimer levels might be dedicated to excessive amount of fibrinogen which converted to fibrin inside blood vessels due to fibrinolytic degradation. Given D-dimer as a fibrin related end product, the vast majority of diseases due to procoagulant stimuli, could be addicted to acute or chronic thrombotic and embolic changes (Mammen 2000). In the present study D-dimer levels (mean ±standart deviation) in healthy control vs. diseased calves [0.09±0 vs. 2.07±2.22) indicated elevated circulatory reaction. It was suggested that high levels of D-dimer that detected in calves with cryptosporidium are indicative of seriously impaired haemostasis and the development of secondary fibrinolysis associated with DIC. Increased D-dimer concentrations are indicative of secondary activation of the fibrinolysis system preceded by clotting activation and thrombin production, which are typical DIC symptoms (Matyszczak et al. 2008). D-dimer measurement is commercially easily available and reasonably priced, thus represents a probably ideal candidate marker for several diseases, at least for the calves enrolled herein. The present researchers could assume that D-dimer analysis may be useful for diagnosis of pre-coagulation status conditions, indicating that inflammatory conditions and coagulation might be in relatiosnship. This was also mentioned in a prior review article, in which concluded that coagulation and acute inflammation follow all types of tissue trauma. The underlying mechanisms of cryptosporodiosis would be underlined beneath (Pottmeyer et al 1986). In clinical practice the usage of D-dimer analysis for interpretation of caogulopathy and inflammatory response, is currently be coming routine, whereas available evidence showed that D-dimer levels may be decreased with anticogulation therapy (Couturaud et al, 2002) which might be the case in calves with cryptosporodiosis presenting high D-dimer values.

The underlying mechanisms for elevated D-imer leves in calves with cryptosporodiosis might be briefly discussed. Tool like receptor signalling over bovine and human epithelial cells existing within *C. parvum* infection resulted within pro-inflammatory cytokine, chemokines and antimicrobial peptide production, in which the latted respond induces protective immunity for combatting (Thomson et al. 2017). Besides elevated expression of the latter co-stimulatory molecules, mouse bone marrow-derived dendritic cells reacting to *C. parvum* existed pro-inflammatory cytokines including TNFα, IL-6 and IL-12 (Perez-Cordon et al, 2014). To those of TNF-alpha levels ($p<0.01$) were independently associated with elevated D-dimer levels in patients with non-metastatic lung cancer, suggesting that elevated concentrations of TNF-alpha could participate in activation of fibrinolysis (Guadagni et al. 2004). All those proposed mechanisms might be involved within the pathogenesis of existing iflammation and pre-coagulatory processing in cryptosporodiosis, in which to the present authors' knowledge this is the first study detecting D-dimer levels in calves with this infection. Obtained results should be taken into consideration, which would thus probably change treatment protocoles for the future with naturally occurring cryotosporidium infection among calves. The present authors have competing interests for further warranted researches.

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