ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

DOI: 10.5336/vetsci.2022-92569

Leaky Gut and *Giardia duodenalis* Infection Associated Serum Zonulin Levels Among Calves: Randomized Clinical Study

Buzağılarda Sızıntılı Bağırsak ve *Giardia duodenalis* Enfeksiyonu ile İlişkili Serum Zonulin Seviyeleri: Randomize Klinik Çalışma

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ABSTRACT Objective: Giardiasis is the most common encountered parasitic disease of a wide variety of animal species and intestinal malabsorption, and diarrhea are long term outcomes of giardiasis. Given elevated gut permeability-within the moneclature synonym-leaky gut- has been suggested as a probable contander to inflammaging and gut dysbiosis, relatively very few data exist in ruminants. In the present study, therefore, field research prompted the present author to seek information on the relationship between a leaky gut and giardiasis (this subject was dept in analysis), through which are influencing growth performance (the special era of interest for her). **Material and Methods:** For this purpose, 11 calves in each group healthy control vs. giardia positive diarrheic were included in the study. Sera samples for zonulin levels were detected to those of giardia positive diarhheic calves. **Results:** Commercially available ELISA tests revealed that serum zonulin concentrations (mean) was higher in diarrheic giardia positive calves (n=11) in comparison to healthy ones (n=11) (63.35±3.73 ng/mL vs. 34.94±3.72 ng/mL). **Conclusion:** Results obtained from this study might have helped detection of leaky gut and intestinal permeability among diarrheic calves with giardiasis in which result should be taken into consideration could change treatment protocols by the veterinary surgeons.

Keywords: Dysbiosis; gut permeability; intestinal parasitic disease

ÖZET Amaç: Giyardiyazis, çok çeşitli hayvan türleri arasında en sık karşılaşılan paraziter hastalıktır ve bağırsaklarda emilim bozukluğu ve ishal, giyardiyazisin uzun vadeli sonuçlarıdır. Artmış bağırsak geçirgenliği göz önüne alındığında -moneklatür eş anlamlısı- sızıntılı bağırsak- yangı ve bağırsak disbiyozunun olası bir taşıyıcısı olarak öne sürülmüş olup, geviş getiren hayvanlarda nispeten çok az veri bulunmaktadır. Bu nedenle bu çalışmada, saha araştırması mevcut yazarı, sızıntılı bağırsak ile büyüme performansını (onun için özel bir ilgi dönemi) etkileyen giyardiyazis (bu konu analize tabi tutulmuştur) arasındaki ilişki hakkında bilgi aramaya sevk etmiştir. **Gereç ve Yöntemler:** Bu amaçla sağlıklı kontrol ve giyardiya pozitif ishalli her gruptan 11 buzağı çalışmaya dâhil edildi. Giyardiya pozitif ishalli buzağıların zonulin düzeyleri için serum örnekleri tespit edildi. **Bulgular:** Ticari olarak temin edilebilen ELISA testleri, ishalli giyardiya pozitif buzağılarda (n=11) sağlıklı olanlara (n=11) kıyasla serum zonulin konsantrasyonlarının (ortalama) daha yüksek olduğunu ortaya koydu (63,35±3,73 ng/mL'ye karşı 34,94±3,72 ng/mL). **Sonuç:** Bu çalışmadan elde edilen sonuçlar, giyardiyazis ile enfekte ishalli buzağılarda, sızıntılı bağırsak ve bağırsak geçirgenliğinin saptanmasına yardımcı olmuş olabilir ve bu sonucun dikkate alınması veteriner hekimler tarafından tedavi protokollerini değiştirebilir.

Anahtar Kelimeler: Disbiyozis; bağırsak geçirgenliği; bağırsak parazit hastalığı

The special protein, zonulin, is gifted to unsettling tight junction decomposition, in which dedicated for arranging mucosal permeability.[1,2] The latter protein initially detected as an endogenous human analogue of the bacterial enterotoxin, zonula occludens toxin, which is harvested by *Vibrio cholerae*.[2,3] In an attempt to install tight junction decomposition, it has been suggested that zonulin trigger epidermal growth factor receptor via proteinase activated receptor 2 (PAR_2) along with G protein-coupled receptor PAR_2, transacting epidermal growth factor receptor.[1,4] Through

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Peer review under responsibility of Turkiye Klinikleri Journal of Veterinary Sciences.

Received: 20 Jul 2022 ***Received in revised form:*** 25 Sep 2022 ***Accepted:*** 07 Oct 2022 ***Available online:*** 19 Oct 2022

activation of these 2 receptors decreases transepithelial electrical resistance, therefore implicating increased intestinal permeability.[1]

Given the gastrointestinal tract for a 1 month old preruminant, milk is the foremost nutritional support, whereas among forestomach abomasum is dominant. As rumen endure behindhand as because of stunted starter intake, following weaning it is prone to development due to elevated starter intake. However at this time point elevated amount of starch existing by the starter might result with ruminal acidosis, bypassing the rumen injuring the lower intestinal compartments. The latter condition results in leaky lower gut, as tight junctions among enterocytes disconnected and elevated permeability permitted antigens launch into circulation.[5] In the present study the author (with the field support of veterinary surgeons) aimed at detecting sera samples for zonulin levels were detected to those of giardia positive diarrheic calves, for preliminary detection of leaky gut and therefore intestinal permeability.

MATERIAL AND METHODS

STUDY AREA

The research was performed (with participation of veterinary surgeons at field conditions who withdraw blood samples for serum zonulin analytes) in a private farm at Aydın Municipality of Türkiye. Each group healthy control vs. giardia positive diarrheic involved 11 calves.

GIARDIASIS DETECTION

Microscopic examination involved diagnostic intervention along with *Giardia duodenalis* positive calves. The latter parasitic infection was microscopically detected (i.e. cysts presence among fecal samples).[6-9] Fecal samples were mixed with a well recognized 33% ZnSO4 solution and centrifuged at 880 x g for 5 min[4] similar to what have been described elsewhere.[6-9]

SAMPLING AND FIELD RESEARCH

One mL blood was withdrawn (whom were experienced veterinary surgeons, were cited by names at the bottom of the paper) from *Vena jugularis* into anticoagulated tubes. Sera samples were stored at -20°C until the analytes were outcome. Commercially available Bovine Zonulin ELISA test kits (MyBiosource ELISA kits, USA) were performed according to the procedure described by the manufacturer. The methodology was similar to previous experience by the present author and her teammate evolved at the relevant researches.[10-12]

ETHICAL APPROVAL

All animals in the study were treated humanely in accordance with the Guide for the Care and Use of Laboratory Animals (www.nap.edu/catalog/5140.html) and the relevant Experimental Animals Ethics Committee Approval Report was obtained. There was any administration that affect the animal welfare in the study.

The study approval was obtained by filling out the information consent form from the owners. This study was approved by Aydın Adnan Menderes University Animal Rights Experiments Local Ethics Committee (date: February 19, 2021, no: 64583101/2021/017).

STATISTICAL ANALYSIS

The data of zonulin levels of healthy and diarrheic calves were tabulated as mean and standard error. After examining the homogeneity of the obtained data, the non-parametric Mann-Whitney U test was used for comparison between groups. All analyses were done by using Graphpad (Prism, IBM) and p value $p<0.05$ were set as statistically significant.

RESULTS

The slides were microscopically examined under 400x power for visualization of Giardia cysts gave positive results for 11 diarrheic calves. An experienced veterinary surgeon microscopically detected cysts.

Circulating serum zonulin levels (ng/mL) was shown on Table 1 and Figure 1 box plot along with statistical analytes. Serum zonulin levels were deemed statistically (significantly altered) in diarrheic giardia positive calves (n=11) in comparison to healthy ones (n=11) (63.35±3.73 ng/mL vs. 34.94±3.72 ng/mL) ($p<0.001$). As p value

TABLE 1: Circulating serum zonulin levels (ng/mL) among diarrheic giardia positive and healthy control calves.

	Control $\bar{X}$±SE	Diarrheic $\bar{X}$±SE
Zonulin (ng/mL)	34.94±3.72	63.35±3.73
p value	0.001	

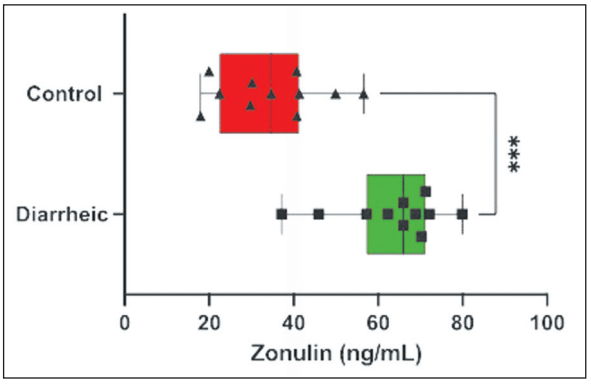

FIGURE 1: Boxplot analytes for circulating serum zonulin levels.

p<0.05 were set as statistically significant, as reported above, this could be briefly explained with the association among leaky gut and giardiasis among calves involved herein.

DISCUSSION

Intestinal permeability (ip) might be altered by several factors. Different researches presented ip changes among clinical conditions [i.e. food allergy/intolerance, rheumatoid arthritis, non-steroidal anti-inflammatory drug therapy, IgA-associated nephropathy, and infectious diseases and tropical malabsorption] were reported to be associated with increased ip.[13-17] In the present study in an attempt to detect ip, serum zonulin levels were deemed available.

Given crucial participation of the intestinal epithelium for maintenance of solute and fluid alterations along with absorption of nutrient, its relationship with gut-associated lymphoid tissue and the neuroendocrine network have been elucidated, in an attempt to understand the crossing of environmental antigens from the intestinal lumen into the sub-mucosa.[3,18] Suggested hypothesis involved, disruption of the mucosal barrier causes elevated antigen passage and relevant macromolecules from the external environment into the host resulting with local/systemic inflammation and immune respond; all of which were dedicated as "gut leakiness."[19] In the present study as evidenced by proof (elevated zonulin levels among diarrheic calves with giardiasis) presence of leaky gut and elevated ip among diarrheic calves with giardiasis in which result should be taken into consideration could change treatment protocols by the veterinary surgeons.

The gravity of bona fide intestinal barrier functionality has been heightened among ruminants exposure to elevated microbial antigens coming from pre-gastric fermentation niche.[20] Arousing proof of evidence dedicated to the gravity of gut health on animal productivity and well-being has been noticed in recent era. Different constituents implanted along with updated management conditions could exist alterations to the animal's gut health, involving its barrier function and leading to leaky gut. Among ruminants the latter conditions comprise heat stress, ruminal acidosis, weaning, diminished feed intake and the transition period among early lactating dairy cows.[11,12,21-30] On the other hand clarifying reasons responsible for leaky gut is not easy as because factors contributing for diminished intestinal barrier integrity thus could influence the metabolism and immune response of other relevant tissues.[29] Examples of these astonishing factors enrolled the periparturient period in dairy cows and heat stress, 2 of that are chaperone by significant homeorhetic adaptations for supporting a novel dominant physiological condition.[21,31] In a prior study (also performed by the present author) performed in August 2021 (41.1˚C with a 36% humidity) serum zonulin levels were assessed by ELISA to those of calves exposure to heat stress. Serum zonulin (ng/mL) levels were elevated (60,07±21,20) at mid night 00.00 am whereas mid-day interpretation at 12.00 pm (34.60±10.90) (p=0.018) were detected. In that study elevated zonulin levels determined intestinal barrier disruption along with elevated ip in relationship with heat stress.[11] Another study involved the hypothesis that heat stress induce dysfunction of intestinal barrier. In that research, Holstein dairy cows (n=7, at the age of 2-5 years)

housed in Aydin municipality with higher temperature conditions in summer were subjected to blood sampling for serum zonulin level detection. Serum zonulin levels were detected to be significantly (p=0.012) altered (at 12 pm and 00 am with temperature records of 44°C and 31°C respectively). In that research heat stress negatively influenced on intestinal integrity among cows.[12] According to the results of this present study as commercially available ELISA tests revealed that serum zonulin concentrations was higher (p<0.001) in diarrheic giardia positive calves (n=11) in comparison to healthy ones (n=11) (63.35±3.73 ng/mL vs. 34.94±3.72 ng/mL), leaky gut and elevated ip should be taken into consideration for veterinarians on the field.

CONCLUSION

In conclusion for the first-time interpretation of leaky gut among diarrheic calves with giardiasis would provide insight into its contribution to the pathophysiology of common on-farm diseases. It should not be unwise to draw preliminary conclusions that multidisciplinary field studies are warranted for futher data. As no comparative data were evident regarding this subject, detailed researches should highlight this era.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

This study is entirely author's own work and no other author contribution.

REFERENCES

1. Tripathi A, Lammers KM, Goldblum S, Shea-Donohue T, Netzel-Arnett S, Buzza MS, et al. Identification of human zonulin, a physiological modulator of tight junctions, as prehaptoglobin-2. Proc Natl Acad Sci U S A. 2009;106(39):16799-804. [Crossref] [PubMed] [PMC]
2. Wang W, Uzzau S, Goldblum SE, Fasano A. Human zonulin, a potential modulator of intestinal tight junctions. J Cell Sci. 2000;113 Pt 24:4435-40. [Crossref] [PubMed]
3. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiol Rev. 2011;91(1):151-75. [Crossref] [PubMed]
4. Cenac N, Chin AC, Garcia-Villar R, Salvador-Cartier C, Ferrier L, Vergnolle N, et al. PAR2 activation alters colonic paracellular permeability in mice via IFN-gamma-dependent and-independent pathways. J Physiol. 2004;558(Pt 3):913-25. [Crossref] [PubMed] [PMC]
5. Fischer AJ, Villot C, van Niekerk JK, Yohe TT, Renaud DL, Steele MA. Invited review: nutritional regulation of gut function in dairy calves: from colostrum to weaning. Applied Animal Science. 2019;35(5):498-510. [Crossref] [PMC]
6. Alic Ural D, Aysul N, Gültekin M. The efficacy of oral administration of clinoptilolite against naturally occuring giardiasis in calves. Kocatepe Veterinary Journal. 2016;9(4):288-93. [Link]
7. Ayan A, Ural K, Aysul N, Gültekin M, Erdoğan H, Balikçi C, et al. Natural cyst shedding in calves infected with giardia duodenalis. Journal of Advances in VetBio Science and Techniques. 2016;1(1):14-9.
8. Toplu S, Ural K, Aysul N, Ayan A, Gültekin M, Balıkçı C. Hypomagnesemia in naturally infected calves with giardia spp. Kocatepe Veterinary Journal. 2016;9(4):386-90. [Link]
9. Gultekin M, Ural K, Aysul N, Ayan A, Balikci C, Toplu S, et al. Prevalence and molecular characterization of Giardia duodenalis in calves in Turkey. Acta Scientiae Veterinariae. 2017;45(1450):6. [Crossref]
10. Wilson JM, Hankenson FC. Evaluation of an inhouse rapid ELISA test for detection of giardia in domestic sheep (Ovis aries). J Am Assoc Lab Anim Sci. 2010;49(6):809-13. [PubMed] [PMC]
11. Alic Ural D, Erdoğan S, Erdoğan H, Ural K. Heat stress, intestinal barrier disruption and calves: multidisciplinary perspective field study. Journal of Advances in VetBio Science and Techniques. 2021;6(3):265-9. [Crossref]
12. Alic Ural D, Ural K, Erdogan H, Erdogan S. Alterations in gut integrity due to heat stress among dairy cattle of Aydin city: analytical interpretation of zonulin levels within repetitive measurements. International Journal of Veterinary and Animal Research (IJVAR). 2021;4(3):111-4. [Link]
13. Forget P, Sodoyez-Goffaux F, Zappitelli A. Permeability of the small intestine to [51Cr]EDTA in children with acute gastroenteritis or eczema. J Pediatr Gastroenterol Nutr. 1985;4(3):393-6. [Crossref] [PubMed]
14. Bjarnason I, MacPherson A, Hollander D. Intestinal permeability: an overview. Gastroenterology. 1995;108(5):1566-81. [Crossref] [PubMed]
15. Davin JC, Forget P, Mahieu PR. Increased intestinal permeability to (51 Cr) EDTA is correlated with IgA immune complex-plasma levels in children with IgA-associated nephropathies. Acta Paediatr Scand. 1988;77(1):118-24. [Crossref] [PubMed]
16. Ford RP, Menzies IS, Phillips AD, Walker-Smith JA, Turner MW. Intestinal sugar permeability: relationship to diarrhoeal disease and small bowel morphology. J Pediatr Gastroenterol Nutr. 1985;4(4):568-74. [Crossref] [PubMed]

17. Cook GC, Menzies IS. Intestinal absorption and unmediated permeation of sugars in post-infective tropical malabsorption (tropical sprue). Digestion. 1986;33(2):109-16. [Crossref] [PubMed]
18. Odenwald MA, Turner JR. Intestinal permeability defects: is it time to treat? Clin Gastroenterol Hepatol. 2013;11(9):1075-83. [Crossref] [PubMed] [PMC]
19. Ajamian M, Steer D, Rosella G, Gibson PR. Serum zonulin as a marker of intestinal mucosal barrier function: May not be what it seems. PLoS One. 2019;14(1):e0210728. [Crossref] [PubMed] [PMC]
20. Mani V, Weber TE, Baumgard LH, Gabler NK. Growth and development symposium: endotoxin, inflammation, and intestinal function in livestock. J Anim Sci. 2012;90(5):1452-65. [Crossref] [PubMed]
21. Baumgard LH, Rhoads RP Jr. Effects of heat stress on postabsorptive metabolism and energetics. Annu Rev Anim Biosci. 2013;1:311-37. [Crossref] [PubMed]
22. Pearce SC, Mani V, Weber TE, Rhoads RP, Patience JF, Baumgard LH, et al. Heat stress and reduced plane of nutrition decreases intestinal integrity and function in pigs. J Anim Sci. 2013;91(11):5183-93. [Crossref] [PubMed]
23. Emmanuel DG, Madsen KL, Churchill TA, Dunn SM, Ametaj BN. Acidosis and lipopolysaccharide from Escherichia coli B:055 cause hyperpermeability of rumen and colon tissues. J Dairy Sci. 2007;90(12):5552-7. [Crossref] [PubMed]
24. Khafipour E, Krause DO, Plaizier JC. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J Dairy Sci. 2009;92(3):1060-70. [Crossref] [PubMed]
25. Minuti A, Ahmed S, Trevisi E, Piccioli-Cappelli F, Bertoni G, Jahan N, et al. Experimental acute rumen acidosis in sheep: consequences on clinical, rumen, and gastrointestinal permeability conditions and blood chemistry. J Anim Sci. 2014;92(9):3966-77. [Crossref] [PubMed]
26. Moeser AJ, Klok CV, Ryan KA, Wooten JG, Little D, Cook VL, et al. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. Am J Physiol Gastrointest Liver Physiol. 2007;292(1):G173-81. [Crossref] [PubMed]
27. Wood KM, Palmer SI, Steele MA, Metcalf JA, Penner GB. The influence of age and weaning on permeability of the gastrointestinal tract in Holstein bull calves. J Dairy Sci. 2015;98(10):7226-37. [Crossref] [PubMed]
28. Zhang S, Albornoz RI, Aschenbach JR, Barreda DR, Penner GB. Short-term feed restriction impairs the absorptive function of the reticulo-rumen and total tract barrier function in beef cattle. J Anim Sci. 2013;91(4):1685-95. [Crossref] [PubMed]
29. Kvidera SK, Horst EA, Abuajamieh M, Mayorga EJ, Fernandez MV, Baumgard LH. Glucose requirements of an activated immune system in lactating Holstein cows. J Dairy Sci. 2017;100(3):2360-74. [Crossref] [PubMed]
30. Abuajamieh M, Kvidera SK, Fernandez MV, Nayeri A, Upah NC, Nolan EA, et al. Inflammatory biomarkers are associated with ketosis in periparturient Holstein cows. Res Vet Sci. 2016;109:81-5. [Crossref] [PubMed]
31. Bauman DE, Currie WB. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. J Dairy Sci. 1980;63(9):1514-29. [Crossref] [PubMed]