Ph.D. THESIS

Applications of electroencephalography in diagnosis of seizures and epileptic syndromes in dogs

(SUMMARY OF THE Ph.D. THESIS)

PhD student **Helga Demény**

Scientific coordinator **Prof.univ. Dr. Laurenț Ognean**

INTRODUCTION

Canine epilepsy is considered the most common neurological pathology, therefore the clinical approach and therapeutic management of the patient with epileptic seizures is becoming an increasingly important topic in veterinary medicine. Epilepsy is a complex pathology, which allows abnormal, sudden and transient electrical discharges in the cerebral cortex, leading to clinical signs of a seizure, characterised by motor, autonomic and/or behavioural manifestations. Knowing the heterogenity of epilepsy with highly variable symptoms and aetiology, a diagnostic protocol is crucial in order to develop an appropriate treatment scheme.

The approach to a patient with epileptic seizures must first include, identification and confirmation of the actual epileptic seizure, followed by investigation of the exact cause by multiple paraclinical methods such as laboratory analysis, advanced imaging or even genetic testing. In the last years of practice, our experience shows that we often encounter difficulties in identifying the nature of the episodes described by owners as epileptic seizures. Unlike in human medicine, in canine epileptology the anamnesis is reduced to the signs observed by the patient's owner and in the best case to some video recording of the episodes. In human medicine, electroencephalography (EEG) is a routine and essential functional investigation in patients with episodes of loss or alteration of consciousness, which plays a major role both in confirming the diagnosis of epilepsy and in monitoring over time the patient's evolution under treatment. On the other hand, in veterinary medicine the EEG is still considered an investigation with insufficiently proven diagnostic value.

Neurophysiological models and EEG recording protocols practiced in human medicine have been a source of inspiration and constant enthusiasm in the objective of this thesis, through which we aimed first of all to demonstrate the usefulness of EEG in veterinary neurology, and at the same time, through the results obtained, to encourage the community of veterinary neurologists towards the use of this valuable neurophysiological exploration.

STRUCTURE OF THE THESIS

The thesis is composed of 8 chapters, comprising a first part literature review, strictly related to published studies, which are contained in the second part.

Literature review

Chapter 1 of the thesis first defines the difference between epileptic seizures and epilepsy. It also systematically presents all the known forms of epilepsy and the variety of clinically defined epileptic seizures in the veterinary neurology literature. Furthermore, it details aspects of structural epilepsy related to quadrigeminal intracranial cysts and rare epileptic syndromes such as progressive myoclonic epilepsy – the Lafora disease.

Chapter 2 aims to summarize the most important aspects related to the source of EEG signals, and the technical features necessary to understand and interpret the registered data. The EEG is the most commonly used method in functional brain investigation, representing an examination method of particular value in understanding the pathophysiology of epileptic seizures in humans. Therefore, the information obtained by EEG plays a major role in confirming the diagnosis of epilepsy and assessing the efficacy of antiepileptic treatment.

Despite the fact that canine epilepsy is the most common neurological pathology in veterinary medicine, EEG is not used as a routine diagnostic method. The "International Veterinary Task Force" recommends EEG evaluation in canine patients for confirmation of idiopathic epilepsy (DE RISIO et al., 2015).

However, in the recent years a number of articles have investigated the usefulness of EEG in veterinary medicine and confirmed the value of this analysis, both in differentiating epileptic seizures from other non-epileptic episodes and in diagnosing brain pathologies manifested by impaired patient consciousness (JAMES et al., 2017; GRANUM et al., 2019; WRZOSEK et al., 2016, DEMENY et al., 2020).

Unlike human medicine, EEG in veterinary medicine faces a number of technical challenges, such as the lack of standardized EEG recording methods, lack of sedation protocols necessary for EEG registration in canine patients, and identifying of epileptic graphoelements. A number of proposals have been published over the previous years, but there is still no universally accepted standard protocol of EEG recording and interpretation in clinical veterinary practice. This is why technical details are still adapted from human medicine.

For this reason, in this chapter we tried to summarize the publications related to the source of EEG, the most important ideas related to the recording technique, ictal and

interictal pathological graphoelements, and the importance of other non-EEG parameters that deserve to be part of routine recordings.

Personal contribution

Chapter 3 aims to define the main objective of the thesis, which consists of the development of a clinical-paraclinical algorithm for the confirmation of epileptic seizures and for the monitoring of the evolution of epileptic syndromes in the canine patients.

Through the published studies we also aimed to:

- ✓ Critically analyse the usefulness of EEG in veterinary neurology, by performing EEG in canine patients with suspected epileptic seizures and correlate EEG recording aspects with the seizure semiology;
- ✓ Assess the need for long-term EEG recordings, both in the detection of non-convulsive status epilepticus and in monitoring of patients under treatment;
- ✓ Identify a particular aspect of epileptic discharges on EEG in patients with progressive myoclonic epilepsy - Lafora disease, and the diagnostic value of EEG in confirming the diagnosis, especially in breeds where genetic testing is not yet available;
- ✓ Confirm the cortical epileptic source of some motor manifestations, such as myoclonic jerks, and correlate the EMG signal with the EEG signal;
- ✓ Analyse the usefulness of EEG in temporospatial correlation of the structural intracranial lesion - quadrigeminal cyst - with the epileptogenic foci and to monitor the patient's evolution under conservative treatment.

Chapter 4 entitled "EEG diagnostic and monitoring protocol in canine epilepsy" is based on recently published data (DEMENY et al., 2018). Starting from the basic idea suggesting that EEG is an analysis difficult to perform and interpret in canine patients, and with questionable diagnostic value in veterinary medicine, we proposed to demonstrate the usefulness of EEG in a significant number of canine patients. The diagnostic value of EEG can be demonstrated by identifying interictal abnormalities in patients with suspected epileptic seizures. The indispensable role of EEG is also worth mentioning in the diagnosis of pure electrical seizures and non-convulsive status epilepticus in patients with altered states of consciousness. Moreover, as there is currently no standard protocol for EEG recording in canine patients, being inspired by human neurophysiology we set out to describe a method of using a minimum number of electrodes and an EEG recording protocol with applicability in veterinary medicine.

56 canine patients with a history of at least one epileptic seizure and/or altered states of consciousness at presentation were examined. Subsequently, the study group included only patients who had epileptiform changes on EEG recording and were not receiving any antiepileptic medication at the moment of presentation (n=31).

Clinical neurological examination and spontaneous EEG recording under sedation was performed in all patients. Other laboratory tests such as haemoleucogram, routine serum biochemistry, ionogram, serological tests, urinalysis and brain imaging were recommended on a case-by-case basis and were performed according to the financial possibilities of the owners.

Thirty-one canine patients presenting with a history of at least one epileptic seizure, and/or altered states of consciousness at the time of presentation were included in the study. All the patients included in the study were without any antiepileptic medication at the time of presentation and all of the subjects had epileptiform changes during EEG recording.

Clinical neurological examination and spontaneous EEG recording under sedation was performed in all patients. Other laboratory tests such as haematology, routine serum biochemistry, ionogram, serological tests, urinalysis and brain imaging were recommended on a case-by-case basis and were performed according to the financial possibilities of the owners.

The EEG results obtained were able to identify and describe interictal (Fig. 1) and ictal (Fig. 2) abnormalities in 31 out of 56 patients (55% sensitivity).

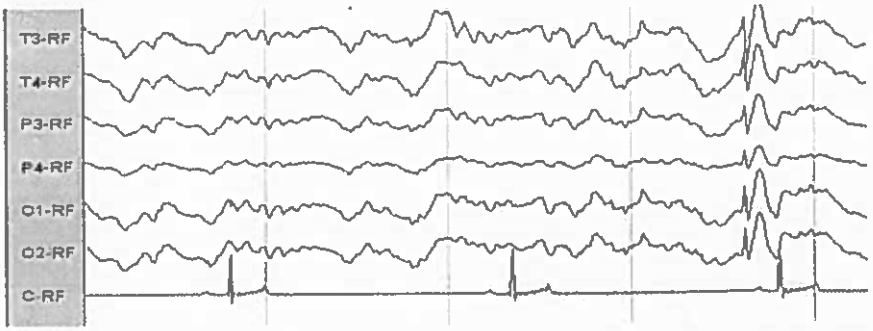

Fig. 1. Generalized spike-slow wave complex, observed in referential montage

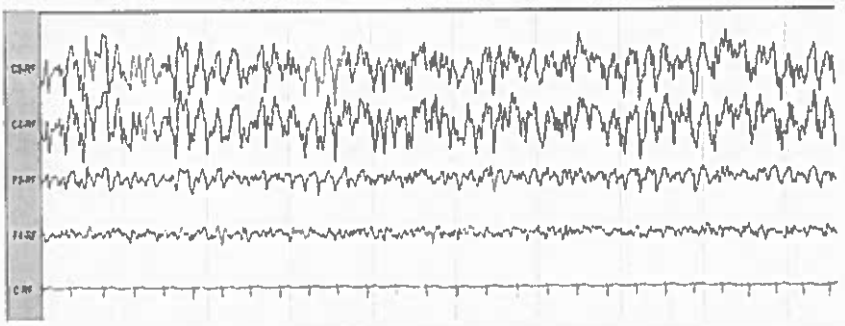

Fig. 2. Generalized non-convulsive status epilepticus with autonomic manifestations, such as mydriasis and hypersalivation, but without any motor activity

The study describes frequent progression to status epilepticus, predominantly in patients who were known to have a history of at least one previous seizure. 63% of patients with status epilepticus experienced refractoriness to first- and second-line treatment and required sedation. In 11 of 31 patients with altered or lost consciousness, EEG confirmed non-convulsive status epilepticus, essential for initiation of emergency treatment. These patients, apart from altered consciousness and possibly subtle motor or autonomic phenomena, showed no other clinical manifestations, suggesting that without EEG, diagnosis would have been impossible.

Following the definition of the cause of epileptic episodes 25.8% of patients were diagnosed by exclusion with idiopathic epilepsy, 41.9% of them were diagnosed with structural epilepsy (including vascular, inflammatory, congenital abnormalities and neoplastic causes), 6.4% of patients had metabolic/toxic causes and in 25.8% of patients, for financial reasons, an exact cause of epileptic seizures could be not identified.

EEG performed in patients with altered consciousness without obvious motor manifestations had a high sensitivity in confirming the diagnosis of purely electrical seizures or nonconvulsive status epilepticus. The use of medetomidine or dexmedetomidine as a method of sedation to prepare the patient for EEG recording, has been shown to be an appropriate method that does not influence the EEG background pattern.

This study highlights the value of the EEG in identifying interictal epileptiform abnormalities and, last but not least, demonstrates the indispensable role of this analysis in detecting and monitoring non-convulsive status epilepticus and purely electrical seizures.

EEG, as a result, is the most useful tool for the evaluation of brain functions and epileptiform discharges.

Chapter 5 is dedicated to the publication entitled "EEG patters orienting to Lafora disease diagnosis - case report in two Beagles.

Lafora disease is a rare, genetic form of myoclonic epilepsy reported in both humans and certain breeds of dogs. In canine patients, the electrodiagnostic features of Lafora disease reported in the literature are quite few. Irregular myoclonus without EEG correlation has been described in a beagle with Lafora disease (GREDAL et al., 2003). The first article describing electroencephalographic pathological aspects correlated with muscle electrical activity during myoclonus is included in this thesis. The case report, based on two Beagle cases, diagnosed with Lafora disease, presents the pathological EEG aspect, namely generalized cortical discharges, correlated with spontaneous or light stimulus-triggered myoclonus (DEMENY et al., 2020).

Starting from the idea that the diagnosis of Lafora disease is complex and difficult for clinicians, especially in breeds with the genetic mutation not yet described and identified, we aimed to demonstrate the usefulness of EEG both in establishing the

cortical source of myoclonus and in guiding the diagnosis by identifying EEG features almost characteristic of the pathology.

Given the rarity of Lafora's disease, this study included two canine patients for a single episode or repeated episodes of generalized epileptic seizures. The two beagle patients, aged 7 and 9 years old, came from different cities, did not have common parents, and no co-breeding could be proven. The two patients were approached in terms of an almost identical diagnostic plan. General and neurological examination, blood tests such as routine haematology and biochemistry, urine analysis, spontaneous and intermittent light stimulation EEG under sedation, genetic PCR testing for Lafora's disease and histopathological examination of skin and muscle tissue taken by biopsy were performed.

Based on the clinical history and the neurological examination, the list of differential diagnoses for both patients included Lafora's disease, ceroid neurolipofuscinosis, myoclonic epilepsy of unknown origin and other structural brain changes. Results of routine haematological, urine and serum biochemistry tests were unchanged in both patients.

Spontaneous and photic-stimulated EEG recording confirmed the cortical source of myoclonus (Fig. 3). Thus, the occurrence of solitary spikes (amplitudes of 50-250 μV), solitary spike complexes followed by slow waves, and generalized poly-spikes complexes with slow waves every 20-40 s were observed.

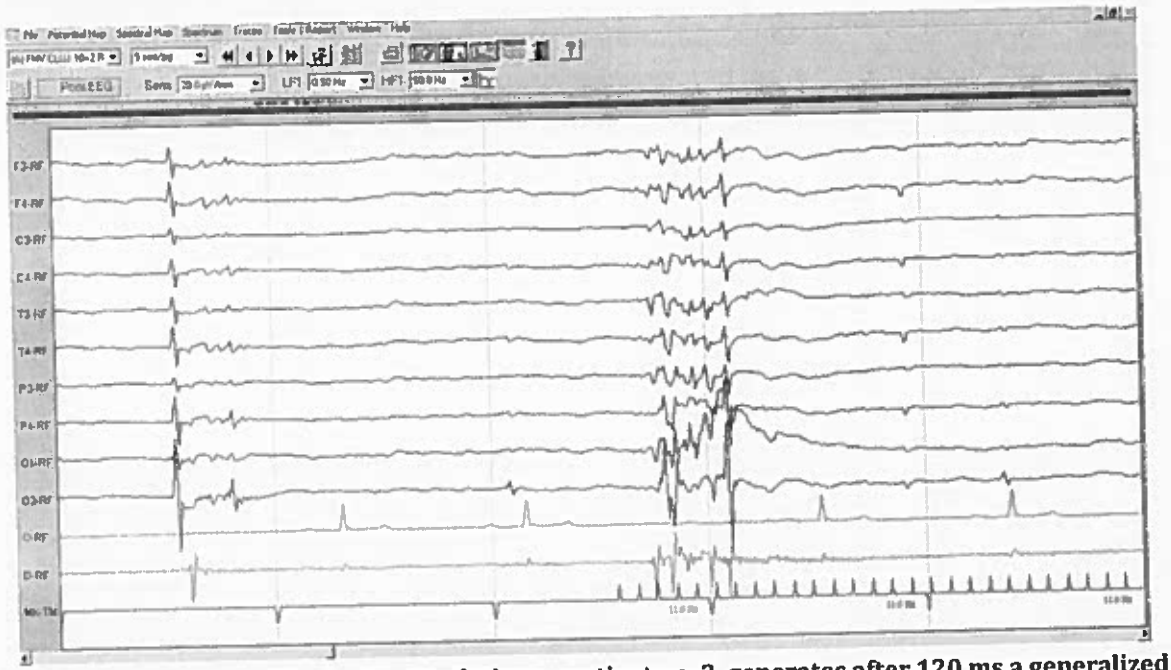

Fig.3. EEG recording with photic stimulations – patient no. 2, generates after 120 ms a generalized polispike-slow wave complex, followed immediately by a myoclonic contraction observable on the EMG channel

Channel C- EKG, Channel D- bipolar surface EMG on the right brachiocephalic muscle, Channel MKT – photostimulation marker.

Histologically, ~10% of skeletal myofibers examined contained one to several poorly demarcated, intensely PAS-positive subsarcolemmal and intracytoplasmic inclusions measuring 3-50μm (Fig. 4).

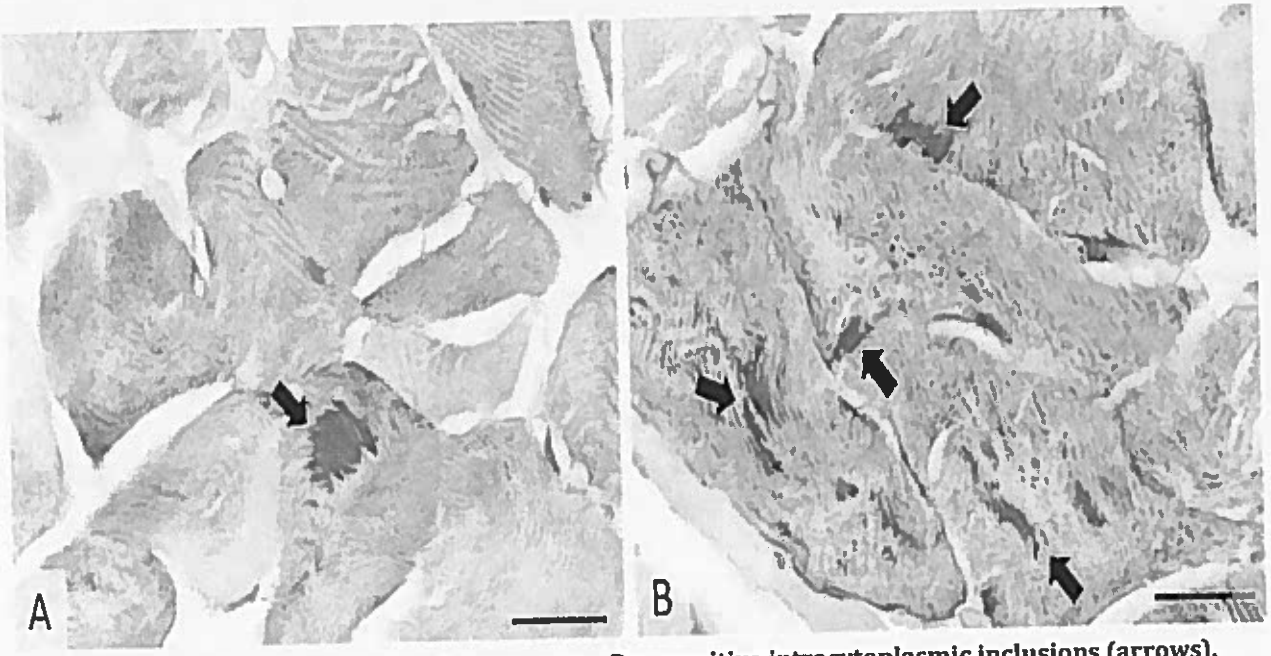

Fig. 4. Cross section of muscle showing numerous Pas -positive intracytoplasmic inclusions (arrows).

In both patients, genetic results confirmed the Laf/Laf genotype as homozygous for the Lafora disease-causing mutation in the NHLCR1 gene.

Although cases from different breeds of dogs with Lafora disease have been reported, the relationship between disease semiology and cortical electrical activity recorded by EEG has not been investigated. The study demonstrates that EEG is useful in guiding the diagnosis of Lafora disease and in confirming the purely cortical origin of myoclonus. In both cases described in this study dogs with Lafora disease showed EEG patterns similar to those described in humans, with spikes or slow solitary spike-wave complexes, occurring spontaneously or elicited by visual stimuli.

This is the first internationally published report with photic stimulated EEG recordings in dogs with confirmed Lafora disease diagnosis, demonstrating that EEG is a very valuable exploration in guiding the diagnosis of Lafora disease, proposing the method also in confirming the diagnosis in breeds where genetic testing is not yet available.

Chapter 6 is based on the third published study, entitled "Epileptic discharges identified in a canine patient with quadrigeminal cyst, but unrelated to the localization or to the patient's evolution under conservative treatment."

Intracranial arachnoid cysts are represented by congenital developmental brain lesions, formed by a focal division or duplication of the leptomeninges resulting in a cerebrospinal fluid-filled diverticulum (DENWEY et al., 2008; SAITO et al., 2001; WANG et al., 2013). In canine patients these congenital anomalies occur almost

exclusively in the caudal fossa, thus being associated with the quadrigeminal cisterna (DENWEY et al., 2008; MATIASEK et al., 2007; WANG et al., 2013). Although epileptic seizures are the most common symptom in patients with quadrigeminal cysts, the relationship between the location of the cyst and the electrical epileptic foci has not yet been investigated.

With this case report we aimed to evaluate the temporo-spatial correlation of the epileptic focus with the quadrigeminal cyst and EEG monitoring of the patient's evolution under conservative treatment (DEMENY et al., 2022).

Following the results obtained in terms of the appearance of the recordings over time, we can confirm that the electrical epileptiform activity of the patient, appeared mainly on the frontal leads, at a distance from the occipital location of the quadrigeminal cyst confirmed by MRI (Fig. 5).

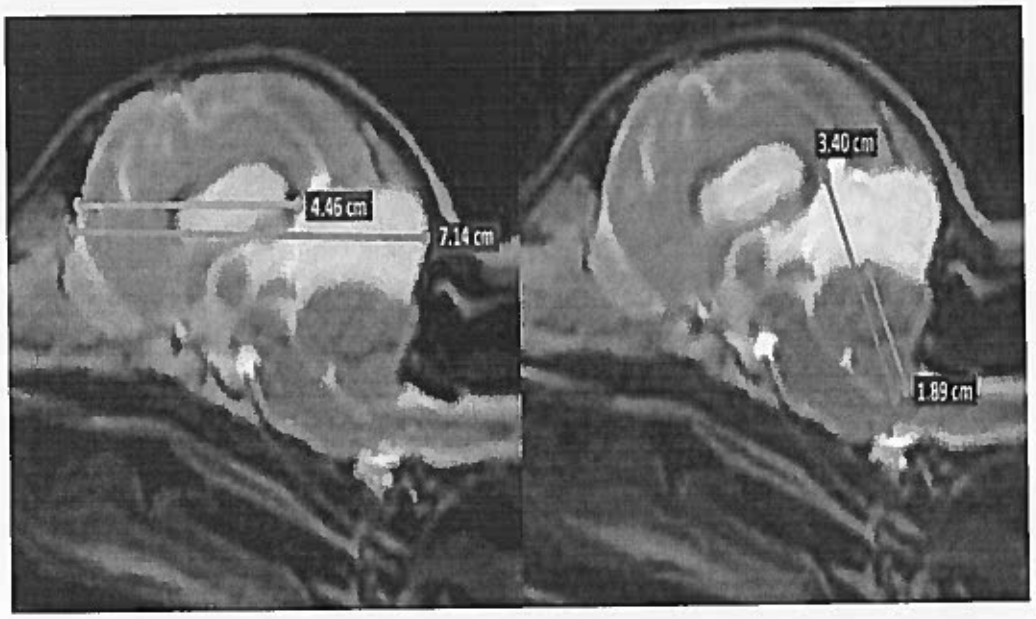

Fig. 5. Measurement of the level of compression of the brain parenchyma (20 A) and cerebellum (20 B) an MRI images, sagittal section, T2- weighted

The surface EEG failed to correlate the epileptic focus with the location of the structural lesion. Also, the worsening of the appearance of repeated EEG recordings, in terms of the frequency of occurrence of epileptiform abnormalities, could not be correlated with the patient's favourable clinical evolution (with the improvement of the symptoms).

Although it represents the first study describing the pathological appearance of the EEG in a dog, diagnosed with quadrigeminal arachnoid cyst, in the described case, surface EEG did not prove to be a valuable test for the localization of the quadrigeminal cyst or for monitoring the clinical evolution of the canine patient under conservative treatment. However, it was possible to confirm the presence of cortical

epileptic electrical activity, further data is still needed to establish the value of EEG examination in these patients.

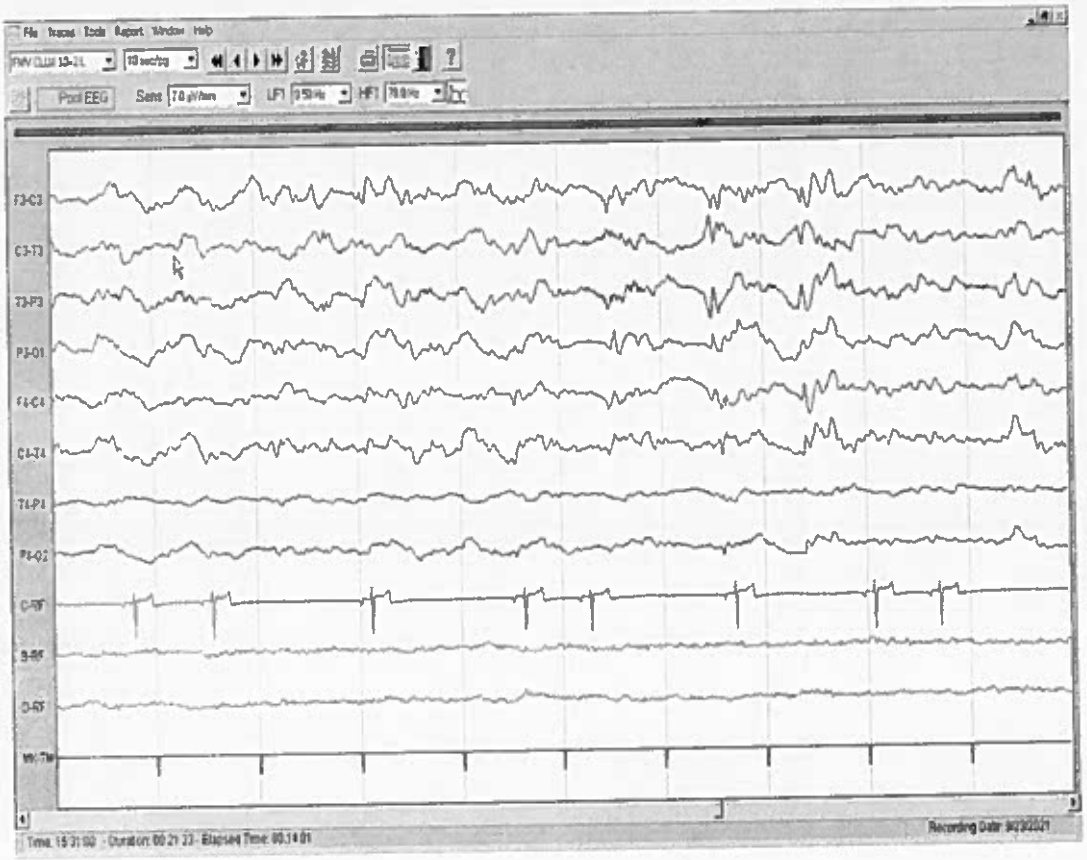

Fig. 6. The appearance of the third EEG recording, 4 months after the first one, in longitudinal montage, showing spike waves with increased amplitude, sharp waves and even spike-slow wave complexes occurring every 20-40 seconds.

Chapter 7 presents the overall conclusions and recommendations of the three studies. It is known that performing a good quality EEG and interpreting the results is relatively difficult and requires a qualified person. This thesis provides a proven effective method of performing EEG and provides the reader with the most common ictal and interictal epileptic EEG features. The three published studies also confirm the value of this exploration in veterinary neurology.

Chapter 8 is devoted to the originality and innovative contribution of the thesis. Essentially, the studies included in this thesis uncover and define the value and limitations of EEG. We hope that the results provided by the studies, encourage the development of electroneurophysiology in general and the expansion of EEG applications in veterinary medicine.

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