**PAROXYSMAL DYSKINESIAS: USE OF ANTIBODY TITRATION (Ig-A TISSUE TRANSGLUTAMINASE AND Ig-G MODIFIED GLIADIN PEPTIDES) IN DOGS OF DIFFERENT BREEDS AND ASSESSMENT OF CLINICAL RESPONSE TO GLUTEN-FREE DIET.**

1. **INTRODUCTION**

Paroxysmal dyskinesias (PD) are a heterogeneous group of movement disorders widely described in human medicine and, more recently, of increasing interest in veterinary medicine.

In dogs, these neurological manifestations, in the past often misinterpreted as epileptic seizures, are characterized by a hyperacute onset of paroxysmal movements, dystonia of the limbs, and consequent difficulty of movement. PDs in dogs are characterized by the absence of autonomic signs, consciousness, pain, and a postictal phase.

Although in recent years some articles have tried to outline the main phenotypic aspects of PD in certain breeds, the therapeutic approach to this group of pathologies is still unclear. Therapeutic trials carried out over the years with various drugs such as antiepileptics (phenobarbital, levetiracetam, or benzodiazepines), fluoxetine or acetazolamide have given inconsistent results.

It is particularly interesting to note that, in a work published in 2017 by Stessen and colleagues, a population of Border terriers suffering from PD presented an improvement in the frequency of episodes once treated with a gluten-free diet. Gluten sensitivity in these patients was tested by titration of anti-transglutaminase-2 and anti-gliadin antibodies. In this paper, gastroenteric signs such as audible borborygmi were found in patients with PD. Although some theories have been formulated on the etiopathogenetic mechanism in human medicine, the effect of these two antibodies on the central nervous system remains to be clarified.

At present, there is little information about the clinical response to gluten-free treatment in other breeds affected by PD.

The project is aimed to verify a possible positive response to the gluten-free diet in dogs of different breeds than the Border terrier, assuming that dogs of different breeds with a clinical diagnosis of PD may have a good response to the dietary trial. At the same time, the project is focused on the investigation of the presence of chronic inflammatory enteropathy in patients affected by PD, to assess the presence of possible GI factors associated with PD.

1. **PURPOSE OF STUDY**

The main objectives of the study are to:

* 1. **Assess** **the phenotypic patterns of PD** with particular attention to identifying a possible correlation with the breed, sex, age, and body weight.
	2. **Identify any trigger for PD**.
	3. **Assess the presence of an underlying gastroenteric disorder.**
	4. **Assess antibodies** (IgA-transglutaminase tissue and IgG-modified gliadin peptides) values at T0 (before starting a gluten-free diet), T1 (90 days after the introduction of the gluten-free diet), and T2 (before the end of the 180-day gluten-free diet trial). This data will then be correlated with the frequency of episodes before and after the introduction of the gluten-free diet.
	5. **Evaluate the clinical response to a gluten-free diet.**
	6. **Characterize and compare the fecal microbiota** of healthy dogs and dogs with PD, before and after the gluten-free diet treatment, and to evaluate whether PD might be associated to intestinal dysbiosis in dogs.
1. **DESCRIPTION OF THE RESEARCH PLAN**

**INCLUSION CRITERIA:**

All dogs meeting the following inclusion criteria will be included in the study:

* Dogs of any breed (excluding Border terrier breed dogs), age, and sex.
* Dogs with at least two PD episodes in the six months before the neurological clinical examination.
* Dogs not under a gluten-free diet before the neurological examination.
* Dogs normal on neurological examination

**STUDY DESIGN:**

**T0:**

* Collection of patient data on signalment (breed, gender, age, and body weight).
* General physical examination with particular attention to the medical history and gastroenteric signs.
* Neurological history and neurological examination (carried out at least 24 hours after the episode and that must be normal).
	+ - In particular, will be recorded all information concerning:
		- Age at the time of the first episode;
		- Frequency of episodes;
		- Any trigger factors;
		- Duration of the episodes;
		- Possible post-episode phase (duration and description of the same);
		- State of consciousness (preserved or altered);
		- Presence/absence of autonomic signs (sialorrhea, urination, defecation, mydriasis);
		- Involvement of one or more limbs (flexion vs extension);
		- Neck and trunk involvement;
		- Tremors (localized to an anatomical or generalized district).
* Complete blood chemistry including folate and cobalamin dosing.
* Abdominal ultrasound.
* Magnetic resonance of the brain. This diagnostic test can help us to rule out focal epileptic seizures cause by prosencephalic structural disease such as vascular, inflammatory, or neoplastic lesions.

In patients that met the inclusion criteria, an initial antibody titration will be carried out at the same time as the neurological examination (T0 SAMPLING). Once the result is obtained (both positive and negative) we will proceed with the prescription of a specific commercial gluten-free diet (Purina HA Hypoallergenic). Further antibody titrations will be performed at 90 (time T1) and 180 (time T2) days from the introduction of the diet.

To standardize the results of antibody titration, all serum samples will be sent to a vet lab (LABOKLIN laboratory). While awaiting shipment to the laboratory, the sample shall be refrigerated and kept away from light.

The response to the dietary trial will be defined in terms of the frequency of the episodes and will be classified as EXCELLENT (absence of further episodes), GOOD (reduction of the frequency of at least 50% of the episodes), MODEST (reduction of less than 50% of the episodes) or NONE (frequency unchanged).

1. **EXPECTED RESULTS**
* **To verify the presence of common PD phenotypic patterns among different breeds**.
* **To recognize any trigger** of PDS.
* **To verify the correlation between the presence of PD episodes, high antibody titers and the clinical response to a gluten-free diet.**
* **To assess the incidence of gastroenteric disorders in patients with PD** and verify the possible correlation between the introduction of diet, the improvement of the frequency of PD episodes, folate, and cobalamin levels, and gastroenteric symptoms.
1. **REFERENCES**
* Lowrie M, Garosi L. Classification of involuntary movements in dogs: Paroxysmal dyskinesias. Vet J. 2017 Feb;220:65-71.
* Cerda-Gonzalez S, Packer RA, Garosi L, Lowrie M, Mandigers PJJ, O'Brien DP, Volk HA. International veterinary canine dyskinesia task force ECVN consensus statement: Terminology and classification. J Vet Intern Med. 2021 May;35(3):1218-1230.
* Lowrie M, Garden OA, Hadjivassiliou M, Harvey RJ, Sanders DS, Powell R, Garosi L. The Clinical and Serological Effect of a Gluten-Free Diet in Border Terriers with Epileptoid Cramping Syndrome. J Vet Intern Med. 2015 Nov-Dec;29(6):1564-8.
* Stassen QEM, Koskinen LLE, van Steenbeek FG, Seppälä EH, Jokinen TS, Prins PGM, Bok HGJ, Zandvliet MMJM, Vos-Loohuis M, Leegwater PAJ, Lohi H. Paroxysmal Dyskinesia in Border Terriers: Clinical, Epidemiological, and Genetic Investigations. J Vet Intern Med. 2017 Jul;31(4):1123-1131.
* Black V, Garosi L, Lowrie M, Harvey RJ, Gale J. Phenotypic characterisation of canine epileptoid cramping syndrome in the Border terrier. J Small Anim Pract. 2014 Feb;55(2):102-7.
* Black V, Garosi L, Lowrie M, Harvey RJ, Gale J. Phenotypic characterisation of canine epileptoid cramping syndrome in the Border terrier. J Small Anim Pract. 2014 Feb;55(2):102-7. doi: 10.1111/jsap.12170.
* Pilla R, and Suchodolski JS. The Gut Microbiome of Dogs and Cats, and the Influence of Diet. Vet Clin Small Anim. 2021 51:605–621.
* AlShawaqfeh MK, Wajid B, Minamoto Y, et al. A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. FEMS Microbiol Ecol. 2017 93:fix136.