CONCEPTS OF FUNCTIONAL ANATOMY
The Peripheral Nervous System (PNS) consists of an efferent part, with motor functions, and an afferent part, with sensory function.

Within locomotion, the PNS transports sensory stimuli from the periphery and drives them through fibers that enter the Central Nervous System (CNS). At this level, information can be conveyed to the upper centers or transported directly to the efferent neurons located in the ventral horns of the spinal cord. In both cases, the result is the activation of the efferent nerve component, whereby the final result is a specific response represented by muscle contraction.

The efferent part of PNS in the clinical practice is identified with the neuromuscular system (or the lower motor neuron system) which, as indicated by the name, includes the muscle producing a morpho-functional unit of great clinical importance. The neuromuscular system consists of multiple motor units, each of them consisting of the nerve cell (α-motoneuron), neuromuscular junction and innervated muscle fibers.

The identification of the efferent part of the PNS with the neuromuscular system is improper, since part of the neuromuscular system is anatomically located in the CNS. The α-motoneuron is a particular efferent neuron whose cell body is located in the grey substance of the spinal cord ventral horns, that is, in the CNS. The shape and size of the grey ventral column reflect the number of motoneurons. Neurons innervating the appendicular muscles are very numerous and therefore cause a sort of spinal cord “swelling”, appreciable in the cervical and lumbosacral intumescence.

The axon of the α-motoneuron exits the spinal cord as part of the ventral nerve root and participates to the constitution of the spinal nerve, in which reaches the neuromuscular junction as part of a specific peripheral nerve (this latter made by the contribution of several spinal nerves). Close to the muscle, the axon of the α-motoneuron is branched proportionally to the type of innervated muscle fibers. The resultant motor unit may consist of a variable number of muscle fibers. In the case of the finely regulated muscle activity, such as that of eye movements, motor units are made up of only 3-6 muscle fibers, whereas muscle for more rough activities such as walking can have hundreds of fibers per motoneuron.

In relation to the localization of the disease, it is difficult, from a clinical point of view, to differentiate between a problem that is selectively affecting the nerve, the muscle or the neuromuscular junction. Therefore, the clinical accuracy relies in defining the complex of characteristic signs in the term "peripheral syndrome" or, as is often the case with veterinary medicine, "neuromuscular syndrome" (or "lower motoneuron syndrome"). In a neuromuscular syndrome is affected selectively the efferent motor part. It should be noted that the vast majority of PNS pathologies affects the efferent part more or less selectively and, for this reason, most of the clinical relevant information in the literature concerns neuromuscular syndrome.

THE “CLASSICAL” CLINICAL PRESENTATION
Patients suffering from PNS disorders manifest extremely variable clinical signs depending on a variety of factors including severity, onset and course, or degree of extension of the pathological process. Regarding the onset and course, most systemic neuropathies have insidious onset and a chronic-progressive course, although some of them may have an acute (such as idiopathic polyradiculoneuritis) or subacute onset.

During generalized peripheral nervous dysfunction, such as polyradiculoneuropathies and polyneuropathies, clinical signs involve the entire body and affect, usually in symmetric form, the different regions of the body. Polyneuropathies involve spinal nerve more frequently than cranial nerves.
It is important to remember once more that in veterinary medicine, polyradiculoneuropathies/polyneuropathies produce dysfunctions that mainly affect efferent fibers and, therefore, selectively the PNS motor component. The resulting clinical presentation is characterized by changes in the normal gait, consisting in weakness and inability to withstand a normal muscle activity. These dysfunctions are properly defined as paresis and exercise intolerance. Unlike human medicine, sensory and mixed polyneuropathies are extremely infrequent in veterinary medicine. Therefore, only rarely ataxia and true proprioceptive deficits are detected. A patient with neuromuscular problems is generally referred with the main complain of weakness and exercise intolerance, peculiar but not pathognomonic signs of a peripheral syndrome. All patients suspected of having a neuromuscular problem should first undergo a thorough physical examination to exclude non-neurological causes that may cause clinical signs similar to those of neuropathies. It is important to remember that even anaemic syndromes and some cardiorespiratory diseases may manifest with "weakness". Next step includes a complete neurological examination, in order to highlight the various neurological signs that allow the clinician to reach a neuroanatomical localization.

For educational purposes, in this abstract, the patient with neuromuscular syndrome is approached following the neurological examination scheme. For obvious reasons of space, only those parts of the neurological examination that may be altered by a neuromuscular syndrome will be described.

**Mental status and behaviour** - In PNS disorders the mentation is usually not altered. However, it is sometimes possible to observe obtundation or lethargy in the course of, for example, endocrinopathies such as hypothyroidism.

**posture** - In case of polyneuropathy, posture changes are observed only for severe cases. Postural alterations reflect generalized neuromuscular weakness: the neck and the head may show ventroflexion (emprosthotonus) for the difficulty to support the weight of the head. Affected animals may have prolonged recumbency that, in the most serious cases, results from the inability to maintain the quadruped stance. In extremely severe cases, patients may lie in lateral recumbency (e.g. in acute idiopathic canine polyradiculoneuritis).

**Gait** – Gait examination plays an important role because highlights a number of changes suggestive of a peripheral syndrome. The main sign is the paresis (weakness). The affected patient shows lack of the strength necessary to normally lift the limbs during movement. The term paresis defines a motor function (descending) deficit, characterized by the inability to perform entirely a voluntary movement. The gait examination highlights, as typical features of paresis, hypometry, short-strided gait, hyperflexion of the joints, dragging of the limbs (with consequent noise of rubbing the nails on the ground), associated with lowered tail, emprosthotonus and exercise intolerance. Typically, exercise intolerance occurs after a more or less short walk and is characterized by the progressive difficulty in walking because of progressive inability to further support its weight. The affected patient is forced to recumbency to rest and recover the strength needed to walk again. Rest usually improves this condition, which will recur every time the dog is making an effort. During the walk, you may also notice generalized tremors that exacerbate with the persistence of the exercise, indicative of weakness. The paresis may be apparent mainly on two (paraparesis) or four (tetraparesis) limbs. In severe cases, tetraparesis can rapidly evolve into tetraplegia. A peculiar feature of paresis and plegia due to neuromuscular syndrome is flaccidity, the peculiar clinical sign of lower motor neuron injury.

Gait changes may be acute (e.g. acute idiopathic polyradiculoneuropathy, or fulminant myasthenia) or chronic-progressive (e.g. in paraneoplastic syndromes or endocrinopathies), depending on the disease. As mentioned above, the patient affected by neuromuscular syndrome does not show ataxia. Ataxia defines a lack of movements’ coordination, due to an abnormal function of the ascending part of the NS. Ataxia is not found in patients with generalized lower motor neuron diseases because the lesion primarily and selectively affects the motor’s (efferent) part of the PNS, sparing the ascending (sensitive) function. While evaluating the gait, is therefore crucial not to mistake the consequences of paresis, such as stumbling due to weakness and inability to bear weight, with a primary form of ataxia.

**Postural and proprioceptive reactions** – For the aforementioned reasons, postural reactions should be normal, as PNS diseases only rarely affect the ascending proprioceptive system. However, since the responses to the postural and proprioceptive reactions test includes the phase of the motor response, sometimes they may appear reduced or even absent not because of a real proprioceptive deficit, but because of the serious weakness preventing the normal response to the test. A patient suffering from a severe neuromuscular syndrome may
have difficulty in quickly repositioning the dorsiflexed paw or supporting his weight during wheelbarrowing and hopping tests. If the animal is able to support adequately its own weight, the tests of the postural and proprioceptive reactions are normal in the face of the marked gait changes.

**Spinal reflexes** - In the classic clinical presentation, spinal reflexes examination highlights a **generalized decrease in all reflexes** in both the front and back limbs. Spinal reflexes examination contributes to orient the neuroanatomic localization to a PNS disorder, since there is no spinal cord lesion that may hesitate in a widespread decrease in reflexes spinal.

In particular, the **flexor reflex**, evoked by gentle pressure on the phalanges should hesitate in a flexion of all the joints of the limb. In the neuromuscular patient, the flexor reflex highlights the weakness of the dog/cat who appears unable, despite attempts, to withdraw adequately the limb because due to the lack of the needed strength.

Examination of spinal reflexes may provide different results depending on whether the pathology is a neuropathy, a junction disorder or a muscular disease. Spinal reflexes are usually decreased only during neuropathy. In the other two forms of affection, the reflected activity is generally preserved.

**Cranial nerves** - In the occurrence of polyneuropathy, dysfunctions of the cranial nerves are relatively rare, with the exception of those affecting the Vagus Nerve which, when present, may result in **regurgitation** due to the presence of **megaeosophagus** (e.g. in myasthenia gravis), **changes in voice, dysphonia** (e.g. in Acute Idiopathic polyradiculoneuritis) and also respiratory problems such as **coughing, inspiratory stridor** and **dyspnoea** related to **laryngeal paralysis** or, in case of frequent complications of the **megaeosophagus**, aspiration pneumonia.

Further deficiency of the cranial nerves can be found in inflammatory systemic diseases primarily affecting the roots of the spinal nerves with the involvement of the cranial nerve roots as well. It is the case of polynuernitis that may affect besides the spinal roots, also the facial nerve, or the motor component of the trigeminal nerve. Other pathologies, such as myasthenia gravis, besides megaeosophagus, can display cranial nerves deficits, particularly reduced/absent palpebral reflex and facial paralysis.

**Muscular tone and trophism** – The neurological examination ends with palpation of the muscular masses, in order to evaluate tone and trophism, which are often abnormal during neuromuscular syndrome. Muscles are often reduced in tone (**muscular hypotonia**) and may be affected by a more or less marked **neurogenic atrophy**. Neurogenic atrophy appears suddenly (in less than 10 days) and causes an extreme volume reduction of the muscle in case the disease causes axonal degeneration and/or destruction, as in some severe forms of acute polyradiculoneuritis.

Neuropathic disorders affecting primarily the integrity of the axon are characterized by reduction in both tone and muscle trophism. If, however, the polynuernopathy is the result of the demyelination process alone, tone and muscular trophism can be almost normal.

During primary myopathy (i.e. not due to the effect of denervation) generally muscular hypo-atrophy occurs. In some cases of hereditary myopathies, the opposite condition of hypertrophy may be detected. Primary myopathies may be associated with a reduced muscle tone; Myotonic myopathy may hesitate in hypertonus due to continuous contraction.

In summary, it is possible to state that the "classic" neuromuscular syndrome is characterized by exercise **intolerance and weakness**, sometimes so severe to make impossible to assume the quadrupedal stance and force the patient in lateral recumbency. Typically, **general reduction in spinal reflexes** can occur, while generally the cranial nerves, except for the Vagus nerve, are spared. The muscular masses of the affected animal are often **hypotonic** and severely atrophic. From the clinical point of view, it’s almost impossible distinguish selectively between nerve, muscle, or neuromuscular junction disorders. In general, it can be stated that when the nerve is affected the clinical picture is characterized by a marked generalized reduction of the spinal reflexes and a decrease in muscle tone. Patients with myopathy may exhibit exercise-related weakness, normal spinal reflexes, either muscular atrophy or hypertrophy and myalgia. The disorders of the neuromuscular junction, however, show exercise intolerance associated with normal spinal reflexes as well as normal muscular tone and trophism.
THE “ATIPICAL” CLINICAL PRESENTATION

Generalized peripheral nervous system pathologies, albeit rarely, may occur in an unusual way, different from what has been described so far.

For example, it is necessary to consider the possibility, not even too sporadic, that disorders occur in some body districts only at the subclinical level, so that the clinical evidence of a peripheral problem sometimes does not correspond to the real extension of the pathological process. This becomes evident in cases where the weakness is markedly different between the front and the hind limbs. The clinical picture of a polyneuropathy can show a simple paraparesis on the hind limbs. A negligent examination might be misleading making the examiner thinking of a spinal cord lesion. More rarely, the exacerbation of clinical signs may originate from the front limbs. Sometimes, especially in the polyradiculoneuropathies/polyneuropathies affecting the myelin sheath, the reflexes may appear quite normal, deceiving the examiner. In these animals, usually is not appreciable hypotonia nor the muscular atrophy, typical of the lower motor neuron affections.

In veterinary medicine, the neurologist is keen to consider PNS disorders as affecting almost exclusively the motor component, that is, the neuromuscular system. However, even sporadically, there may be cases of mixed polyneuropathies and/or even of sensory polyneuropathies. In these cases, the clinical presentation may include proprioceptive ataxia, due not to a spinal cord lesion but to an impaired conduction capacity of the peripheral nerve. Spinal reflexes may be decreased not because of the efferent dysfunction but, rather, because of the afferent nerve dysfunction. Along with ataxia, the examiner can detect analgesia of the periphery of the limbs, documented by the animal inability to perceive the pain of wounds arising, for example, from the dragging of the toes, up to the loss of the nails and the exposure of the bones. An opposite phenomenon, however, caused by the same dysfunction of the sensory perception, is the lick and/or the bite of the extremities, leading sometimes to self-mutilation. Probably, this phenomenon is related to the onset of paraesthesia similar to those producing burning and tingling sensations in humans. Compulsive licking of the paws can be considered a sign of paraesthesia and has to be differentiated from pruritus/discomfort originating by allergy and inflammatory lesions. Proprioceptive loss due to sensory neuropathies produce ataxia of the limbs and true postural reaction deficits. In addition, these patients may have reduced or absent reflexes owing to the loss of the sensory part of the reflex arc. In sensory neuropathies, muscle strength, tone, and mass are usually preserved.

**Muscle diseases** may present with clinical frameworks that have little to do with the typical clinical presentation of PNS affections. Some examples are parasitic myositis, such as Neosporosis, affecting younger dogs and causing severe muscle fibrosis resulting in permanent contractures. Typically, a dramatic contracture of the hind limbs is observed, which ultimately can revert the knee angle.

Sudden and extremely painful cramps, tremors, fasciculations and muscle stiffness may occur during Hypocalcaemia.

Myokymia and neuromyotonia, described in the Jack Russell Terrier and observed sporadically in the dachshound, seem to be related to an altered axonal excitability of genetic origin. The clinical presentation is characterized by stiffness of all muscles (neuromyotonia), while myokymia describes worm-like movements of the muscles visible under the skin. Neuromyotonic attacks can be exacerbated by stress and fatigue and are often accompanied by severe hyperthermia. Such episodes can be fatal.

Pseudomyotonia is well-known in a modest percentage of dogs with hypercortisolism. This rigidity can also be observed after several months of remission of the signs following trilostane therapy. The reason for this generalized hypertonia when other typical signs of hypercortisolism are controlled by therapy are still unknown.

DIFFERENTIAL DIAGNOSES AND DIAGNOSTIC WORK-UP

Once assessed the neuroanatomical localization of the lesion at the level of the peripheral nervous system, it is essential to list the possible differential diagnoses considering all the diseases that, at least in theory, can affect this part of the nervous system. The importance of this procedure lies in that the veterinarian has to set up, based on the different diagnostic-differential hypotheses, a diagnostic protocol including the most appropriate investigations to reach a definitive diagnosis and, thus, start the best therapy, whenever possible.

An exhaustive discussion of the possible differential diagnoses of the neuromuscular patient is not the goal of these notes. In order to draw up the list of possible differential clinical diagnoses, should be considered the information gathered with the signalment and history.
Regarding signalment, age and breed are important factors to consider. Some diseases are typical of certain breeds, such as the muscular dystrophy in the Golden Retriever or the hereditary polyneuropathy in the Alaskan Malamute. In relation to age, paraneoplastic syndromes tend to affect older subjects, whereas congenital disorders manifest themselves since birth. Numerous neuromuscular genetic breed-related disorders affect young patients. Nevertheless, their incidence is quite low. Older patients may suffer from endocrinopathies or paraneoplastic disorders.

The onset and progression of clinical signs should be carefully evaluated when collecting the history of the patient. Acute-onset disorders of the LMN system are relatively few, including AICP, botulism, tick paralysis and fulminating myasthenia gravis. The list of chronic disorders affecting the LMN system is much longer and includes degenerative, metabolic, neoplastic, toxic and infectious disorders. In addition to gait abnormalities, information on signs such as regurgitation, coughing or loss of the voice play an important role. The vaccination history of the patient may have important consequences: in younger dogs, an acute polyradiculoneuritis associated with infectious diseases was described.

In clinical practice, the VITAMIN D acronym is used to help listing the differential diagnoses. VITAMIN D scheme is aimed to schematically divide the different categories of diseases, in which V is for vascular disease, I for inflammatory-Infectious T for traumatic, A for anomalous-congenital, M for metabolic, I for idiopathic, N for neoplastic and D for degenerative. The list of clinical differential diagnoses should be ranked according to a probability order: diseases that are more likely to be the cause of the signs shown by the patient should be considered first, leaving the least likely for the last. All this is done with the idea of drawing up an appropriate diagnostic protocol that considers the necessary priorities without becoming too expensive for the owner. In the clinical perspective, despite a thorough and complete diagnostic investigation, it is not always possible to achieve a final diagnosis.

Clinical Pathology and Diagnostic Imaging - Laboratory and Image Diagnostics - The diagnostic protocol for neuromuscular diseases always involves performing CBC and haematobiochemical profile, eventually associated to specific serological or functional tests. Primitive diseases of the nervous system rarely have reflections on the blood tests. Nevertheless, it is possible to highlight abnormal values in presence of metabolic or inflammatory-infectious diseases.

Hypothyroidism can produce hyperlipemia, hypercholesterolaemia and mild non-regenerative anaemia. In a patient affected by hyperadrenocorticism, hematobiochemical tests show leukopenia characterized by lymphopenia and neutrophilia ("Stress leukogram"), increase of Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), hyperlipemia and hyperglycaemia. The demonstration of hyperglycaemia and glycosuria allows the diagnosis of diabetes mellitus. Concerning the inflammatory-infectious diseases, although sporadically, the CBC may show lymphocytosis, neutrophilic or eosinophilic leukocytosis, or, more rarely, monocytosis.

In all these cases, the haematobiochemical profile allows to direct the diagnostic work-up to more specific investigations, such as thyroid hormone dosing or ACTH stimulation test, in the case of suspected endocrinopathies, or to serological tests or molecular biology assays (PCR) to assess the presence of infectious/infestive agents.

Assessment of endocrinopathies includes the evaluation of specific blood parameters. Fructosamine assessment is important in the diagnosis of diabetic neuropathy. Low serum glucose and high insulin levels in fasted patients support the diagnosis of insulinoma.

Confirmation of hypothyroidism includes assessment of total or free T4 (tT4 or fT4, respectively) and canine thyrotropin (or thyroid stimulating hormone: TSH). In doubtful cases, TSH stimulation test (evaluation of tT4 levels immediately before and 4-6 hours after the injection of 75-100 µg of human recombinant TSH [rh-TSH]) is considered the gold standard for the diagnosis.

The biochemical profile also allows to document the involvement of muscles. In myositis a serum increase of enzymes such as aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and, above all, creatinphosphokinase (CPK) may occur.
The search for serum antibodies (immunoglobulins G and M) through serological examinations is useful for the diagnosis of infectious diseases as toxoplasmosis and neosporosis. However, the interpretation of serological assays has several limitations because of false-positive tests due, for example, to the high presence of certain pathogens in the population or the long persistence of IgGs in serum. Over the last few years, it has become increasingly important to confirm or exclude neosporosis or toxoplasmosis through PCR techniques.

To confirm suspected autoimmune diseases such as masticatory muscles myositis (MMM) and myasthenia gravis antibodies against 2M type fibres and those against acetylcholine receptors are sought. Hematobiochemical tests are necessary also because anaesthesia is required to perform more specific tests, such as electrodiagnostic tests and neuromuscular biopsies and, therefore, a thorough assessment of the general state of the patient is essential.

For the investigation of PNS disorders, except for rare cases, diagnostic imaging such as X-ray, ultrasound, computed tomodraphy (TC) and Magnetic Resonance Imaging (MRI) is not of primary importance. In patients with regurgitation, chest radiographs are crucial to demonstrate the presence of megaoesophagus and/or aspiration pneumonia. The suspicion of paraneoplastic neuropathies may require a complete investigation of the body of the patient, including abdomen ultrasound and chest radiographies. Ultimately, although laboratory tests can provide important diagnostic aid, the confirmation of PNS pathologies require electrophysiological studies and histologic examination of the muscle and nerve.

**Electrodiagnostic Test** - Electrodiagnostic procedures provide useful information in diagnosing peripheral and muscular pathology. Although rarely lead to the attainment of an etiologic diagnosis, they allow to define the characteristics of a disease that sometimes remains subclinical. Electrodiagnostic tests help identifying the part of the motor unit involved and also provide a valuable help in finding the area where to perform an appropriate biopsy. The most common electrodiagnostic tests include electromyography, nerve conduction studies (motor and sensory) and evoked potentials.

**Neuromuscular Biopsies** - The last part of the diagnostic work-up of a neuromuscular disease is represented by muscle and nerve biopsy. Neuromuscular biopsy helps to obtain more detailed information on the pathogenesis of the lesion, to distinguish a neuropathy from a primary myopathy and, whenever possible, to achieve an etiologic diagnosis. However, it is important to point out that not always histopathological findings permit the identification of a specific disease. Unfortunately, much more often, neuromuscular biopsies only detect pathological phenomena common to many diseases.

**BIBLIOGRAFIA**