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Assemblage Characterization of *Giardia duodenalis* and Comparison of the Pathogenicity of Intermittent Spreading Among Cattle

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Abstract

It was aimed with present study to determine the assemblage's characterization of *Giardia duodenalis* and to comparison of pathogenicity of intermittent spread agent with microscopic faecal examination and Nested PCR in cattle. Assemblages A and E were found to be major assemblage of *G. duodenalis* detected by targeting the amplication of β-giardin with nPCR. Furthermore, it was observed that 7/9 calves that were nPCR negative in the 1st month returned to positivity in the following months (100%). Negative microscopy and rapid test kit evaluations could be detected despite periodic nPCR positivity. In conclusion this study might be represented baseline date for following of faceal excreation period and importance of preventive treatment in cattle.

Keywords: Cattle, Giardiasis, Nested PCR.

INTRODUCTION

Giardia is a unicellular protozoan and causes diarrhea in many animal species including ruminants. (Adam, 2000). Transmission can occur through human-to-human or animal-to-human contact with contaminated water or food. (Rendtorff, 1978; Mark-Carew et al., 2010). *Giardia* has been studied extensively and has been found to be an important cause of zoonosis. (Craun, 1979; Farthing, 1992; Jephcott et al., 1986). In addition to the zoonotic importance of *G. duodenalis*, it causes serious economic losses due to the decrease in feed efficiency, growth retardation and diarrhea, thus increasing the awareness of Giardiasis in farm animals today. (O'Handley et al., 2003). Nowadays, the sometimes subclinical course and sensitive molecular detection of Giardiasis is becoming increasingly important. (Gultekin et al., 2017). Many clinical-pathological findings that occur; growth retardation, diarrhea, progressive weight loss, and sometimes death are associated with the proliferation of the agent. (Aloisio et al., 2006). Giardiasis caused by *G. intestinalis* should be considered as a zoonotic disease because it causes contamination in human and animal life systems. Today, there are 8 different assemblages (A-H) of *G. intestinalis* according to its molecular character. (Feng and Xiao, 2011). E assemblage, commonly known as 'hoofed assemblage', is used in calves. (O'Handley et al., 2000; Trout et al., 2005; Trout et al., 2007), in sheep and goats (Santín et al., 2007; Ruiz et al., 2008; Lim et al., 2013) Although it is the most common assemblage of *G. intestinalis*, studies related to assemblage A and B in both ruminant species have been reported. (Trout et al., 2005; Castro-Hermida et al., 2007; Trout et al., 2007; Lim et al., 2013). Species with the main genotypes A and B assemblage responsible for human infection have also been isolated from dogs, cats, livestock and wild animals. (Ayan et al., 2019). Today, there are many studies on the epidemiology of Giardiasis in ruminants. (Trout et al., 2006; Santín et al., 2007). In addition to causing economic losses with diarrhea, it is thought that determining the regional distribution of the agent due to its zoonotic character may be a pioneer for further studies. (Ayan et al., 2016).

In this study, it was aimed to determine the characterization of *G. duodenalis* in cattle and to compare the pathogenicity of the intermittent spreading agent with microscopic stool examination and Nested PCR.

MATERIALS AND METHODS

Collection of samples

Stool samples were manually taken from the rectum of each animal with disposable latex gloves and placed in stool containers. The sex and age of the animal were recorded for each sample collected. Afterwards, the samples were brought to the laboratory and stored at +4°C until analyzed.

Microscopic Examination

Microscopic examination was also examined under the microscope with the native-lugol method.

DNA extraction

DNA extraction was performed from all 9 samples using the GeneMATRIX Stool DNA Purification Kit according to the company protocol. The obtained DNAs were stored at -20°C until the next steps.

Nested PCR reaction

In the first step of Nested PCR, primers defined by Caccio et al. (2002) G7 F5'- AAGCCCGACGACCTCACCCG CAGTGC-3' forward and G759R 5'-GAGGCCGCCCTGGATCTTCGAGACGAC-3' reverse primers were used. In the second step of Nested PCR, amplification of the β-giardin gene region was performed using primers defined by Lalle et al. (2005) BG1F 5'-

GAACGAGATCGAGGTCCG-3' forward and BG2R 5'-CTCGACGAGTTCGTG TT-3' reverse. In both reactions, the protocol was applied according to Ayan et al. 2019. The reaction was performed on a Kyratec brand Gradient PCR, SuperCycler device. Then, 1.5% agarose gel was prepared and stained with RedSafe™ Nucleic Acid Staining Solution. Then, PCR products were run on agarose gel and images were obtained on the gel imaging device (Syngene bio imaging system). DNA sequence analysis of the beta-giardin gene region of each of the PCR positive samples was performed. Then, blasting was performed on the sequence analyzes in the gene bank (M36728 for sub-genotype A1, AY072723 for sub-genotype A2, AY072724 for sub-genotype A3, AY072725 for sub-genotype B1, AY072726 for sub-genotype B2, AY072727 for sub-genotype B3, AY072728 for sub-genotype B4, AY072729 for Assemblage E) accession numbers.

RESULTS

Microscopic Examination Results

Giardia spp. cysts were found in 1 sample in the (400x) examinations of the 9 stool samples examined under the microscope.

Nested PCR Results

Nested PCR was performed on all 9 samples. Specific bands of 511 bp were obtained in 2 of the samples. Assemblage A was detected in 1 of the positive samples and Assemblage E was detected in 1 of the positive samples.

As presented in Table 1, 7/9 calves that were nPCR negative in terms of Giardiasis in the first examinations (within the first month) turned positive in the following months (100%), and second month 6/9, third month 8/9, fourth month 7/9 positivity was detected with nPCR. Despite periodic nPCR positivity, microscopic (mic) and rapid test kit (rtK) evaluations were negative.

Table 1. Monthly follow-up of giardiasis in calves with different diagnostic methods.

Calve no	1. month			2. month			3. month			4. month		
	nPCR	**mic**	**rtK**	**PCR**	**Fs**	**Rdtk**	**PCR**	**Fs**	**Rdtk**	**PCR**	**Fs**	**Rdtk**
I	-	-	-	-	-	-	†	†	†	†	†	†
II	†	-	-	†	†	†	†	†	†	-	-	-
III	-	-	-	-	-	-	-	-	-	†	†	†
IV	-	-	-	-	-	-	†	†	-	†	†	†
V	†	†	†	†	†	†	†	†	†	-	-	-
VI	-	-	-	†	†	†	†	†	-	†	†	†
VII	-	-	-	†	†	†	†	†	†	†	†	†
VIII	-	-	-	†	†	†	†	†	†	†	†	†
IX	-	-	-	†	†	†	†	†	†	†	†	†

nPCR: nested PCR mic: microscopic evaluation rtK: evaluation with rapid test kit

DISCUSSION AND CONCLUSION

Giardia infections have an important place among the important parasitic diseases that can be detected all over the world. *Giardia* agents cause intestinal infections with extracellular involvement and can lead to chronic diarrhea, dehydration and malabsorption. (Allain et al., 2017; Gutierrez-Gutierrez et al., 2017). Different field studies have been carried out in our country on this subject (Alıç Ural et al., 2014; Alıç Ural et al., 2016; Ayan et al., 2016; Karademir et al., 2016; Alıç Ural et al., 2017; Ayan et al., 2017; Gültekin et al., 2017; Çamkerten et al., 2019). Gültekin et al. (2017) also detected the zoonotic potential A3 assemblage (lower genotype) with a high prevalence in calves of Aydın region. The researchers has publications on Giardiasis with different researchers at different times (Alıç Ural et al., 2016; Alıç Ural et al., 2017; Ayan et al., 2017, Ural et al., 2017; Ayan et al., 2018; Ayan et al., 2019; Çamkerten et al., 2019, Erdoğan et al., 2020). Since the lack of longitudinal studies was observed in this field, a planning was made for our country, and it was especially aimed to draw the attention of our field physicians. In this context, calves were followed for 4 months with a longitudinal (longitudinal, longitudinal) study, and analyzes were carried out within 3 different methods, taking into account intermittent cyst scattering, including calves with previously detected negativity. In other words, considering that the rapid test kits used in the study can detect 125 cysts per microliter and above, mic negativity can be understood despite nPCR positivity. Again, in Giardiasis, intermittent cyst scatter should be considered for diagnosis in field conditions, and patients or subclinical cases should be detected in the longitudinal period.

To determine the prevalence of *G. duodenalis* genotypes in cattle of different ages, faecal samples were collected from 30 calves from birth to 24 months at a dairy farm in Maryland. Fecal samples were subjected to density gradient centrifugation to remove debris and concentrate cysts. Samples were analyzed by immunofluorescence microscopy and polymerase chain reaction (PCR). All PCR positive samples were sequenced using the SSU-rRNA gene of *Giardia*. All 30 calves shed *G. duodenalis* cysts during the study. Of 990 samples, 312 were positive for *G. duodenalis* (31.5%). The highest prevalence of infection occurred at weeks 4 and 5, when 25 of the 30 calves shed cysts at these sampling times. Overall, weaned calves (<8 weeks old) exhibited the highest prevalence (60.8%), followed by weaned calves (3-12 months) (32.1%) and heifers (12-24 months). (11.4%). Sequence analysis of 312 PCR-positive samples revealed the presence of both A and E, *G. duodenalis* communities with cumulative prevalences of 70% and 100%, respectively. Community A was not detected in preweaning calves, but was detected in 6.9% and 4.7% of weaned calves and heifers, respectively. These data suggest that not only are calves infected simultaneously with Groups A and E, but also that zoonotic community A, *G. duodenalis* infections are more common than previously reported. Therefore, calves appear to be a more important reservoir for the human infectious *G. duodenalis* than previous data suggest. (Santin et al., 2009). In our study, however, Assemblage A and E were found to be major assemblages of *G. duodenalis*, whose β-giardin amplification was targeted by nPCR. In addition, it was observed that 7/9 calves that were nPCR negative in the 1st month turned positive (100%) in the following months and could be detected with negative microscopic and rapid test kit evaluations despite periodic nPCR positivity.

As a result, natural probiotic (Alıç Ural et al., 2020) or clinoptilolite (Alıç Ural and Ural, 2017) etc. feed additives and nutraceuticals, if possible, against *Giardia* disease existing in our country. Preventive treatment with clinoptilolite in ruminants with high risk of clinical manifestation of *Giardia* infestation have favorable effect of mitigation economic loss. It can be said that it is necessary to increase the studies on its use for anti-Giardial purposes.

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Conflict of Interest
The authors declare that they have no conflict of interest.

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