UNIVERSIDADE DE TRÁS-OS-MONTES E ALTO DOURO

Prognostic Indicators in Foals with Neonatal Encephalopathy

Dissertação de Mestrado Integrado em Medicina Veterinária

Ana Alexandra Vieira Vilela

Orientador: Professor Doutor Mário Pedro Gonçalves Cotovio

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TÍTULO DA DISSERTAÇÃO DE MESTRADO EM MEDICINA VETERINÁRIA: Prognostic Indicators in Foals with Neonatal Encephalopathy

ORIENTADOR: Mário Pedro Gonçalves Cotovio

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Declaro que esta Dissertação de Mestrado é resultado da minha pesquisa e trabalho pessoal e das orientações dos meus supervisores. O conteúdo é original e as fontes consultadas estão devidamente mencionadas no texto e na bibliografia final. Declaro ainda que este trabalho não foi apresentado em nenhuma outra instituição para obtenção de qualquer grau académico.

Vila Real, 2019

Ana Alexandra Vieira Vilela

"The love for all living creatures is the most noble attribute of man."

Charles Darwin

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ABSTRACT

Neonatal Encephalopathy is the most common neurological disorder in neonatal foals. It is usually associated with peripartum events that can lead to acute or chronic hypoxia, but neither the etiology nor the pathophysiology are still fully understood. Clinical signs often observed include recumbency, abnormal nursing behavior, loss of awareness of the environment, and seizures. However, a multi-system evolvement can also be found. There is no definitive ante-mortem diagnostic test, so diagnosis is based on history, clinical presentation, elimination of other differentials, and supplementary diagnostic tests. Treatment is mainly supportive, relying on resolution of secondary complications. Prognosis is usually good (60%-80%) in uncomplicated cases.

The purpose of this dissertation is to stablish prognostic indicators in a population of foals with Neonatal Encephalopathy. Medical records from 61 foals with the diagnosis of Neonatal Encephalopathy between 1982 and 2018 were collected from the Equine Hospital of the University of Tennessee, College of Veterinary Medicine, in the United States of America and from the Hospital La Equina, in Málaga, Spain. Variables included in the study were surviving rate, animal identification, predisposing factors of the mare and foal, physical exam and laboratory findings on admission, concurrent diseases, and treatment. In order to determine factors associated with the outcome, these variables were statistically compared between survivors and non-survivors.

The overall surviving rate was 57.4%. Neither gender nor breed were associated with the surviving rate. There were no predisposing factors associated with survival as well. This study demonstrated that normal body temperature, normal glycemia levels, normal creatinine concentration, or absence of recumbency were associated with a good surviving rate, while hypothermia, abnormal glycemia levels, creatinine levels >4 mg/dL, pneumonia, anemia, or sepsis were associated with mortality. Foals with hypothermia, hypoglycemia or hyperglycemia had greater odds of non-survival. The use of antibiotics or non-steroidal anti-inflammatory drugs were positively associated with survival.

In conclusion, body temperature, glycemia, creatinine concentration, absence of recumbency, presence of pneumonia, anemia, and sepsis are the main prognostic indicators in the population in study. Given its influence in survival, using antibiotics or non-steroidal anti-inflammatory drugs are recommended in foals with NE.

Keywords: Neonatal Encephalopathy, Equine, Neonatology, Foal.

RESUMO

A Encefalopatia Neonatal é a doença neurológica mais comum em poldros neonatos. Está, normalmente, associada com problemas no pré-, durante e no pós-parto que podem conduzir a episódios agudos ou crónicos de hipóxia. No entanto, atualmente, nem a etiologia nem a fisiopatologia estão completamente compreendidas. Os sinais clínicos observados mais frequentemente incluem decúbito, perda do reflexo de sucção, perda de afinidade pela mãe, alteração do estado de alerta e convulsões. No entanto, também pode ocorrer um envolvimento multissistémico. Uma vez que não existe um teste *ante-mortem* definitivo, o diagnóstico é feito com base na história clínica, sinais clínicos, eliminação de diagnósticos diferenciais e exames diagnósticos complementares. O tratamento é de suporte, baseando-se na resolução de complicações secundárias. O prognóstico é normalmente bom (60%-80%) em casos não complicados.

O objetivo desta dissertação é definir fatores de prognóstico numa população de poldros com Encefalopatia Neonatal. Foram recolhidos os registos médicos de 61 poldros com o diagnóstico de Encefalopatia Neonatal do Hospital Equino da Universidade do Tennessee, Faculdade de Medicina Veterinária, Estados Unidos da América e do Hospital La Equina, em Málaga, Espanha, datados desde 1982 até 2018. As variáveis incluídas neste estudo foram a taxa de sobrevivência, identificação do animal, fatores predisponentes da égua e do poldro, exame físico e exames laboratoriais, doenças concorrentes e tratamento. De forma a determinar os fatores associados com o prognóstico, estas variáveis foram estatisticamente comparadas entre sobreviventes e não-sobreviventes.

A taxa de sobrevivência desta população foi de 57.4%. Nem o género, nem a raça tiveram influência na taxa de sobrevivência. Os fatores predisponentes relativos à égua ou poldro também não mostraram nenhuma associação com a sobrevivência. Este estudo demonstrou que temperatura corporal normal, níveis de glicémia normais, concentrações de creatinina normais e ausência de decúbito tiveram uma influência positiva na sobrevivência. Por outro lado, a presença de hipotermia, níveis de glicémia anormais, valores de creatinina superiores a 4 mg/dL, pneumonia, anemia ou septicémia estiveram associados a mortalidade. De facto, poldros com hipotermia, hipoglicémia ou hiperglicémia tiveram mais chances de morrer. O uso de antibióticos ou de anti-inflamatórios não esteroides tiveram uma associação positiva com a taxa de sobrevivência.

Em conclusão, a temperatura corporal, glicémia, concentração de creatinina, ausência de decúbito, presença de pneumonia, anemia ou septicémia são os indicadores de prognóstico desta população. Dada a sua influência na sobrevivência, o uso de antibióticos e anti-inflamatórios não esteroides estão recomendados em poldros com Encefalopatia Neonatal.

Palavras-chave: Encefalopatia Neonatal, Equino, Neonatologia, Poldro.

CONTENTS

FIGURE INDEX

TABLE INDEX

GRAPHIC INDEX

Abbreviations

ATP	Adenosine Triphosphate
CI	Confidence Interval
CNS	Central Nervous System
CT	Computed tomography
DMSO	Dimethyl sulfoxide
EEG	Electroencephalography
FPTI	Failure of passive transfer immunity
GI	Gastrointestinal
HIE	Hypoxic-Ischemic Encephalopathy
HPA	Hypothalamic-Pituitary-Adrenal Axis
IgG	Immunoglobulins G
IV	Intravenous
Mg	Magnesium
MRI	Magnetic Resonance Imaging
NE	Neonatal Encephalopathy
PAS	Perinatal Asphyxia Syndrome
pNF-H	Plasma Concentration of the Phosphorylated Axonal forms of Neurofilament H
PRP	Platelet Rich Plasma
REE	Resting Energy Requirements
OR	Odds Ratio
TH	Thyroid Hormones
T3	Triiodothyronine
T4	Total Thyroxine
UTCVM	University of Tennessee, College of Veterinary Medicine
UCHL 1	Ubiquitin C-Terminal Hydrolase 1

CHAPTER I – BIBLIOGRAPHIC REVIEW

1. INTRODUCTION

Normal neonatal foals, as precocious species (Gold, 2017) are born with an immediate post-birth capacity for survival in the wild (Mellor, 2015). The foal is delivered in 20-40 minutes of active labor and it is essential to achieve a successful transition from the intrauterine state to an extrauterine state of consciousness, which includes the ability to stand up, ambulate, and suck from the mare (Aleman, 2017). It is expected that foals will stand and nurse within 2-3h after birth and stay close to the mare for protection and to bond (Grogan, 2005). It has been reported that skin-to-skin contact, similar as in humans, benefits newborns in short and long-term outcomes (McCallie, 2017), with a reported more accelerated neurophysiological development (Scher, 2009). These critical milestones must be achieved within the first few minutes and hours after birth, otherwise, significant problems associated with inadequate colostrum and milk intake may occur, leading to energy depletion, weakness, difficulty to rise, hypothermia, failure of passive immunoglobulin transfer, infection and death (UC Davis International Animal Welfare Training Institute, 2018).

Neonatal encephalopathy (NE) is a very broad syndrome that includes any neonate with neurologic signs, regardless of etiology. Such signs include difficulty with the initiation and maintenance of respiration, depression of tone and reflexes, subnormal levels of consciousness and frequently seizures (McKenzie III, 2018; Bernard, 2018a). It is a noninfectious syndrome and is primarily characterized by central nervous system (CNS) dysfunction (MacKay, 2005; Tennent-Brown, 2015). Besides that, it is now known that it can affect many organ systems beyond the neurologic one, such as the cardiopulmonary, endocrine, gastrointestinal and renal systems and can cause behavioral dysfunctions (Gold, 2016).

It is recognized as the most common neurological disorder in neonatal foals (Lyle-Dugas, 2017) with an estimated incidence of 1-2% of all births (Bernard, 1995; Bernard, 2018a). It is usually associated with peri-partum events that may result in acute or chronic hypoxia, such as dystocia, cesarean section, premature placental separation, or placentitis (Tennent-Brown, 2015). Other terms commonly used are hypoxic-ischemic encephalopathy (HIE), perinatal asphyxia syndrome (PAS), neonatal maladjustment

syndrome, dummy foal syndrome, wanderer, convulsive and barker foal (Vaala, 2003; Mackay, 2005; Wong, 2011; McKenzie III, 2018).

The confusion associated with the nomenclature is based on the fact that:

- Clinical signs are unspecific for NE;
- Diagnosis is made based on elimination of other potential etiologies;
- It is difficult to determine the exact cause of neurologic dysfunction in foals;
- Elevations in neurosteroids concentrations may be associated with NE, particularly in those without a hypoxic episode.

Therefore, to overcome that, there has been an effort to restrict the term HIE to the subset of cases of NE in which a hypoxic-ischemic episode occurred (McKenzie III, 2018). Although this disorder has often been compared with PAS in human infants, it seems likely that oxygen deprivation may not be the only pathophysiologic mechanism by which NE occurs. An inflammatory insult or failure to correctly transition from intrauterine to extrauterine life can also cause NE.

Since the etiology and pathophysiology are still not fully understood, the vast part of information available is extrapolated from human and other animal models (Gold, 2017). However, some situations differ significantly from their human counterparts, like the fact that many foals recover quickly and demonstrate no neurologic sequelae (McKenzie III, 2018), while about 60% of human infants with PAS die, and 25% of the survivors have some neurological disability, like cerebral palsy (Ballet, 2010). Additionally, long-term outcome for foals is significantly better than for infants (Gold, 2017).

The clinical signs associated with NE can range from mild depression with loss of the suck reflex (Wilkins, 2015a) to severe neurologic signs including central blindness, seizures, coma and death (McKenzie III, 2018). In fact, equine neonatal sepsis and perinatal asphyxia syndrome are the most important causes of morbidity and mortality in newborn foals (Galvin, 2010).

2. PREDISPOSING FACTORS

In order to understand the causal pathways and develop preventive strategies, it is essential to identify the risk factors for NE (Kurinczuk, 2010).

Conditions associated with neonatal encephalopathy include maternal diseases, such as endotoxemia, respiratory disease, hemorrhage/anemia, fescue toxicity, placentitis, chronic or acute/premature uteroplacental separation, and surgery (McSloy, 2008; Gold, 2017), meaning that the health of the mare during gestation is an important key point in the survival of foals with this condition (Gold, 2016). Furthermore, advanced mare's age and multiple parity can also contribute to placental insufficiency and, subsequently, to foal's disease and gross abnormalities in the placenta (Wilsher, 2003).

Problems during parturition, such as induced parturition, dystocia, caesarean delivery and post-term pregnancy can also lead to hypoxia and ultimately to NE (Wong, 2011; Hahn, 2008; Gold, 2017; McKenzie III, 2018).

Conditions associated with the fetus/foal include meconium aspiration, twinning, fetal infection (Wong, 2011; McKenzie III, 2018), congenital abnormalities, umbilical cord compression, sepsis, prematurity, and dysmaturity (Gold, 2017).

Nevertheless, it is still not fully understood how these factors contribute to non-survival in foals with NE, which complicates the determination of prognosis and decision making (Gold, 2016).

3. PATOPHYSIOLOGY

The underlying pathophysiology of NE is complex (McKenzie III, 2018) and likely multifactorial (Wilkins, 2015a). It is still not completely understood in the foal or in human infants (Gold, 2017), being most information regarding the pathophysiology extrapolated from human studies to veterinary medicine (Wong, 2011).

A primary hypoxic or anoxic insult, as a result of a reduction in the uterine and umbilical circulation, leads to the activation of the sympathetic adrenergic nervous system (Wilkins, 2004a). This causes a critical redistribution of cardiac output, resulting in preferential blood flow to the heart, brain and adrenal glands and decreased perfusion to other organs like the lungs, gastrointestinal tract, liver, spleen, kidneys, muscles and skin (Hahn, 2008; Harteman, 2013; Magdesian, 2014); hence the likelihood of multisystem evolvement (Hahn, 2008; Wilkins, 2015a). If the initial insult continues to a point where the fetus cannot maintain this centralization of circulation, the cardiac output falls, and cerebral circulation decreases (Wilkins, 2004a). An alteration of the blood flow can cause a decrease in cerebral oxygenation (Gold, 2017) which initiates a cascade of deleterious events (McKenzie III, 2018) leading to neuronal cell death and injury (Wilkins, 2015a), either during the ante-, peri- or post-partum period (Gold, 2017).

Furthermore, studies in human infants have suggested that inflammatory mediators may contribute to the cascade of events that leads to neuronal brain injury (Shalak, 2002), meaning that mares with placentitis or that experienced some illness during pregnancy, will induce an inflammatory response from the fetus, that may be an important contributor to the pathogenic cascade (Hagberg, 2015; Gold, 2017). Thus, NE might occur after in utero exposure to infection or inflammation, leading to the cascade of inflammatory cytokine and causing CNS injury through direct cytotoxicity, changes in vascular tone and tissue perfusion and increased blood-barrier permeability and release of excitotoxic neurotransmitters (Tennent-Brown, 2015). Besides that, microglia can also be activated by hypoxic-ischemic events and consequently produce proinflammatory cytokines, leading to an increase in cerebral blood flow, with alteration of neuronal and microglial function, causing further brain injury and cytotoxic edema (Wong, 2011; Hagberg, 2015).

The pathogenesis of NE can be characterized in 2 different phases:

1) **Primary neuronal cell death**: the hypoxic/anoxic event results in a significant decrease in oxidative phosphorylation in the brain, with concomitant shift toward anaerobic metabolism. This leads to the depletion of adenosine triphosphate (ATP) reserves, accumulation of lactate, and a failure of cellular homeostasis. (Wassink, 2014; McKenzie III, 2018). The Na/K transcellular pumps cannot maintain the ionic gradient, the membrane potential is lost (Wilkins, 2004a) and an intracellular accumulation of sodium, calcium and water occurs (Hahn, 2008; Wassink, 2014; McKenzie III, 2018). This increased intracellular concentration of calcium in the neurons activates calcium-dependent lipases, proteases, and endonucleases (Figure 1). Also, protein synthesis is ceased (Wilkins, 2004a). Following the anoxic cellular depolarization there is the release of the potent excitatory neurotransmitter glutamate from presynaptic vesicles. Glutamate allows calcium to enter the cells by acting on the N-metyl-D-aspartate receptor and opening its channels, leading to neuronal injury by potentiating calcium influx into the neurons. Ultimately, there is a perpetuation of excitotoxicity, causing neuronal cell death and brain injury (Ferriero, 2004; Hahn, 2008; Johnston, 2001; McKenzie III, 2018). When the insult is over, protein synthesis returns to normal in less vulnerable areas of the brain but remains inhibited in specific areas. It seems likely that the loss of protein synthesis can be an early indicator of cell death associated with the primary hypoxic/anoxic insult (Wilkins, 2004a);

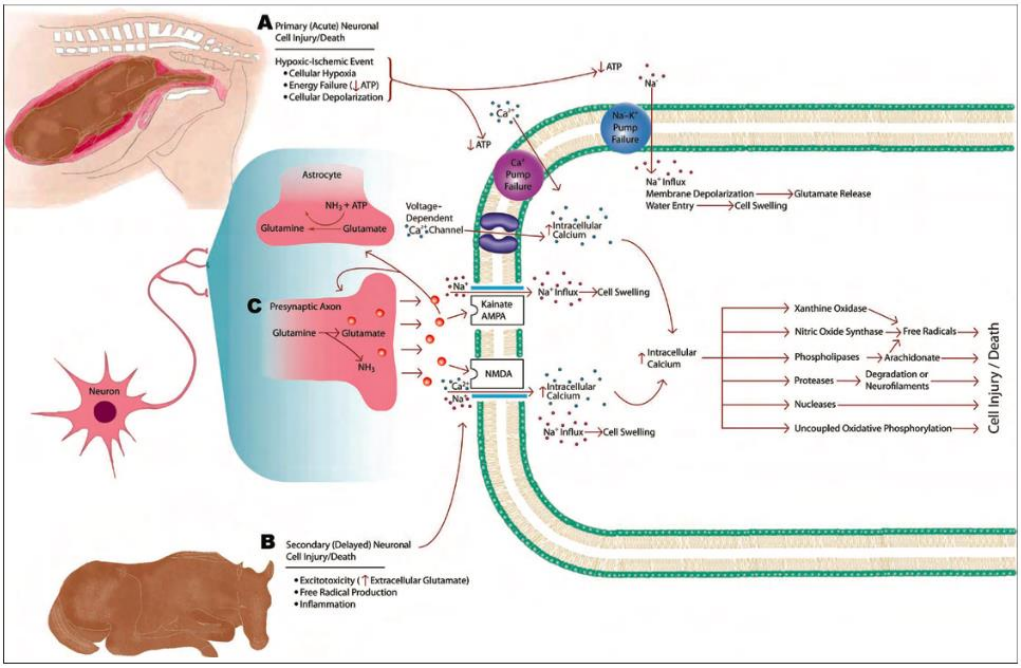


Figure 1 **- Schematic representation of the pathogenesis of NE, as a result of a hypoxic-ischemic event (HIE).** Retrieved from Wong, D., 2011.

2) **Delayed neuronal cell death**: When the initial acute phase injury is stabilized, it is followed by the second wave of delayed neuronal cell death (McKenzie III, 2018), which is associated with reperfusion injury and can occur during the hypoxic-ischemic episode or hours to days after (Gold, 2017). It is caused by the increased production of oxygen radicals, nitric oxide and inflammatory cell infiltration (McKenzie III, 2018). Also, there's an imbalance between excitatory and inhibitory neurotransmitters (Wilkins, 2004a). This ultimately leads to cell death and activation of apoptotic cascades, resulting in further tissue damage (Johnston, 2001; McKenzie III, 2018). Besides that, an inflammatory response causes an increase in the blood flow and vascular permeability, which can be associated with the development of edema. It is documented that this excitatory cascade extends over several days from the time of insult and is modifiable (Wilkins, 2015a). In fact, after restoration of oxygenation and circulation, the destructive processes continue creating damage in the brain, over hours, days and possibly weeks (Whitelaw, 2000; Ferriero, 2004). This period gives a therapeutic window during which the full extent of the brain's damage may be reduced (Whitelaw, 2000). Moreover, it is believed that neurochemical changes (excessive neurotransmitter release), are pivotal in the pathophysiology of secondary neuronal death (Wilkins, 2004a).

Also in human literature, we can find an association between the nature of the event and the severity and distribution of the neurologic damage. For example, a chronic hypoxic-ischemic insult was related with periventricular leukomalacia, whereas an acute hypoxic episode affected mainly the basal ganglia and thalamus. This can be useful in defining neuroprotective strategies and therapies for neonates with NE (Wilkins, 2004a).

In situations of decreased oxygen availability, the fetus decreases its rate of growth, being this the reason why some of these foals are born with physical disproportions, such as large head, little muscle mass, small frail body, and little to no fat (Hahn, 2008).

Some mild cases of foals with NE were born without any complications and had a fast recovery, with no behavioral or neurologic deficits (Gold, 2017). Some of these foals have persistence of neuroinhibitory steroids, such as progesterone, pregnenolone, androstenedione, dehydroepiandrosterone, and episterone, which could possibly indicate an unsuccessful transition from intrauterine to extrauterine life (Aleman, 2013). This is

suggestive that other factors may contribute to the pathogenesis of this disease (Gold, 2017). It is hypothesize that a post-natal persistence of fetal physiological conditions linked to aberrant activity of the fetal hypothalmic-pituitary-adrenal axis (HPA) and/or elevated pregnane concentrations may also play a role in the pathogenesis of NE (Diesch, 2013).

The HPA function is dynamic in the neonatal foal, being responsible for the maintenance of water homeostasis and cardiovascular, immunologic, and metabolic functions, helping the transition from intra to extrauterine life (Ousey, 2004; Hart, 2009; Barrett, 2010). Before 290 days of gestation, the HPA axis has a basal, unresponsive to stimuli activity, being activated after 300 days of gestation. After this time, HPA axis's activity increases, leading to the release of cortisol in fetal circulation immediately before birth (Fowden, 2012). Cortisol has a key-role not only in the terminal maturation of the fetus but also for neonatal adaptation for the extrauterine life (Hillman, 2012). This is important in the perinatal period because it integrates a series of events, such as the onset of labor, the acceleration of fetal tissue maturation immediately before birth, the timely onset of colostrum/milk production by the mother, and the impetus to engage in mother-young bonding (Mellor, 1988). All of these processes are of major importance for foal survival and any alteration can seriously jeopardized it (Diesch, 2013).

While in utero, the fetus is under the neuroinhibitory effects of high concentrations of adenosine, progesterone, allopregnanolone, pregnanolone, prostaglandin D2 and a placental neuroinhibitory peptide. This combined with buoyancy, warmth and cushioned tactile stimulation produces somnolence, contributing to the sleep-like state of the fetus (Mellor, 1988; Mellor, 2006; Mellor, 2010; Diesch, 2013). After birth, the onset of consciousness is achieved by the reduction in cerebral cortical inhibition by factors that are unique to life in utero, as well as to an increase in cerebral cortical activation associated with birth (Diesch, 2013). Normal healthy newborn foals have high concentrations of pregnanes at birth that decrease promptly during the first 48 hours of life. A study performed by Aleman *et al.* (2013) demonstrated that pregnane concentrations in foals with NE remained increased over the normal 48 hour time period, while in healthy neonatal foals declined rapidly to, essentially, zero in the same time frame. Additionally, sick foals affected with other disorders than NE, also had significantly lower progesterone and pregnenolone concentrations at 48 hours compared with birth. This supports the theory that a lack of the normal transition from synthesis to

inhibition of specific neurosteroids for readiness for birth may have a role in the pathogenesis of NE (Aleman, 2013).

As seen in another study, an infusion of allopregnanolone in a healthy foal resulted in signs of sedation and decreased responsiveness to the environment. As the concentration was increased, these signs were more notorious, resulting in dramatic neurobehavioral effects, with the foal in recumbency, stupor, unresponsive to the mare, environment, sound and tactile stimulation. These signs persisted during the infusion and began to fade within 8 minutes after cessation. They kept fading until completely disappear at around 30 minutes after cessation of the infusion, with no long-term effects observed following the infusion (Madigan, 2012).

With this being said, it is thought that NE may comprise of more than one phenotype: foals that suffered hypoxia and ischemia and foals with persistence of fetal hypothalamic-pituitary-adrenocortical axis and increased pregnanes (pregnenolone, progesterone and metabolites) concentrations (Aleman, 2013).

4. CLINICAL SIGNS

Neonatal encephalopathy can produce a wide range of clinical signs (Magdesian, 2014). Most foals may appear healthy at birth but will typically develop central nervous system abnormalities within the first 72 hours of life (McKenzie III, 2018; Bernard, 2018a). In fact, two major categories of NE-affected foals can be distinguished:

- Category 1: includes foals that are normal at birth but develop signs within the first 48 hours of life (MacKay, 2005; Wong, 2011; McKenzie III, 2018);
- Category 2: includes foals that are abnormal at birth, usually associated with documented predisposing factors for NE (MacKay, 2005; McKenzie III, 2018).

Foals from Category 1 usually have an excellent prognosis while foals from Category 2 are associated with worst prognosis for survival (Gold, 2017). The clinical presentation is extremely variable and can include:

- Behavioral changes (Figure 2 and 3): loss of affinity for the mare, inability to find the udder (Wilkins, 2015a), loss of awareness of the environment, inappropriate nursing behavior, head-pressing, and abnormal vocalization (Hahn, 2008; Wong, 2011; McKenzie III, 2018);

Figure 2 and 3– **Foal with NE, showing signs of loss of awareness of the environment and abnormal nursing behavior by nursing the fence (left).** Images kindly provided by the University of Tennessee.

- Altered mentation (Figure 4): varying from depression, stupor, somnolence, difficult to arouse or coma to hyperresponsiveness (Hahn, 2008; Wong, 2011; McKenzie III, 2018);
- Cranial nerve dysfunction (Figure 5): loss of suckle reflex, weak tongue tone, tongue protrusion, and dysphagia (Hahn, 2008; McKenzie III, 2018);
- Central nervous system (CNS) dysfunction: hypotonia, tremors, hypertonia, proprioceptive deficits, central blindness, irregular respiratory patterns (Wong, 2011; McKenzie III, 2018) (sometimes leading to

Figure 4 - **Foal with somnolence and difficulty to arouse, often seen in foals with NE.** Image kindly provided by the Hospital La Equina.

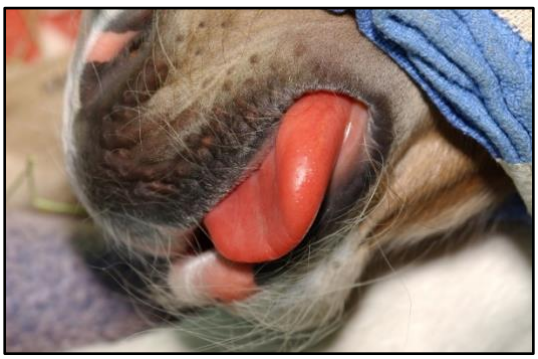


Figure 5 - **Tongue protrusion in foal with NE.** Image kindly provided by the University of Tennessee.

respiratory acidosis, hypoxemia and/or hypercapnia (Giguère, 2008; Tennent-Brown, 2015), opisthotonus, and seizures (McKenzie III, 2018). These can range from mild, abnormal movement of the face and jaw to generalized seizures with recumbency and paddling (MacKay, 2005; Tennent-Brown, 2015). NE is the most common cause of neonatal seizures. They usually manifest within 2 days of life and are associated with long-term seizure risk (Shetty, 2015).

- Other common clinical signs include loss of menace response, fixed dilated pupils, nystagmus, dysuria and wandering (Magdesian, 2014).

Clinical signs are usually related to the cerebrum, but some foals also show signs of brain stem or spinal cord dysfunction, (Tennent-Brown, 2015) often from Category 2 (MacKay, 2005).

The gastrointestinal tract and renal system are commonly affected but the cardiovascular, respiratory, and endocrine systems may also be involved (Wilkins, 2015a). In fact, foals with NE regularly have gastric reflux, feeding intolerance, bloat, meconium retention, colic, and persistent increases of creatinine concentration (Tennent-Brown, 2015; Bernard, 2018a). Oliguria with peripheral edema formation can also be found (Vaala, 2003). As shown in one review of PAS in human neonates, CNS was the most affected body system (82%), followed by the renal system (42%), cardiac (29%), gastrointestinal (29%) and respiratory (26%) systems (Wong, 2011). Limb deficits and generalized spasticity are rare (Vaala, 2003).

5. DIFFERENTIAL DIAGNOSES

The most common differential diagnoses for NE include sepsis-associated encephalopathy, prematurity/dysmaturity and hypoglycemia (Tennent-Brown, 2015). Less common differentials include bacterial meningitis, cerebral trauma, cerebral vascular accidents, congenital abnormalities such as hydrocephalus (Wilkins, 2015a), cerebellar abiotrophy and occipitoatlantoaxial malformation, epilepsy, narcolepsy and cataplexy, botulism, tetanus, metabolic encephalopathies and seizures disorders (McKenzie III, 2018). Hypothermia and hypovolemia should also be considered (Wilkins, 2006).

6. DIAGNOSIS

It is important to clarify that there is no definitive diagnostic test for neonatal encephalopathy (Volpe, 2012; Mackenzie, 2010), so diagnosis is made based on historical information, clinical signs, neurologic examination, elimination of other differentials, and supplementary diagnostic exams (MacKay, 2005; Wong, 2011; McKenzie III, 2018; Bernard, 2018a). These include electroencephalography, brain imaging (computed tomography and magnetic resonance imaging), complete blood count, serum biochemistry, arterial blood gas analysis, blood culture, urinalysis, and measurement of IgG (Wong, 2011).

The clinical signs associated with NE are not specific of this disorder and share similarities with other causes of NE, like sepsis, hypoglycemia and prematurity (Wong, 2011; Gold, 2017). Also, NE can act alone or be complicated by other problems (Gold, 2017), making the diagnosis of NE challenging and very limited (Cruz, 2017; McKenzie III, 2018).

If the birth is attended, gross evaluation of the placenta after delivery can give information regarding fetal well-being. If the membranes are markedly lighter (less than 11% of the foal's birth mass), it means that their surface area was smaller than normal, therefore not being able to give the adequate nutritional support to the fetus (Pirrone, 2014).

1. History

Foals with history of known risk factors of NE, such as dystocia, delivery by caesarian section, premature delivery, abnormal placenta of the mare, premature placental separation or that required resuscitation after delivery, are expected to develop this condition (Hahn, 2008).

2. Neurologic Exam

A concise neurologic examination is indispensable during the initial evaluation of foals with neurologic signs and when monitoring their progression (MacKay, 2005; Tennent-Brown, 2015). Before initiating this exam, objective history-taking and a complete physical examination should be done (MacKay, 2005; Bernard, 2018a). Some

characteristics that can give the clinician early insights into the neurologic status of the newborn foal are:

- Normal foals should be sternal within the first 5 minutes of life and have a suckle reflex within 20 minutes (it is usually present at birth);
- They should stand within an hour, except if the floor is hard and/or slippery or if the foal is very large (in these cases, a little extra time may be needed);
- They are expected to find the mare's udder and nurse by 2h of age;
- It is expected newborn foals to be inquisitive about the environment surrounding them and to remain close to their dams. Their movements are also typically swift and may appear jerky;
- Normal foals usually defecate soon after standing or following their first meal but some do not defecate for 24 hours;
- When the foal is manipulated or stimulated, it is expected to observe an exaggerated response that must not be confused with the hyperresponsiveness associated with NE. When manipulating the head, they can also have an exaggerated physiologic, horizontal nystagmus, with the fast phase in the direction of head movement;
- Healthy foals may allow their tongue to hang out of the mouth but are able to completely withdraw it when stimulated;
- During the ophthalmologic exam, the clinician must take into consideration that pupils in foals 1 to 2 days old should be equal in size, large, and circular. Over the first week of life, they should decrease in size and becoming more ovoid. Newborn foals do not have menace response until about 2 weeks of life, since it is a learned behavior. Also, direct and indirect pupillary light reflexes should be present, with its rate of constriction being influenced by the degree of excitement;
- A crossed extensor reflex that can last for up 3 weeks and an abrupt patellar reflex may also be present in normal newborn foals (Tennent-Brown, 2015).

3. APGAR Score

The modified APGAR Scoring System (Table 1) is a visual classifying system used to evaluate neonatal vitality. It can range from zero to ten (Cruz, 2017) and has been developed as a guide of probable level of fetal compromise, identifying the need for medical intervention and defining when resuscitation should be initiated. This method does not alleviate the need of further monitoring (Wilkins, 2006). A low APGAR score and metabolic acidosis are associated with neurologic dysfunction (Volpe, 2012).

Table 1 - **APGAR Scoring System**. Adapted from Palmer, J. E., 2009.

Score	2	1	0
Heart rate	>60 bpm and regular	<60 bpm or irregular	Absent
Respiratory rate	Regular	Irregular	Absent
Muscle tone, postural responses	Maintains sternal recumbency and attempts to stand	Some flexion of limbs and muscle tone	Laterally recumbent, no muscle tone
Response to stimuli	Head shake, sneeze, or cough	Weak ear movement when stimulated, facial grimace	No response to stimulation

*The scores of the parcels are summed to derive the APGAR score, which is interpreted the following way: Score 7-8: normal; Score 4-6: mild to moderate asphyxia. The foal should be stimulated and oxygen therapy should be implemented; Score 0-3: severe asphyxia. Aggressive cardiopulmonary resuscitation must be done.

4. Laboratory parameters

Careful analysis of the biochemistry panel in critically ill foals, particularly those with neurological signs, is imperative in the assessment of these patients (Johnson, 2012).

We can identify some alterations that are usually present in foals with NE, such as low blood glucose before suckling (normal range values are 93.8-185.6 mg/dL (McAuliffe and Slovis (2008)), hyperlactatemia, hypoxemia and respiratory acidosis (PO2 <60 mmHg; PCO2 >65 mmHg), hypercapnia, metabolic acidosis (ph <7.3;

bicarbonate concentration <20 mEq/L), hypocalcemia and increased serum concentrations of creatinine (normal range values for creatinine are <2 mg/dL and for BUN are <22 mg/dL (Stoneham (2004)) and creatinine kinase (Vaala, 2003; Chaney, 2010). In fact, a study reported that among a 28 foals population with spurious hypercreatininemia included in the study, 20 (71%) had clinical signs of NE. This suggests that these foals did not suffered from renal damage, but rather adverse maternal and/or placental conditions (Chaney, 2010). However, all of these alterations can also be present in a large number of other neonatal diseases (Gold, 2017).

The pancreas and the liver can also be affected, so an insulin-responsive hyperglycemia and increased concentrations of bilirubin and hepatocellular enzyme sorbitol dehydrogenase can also be present (Vaala, 2003).

It has been reported that foals with NE have significantly higher Mg concentration at admission, comparing with healthy, septic, premature/dysmature foals or with other pathologies. This can be explained by the extracellular movement of Mg (an intracellular ion), as consequence of hypoxia, acidosis and probable cellular injury or competition of hydrogen ions with Mg for protein binding (Mariella, 2016). With this being said, plasma total Mg may be helpful in the diagnosis of NE, in addition to historical information and clinical signs (Mariella, 2016).

Thyroid hormones (THs) concentrations are higher in healthy neonatal foals than in adults (Breuhaus, 2014). Total thyroxine (T4) and triiodothyronine (T3) concentrations in normal foals are 14 times higher and free T4 and T3 concentrations are 5 times higher than those in adults (Irvine, 1975). They slowly decrease over the first weeks of life until the adult's concentrations be achieved (Breuhaus, 2014). Pirrone *et al.* (2013) studied plasma THs concentrations (T3 and T4) in critically ill foals suffering from NE during their first 7 days of hospitalization and compared those values with those in healthy foals. This study suggests that NE may cause lower THs concentrations than the healthy ones (Pirrone, 2013). However, lower THs concentrations have also been reported for other systemic diseases, such as sepsis and prematurity. In fact, a prognostic value of these hormones in these illnesses has been reported, since there are lower THs concentrations in non-surviving septic and premature foals than in surviving septic and premature foals (Himler, 2012).

5. Imaging modalities

In human medicine, other exams are also made, including electroencephalography (EEG), computed tomography (CT) and magnetic resonance imaging (MRI) (McKenzie III, 2018). MRI is one of the best diagnostic and prognostic modalities used in human infants with neonatal encephalopathy, however, there is a lack of published reports of MRI findings in foals with this condition. In 2017, D. M. Wong published the first report describing the MRI findings associated with a suspected diagnosis of neonatal encephalopathy in a foal and compared these images with those described in human literature. This study showed imaging changes consistent with the counterpart disease in human neonates, such as hyperintensity of the basal nuclei, superficial layers of the ventral cerebral hemispheres, ventral thalamic nuclei and rostral aspect of the ventral midbrain (Wong, 2017). Nevertheless, MRI machines are usually limited to referral centers (McKenzie III, 2018).

The EEG has been used as a diagnostic tool in human medicine to assess cerebral cortical function (Williams, 2008). It might provide information regarding the pathophysiology and the prognosis of this condition. Besides that, it has the advantage that it can be performed continuously to detect seizure activity (Tennent-Brown, 2015). However, an EEG is difficult to perform in an unsedated and unrestrained foal and the sedation and movements can derail the results. (McKenzie III, 2018).

This led to the search of non-invasive alternatives like ultrasonography of the optic nerve sheath diameter and ultrasonographic assessment of the atlanto-occipital space. A recent study showed that the ultrasonographic assessment of the atlanto-occipital space was easier to perform and appeared to offer more potential for obtaining standardized image planes, comparing to the ultrasonography of the optic nerve sheath diameter. It compared the ultrasound measurements in healthy foals with the ones obtained in foals with NE and the results were: the dimension of the spinal cord was smaller in foals with neonatal encephalopathy than healthy foals, with no significant change in the overall size of the spinal canal; the dorsoventral diameter of the ventral spinal artery in longitudinal images was also smaller in foals with NE than in the healthy ones; and spinal canal to spinal cord ratios for both cross sectional area and width were significantly larger in foals with NE than in healthy foals; which shows that the differences between the two groups were independent of the size of the foal. It is also possible that the differences in spinal cord dimensions could be related to increased

intracranial pressure, usually found in some critically ill foals. This approach has been described in the past to be useful to aid the diagnosis of subarachnoid hemorrhage or meningitis (Mackenzie, 2010).

6. Biomarkers

Because of the limitation associated with clinical imaging, the use of body fluid analysis in foals with NE to monitor brain injury and evaluate neuroprotective effects may allow early diagnosis and interventions (Lv, 2015). Two potential biomarkers of NE have been recently documented in a study where the plasma concentration of the phosphorylated axonal forms of neurofilament H (pNF-H) and ubiquitin C-terminal hydrolase 1 (UCHL1) were measured. It was found that the diagnostic performance of UCHL1 was significantly higher than that of pNF-H, with sensitivity of 70% and specificity of 94% for diagnosis of NE. This demonstrates that UCHL1 may have potential as a biomarker of neuronal injury in neonatal foals with signs consistent with NE. This study also referred that pNF-H was heavily concentrated in the white matter and in deep regions of the brain, like the medulla and midbrain, while UCHL1 was more concentrated in the gray matter. This suggests that a blood UCHL1/pNF-H ratio can be useful in localizing the region of the brain damage in foals with NE and other kinds of brain injury (UCHL1 should be increased in any neurologic disease with marked neuron cell death, being unspecific for NE). Despite that, there are some limitations in this study, like the fact that only 33 foals with clinical diagnosis of NE and 17 healthy foals were included and that the measurement of neurobiomarkers is still not available for clinical practice. Nevertheless, this can be a future aid in the *ante-mortem* diagnosis of NE (Ringger, 2011).

7. Other alternatives

Cerebrospinal fluid collection and analysis is not commonly done but it can help rule out meningitis (Tennent-Brown, 2015). It can be either normal or xanthochromic, with an increase in protein and red blood cells concentration (MacKay, 2005).

Measurement of intracranial pressure has been a diagnostic tool used in infants with NE. There were attempts to adapt this procedure to foals, however, since it is an

invasive technique (requires placement of a subdural catheter) and is associated with certain risks, it led to the search of non-invasive alternatives (Mackenzie, 2010).

Furthermore, measuring the plasma concentrations of neuroactive progestogens derivatives may also be helpful in the diagnosis of NE, but these are also increased in foals with other diseases (Aleman, 2013).

8. Necropsy findings

There are still no *post-mortem* findings specific of NE, however, neuronal necrosis and/or degeneration within the central nervous system are consistent with ischemia (Lyle-Dugas, 2017). Some lesions usually associated with this condition are epidural subarachnoid, parenchymal and nerve root hemorrhage of the brain and spinal cord, as well as central nervous system edema and necrosis, and hepatic and renal lesions (Gold, 2017). However, in many cases, an obvious cause of hypoxia is not apparent and there is no evidence of hypoxic-ischemic or hemorrhagic brain injury or cerebral edema in foals clinically diagnosed with NE (Tennent-Brown, 2015).

7. TREATMENT

It is difficult to define one single treatment strategy since a wide range of disease processes are associated with NE in foals (McKenzie III, 2018). The treatment of these foals can be not only difficult and very costly (Gold, 2016), but also stressful for the owner (Wilkins, 2015b).

It usually relies on supportive care and treatment of other complications that may arise (Gold, 2017; Bernard, 2018a), with prevention of CNS injury and normalization of nervous system function as the main treatment goals (McKenzie III, 2018). Some standard therapies include oxygen supplementation, maintenance of blood pressure and glucose, control of seizures, general cerebral support (neuroprotectants), correction of metabolic abnormalities, maintenance of tissue perfusion, maintenance of renal function, treatment of gastrointestinal dysfunction, prevention and early treatment of secondary infections (Wilkins, 2004a; Gold, 2017). Excellent nursing care and monitorization are essential to ensure good outcomes (Tennent-Brown, 2015).

1. Supportive care

A very important thing to be kept in mind is that outside stimuli need to be minimized, especially if the foals are convulsing or with hyperexcitability (Galvin, 2004). Wrapping the limbs may be necessary to protect the foal from self-trauma during seizures (Vaala, 2003). Recumbent foals require a dry and warm place, often maintained in sternal recumbency and turned in order to avoid pressure sores (Galvin, 2004) (Figure 6). To prevent secondary corneal ulceration, artificial tears can be applied to the eyes of the foal (Vaala, 2003).

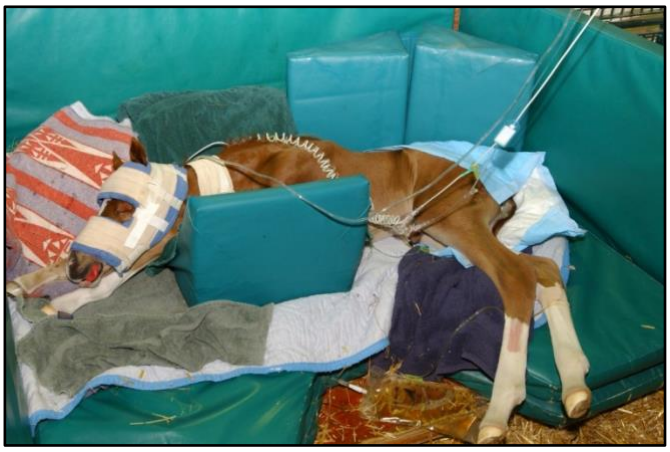


Figure 6 - **Head protection and environment adaptation to a recumbent, convulsive foal with NE.** Image kindly provided by the University of Tennessee.

2. Stabilization of the patient systemically

This is the first thing to do, in order to restore CNS perfusion and ensure an adequate delivery of oxygen and glucose (Douglas-Escobar, 2015; McKenzie III, 2018). Identifying the cause(s) of hypotension and correct it with judicious intravenous fluid support is one of the main goals of treatment. If the foal is unable to maintain tissue perfusion with IV fluids alone, administration of inotropes and vasopressors may also be necessary to support the cardiovascular function and to normalize perfusion pressures. (Wilkins, 2004a; Wong, 2011; Tennent-Brown, 2015). Attention to avoid overhydration and hypertension should be kept, because these may cause cerebral edema and potentiate CNS further injury (Wong, 2011; McKenzie III, 2018). These foals need maintenance-type fluids with lower sodium and chloride concentrations and higher potassium concentrations (Tennent-Brown, 2015). Maintenance requirements should be 5 to 6 ml/kg bwt/h of a balanced polyionic fluid. If there is hypokalemia, potassium supplementation is necessary (Galvin, 2004).

Blood glucose concentration also need to be normalize without causing hyperglycemia, since it is detrimental in foals with NE (Corley, 2005) and critically ill foals may be intolerant to dextrose infusions (McKenzie III, 2018).

If the foal is anemic, blood transfusion may be needed (McKenzie III, 2018). Also, if there is failure of passive transfer of immunoglobulins (IgG levels <800 mg/dL),an intravenous hyperimmune plasma may be required (Vaala, 2003; Galvin, 2004). Bottle, bucket or nasogastric tube feeding of colostrum or artificial IgG supplement can also be done (Vaala, 2003; Grogan, 2005). Foals in need of colostrum can be fed 250 mL/h for the first 6h-10h. To ensure its quality, the specific density has to be greater than 1.060 (Grogan, 2005).

In mild to severe cases of NE, acidosis may be present with a pH less than 7.3 and bicarbonate concentration less than 20 mmol/L, which needs to be fixed (Galvin, 2004).

The most accurate way to determine the foal's fluid status is thought to be with urine output paired with non-invasive blood pressure monitoring. If the urine output falls below 66% of all fluids administered, there should be an intervention in order to restore circulating volume and tissue perfusion. Also, the normal reference range of specific gravity of the urine is 1.001-1.009, which can be higher in cases of dehydration or hypovolemia (Galvin, 2004).

3. Broad-spectrum antimicrobial therapy

Because foals with NE can be predisposed to sepsis, prompt and aggressive broad-spectrum antimicrobial therapy is indicated. (Tennent-Brown, 2015; Bernard, 2018a). Attention must be paid to renal function because some antimicrobials have nephrotoxic effects (Tennent-Brown, 2015).

4. Respiratory support

Lower respiratory rates and/or periods of apnea will cause hypoxemia (PaO_2 <60 mmHg) and hypercapnia ($PaCO_3$ > 65 mmHg), that need to be corrected (Galvin, 2004). Intranasal oxygen therapy can either be used as a preventive measure or as a direct treatment (Tennent-Brown, 2015). It is often used an indwelling nasal cannula, at an initial rate of 9-10 L/min of oxygen (Corley, 2005).

In weak or recumbent foals, or with centrally mediated hypoventilation, periods of apnea and abnormal breathing patterns, with documented arterial hypoxemia, may need additional respiratory support. This can be done with doxapram (0.02-0.05 mg/Kg/h as a constant rate infusion) (Tennent-Brown, 2015). Although both doxapram and caffeine have been recommended and used as respiratory stimulants in foals with NE, there is not much data regarding its efficacy, being the dosage regimens extrapolated from human studies. Besides that, a study that compared the effects of caffeine and doxapram on respiratory and cardiovascular function in foals with induced respiratory acidosis showed that caffeine is unlikely to improve ventilation and decrease hypercapnia in neonatal foals, since its effects were undistinguishable from those of the placebo group (Giguère, 2007). It has also been demonstrated that doxapram is more effective than caffeine for the fast correction of hypercapnia in foals with NE (Giguère, 2007; Giguère, 2008).

Short-term positive pressure ventilation may also be required in foals with persistent or severe hypoxemia and hypercapnia (Wilkins, 2004a; Tennent-Brown, 2015).

Other therapies that have shown some results are the use of melatonin and desferrioxamine, which are free radical scavengers, and the hyperbaric oxygen therapy, which can reduce apoptosis, enhance oxygen radical scavengers, increase brain oxygenation and promote neuronal stem cells in rats with NE (Gold, 2017).

5. Seizure control

In foals with rare or very subtle signs of seizures, specific treatment may not be necessary, however, when there is repeated, generalized seizure activity, it is undoubtedly indicated (McKenzie III, 2018). For seizure control:

Benzodiazepines (diazepam and midazolam): the first-choice therapy in acute seizures because they have a quick onset of action with minimal depressive effects. IV bolus of Diazepam are ideal to control of emergency and single episode seizures (Morresey, 2009; McKenzie III, 2018). If it show no effect or if there is more than two seizures, then it should be replaced by a constant rate infusion of midazolam (McKenzie III, 2018). This latter has the advantage of allowing frequent assessment of neurologic function, since it has a short half-life and can be reversed if necessary (Tennent-Brown, 2015).

Phenobarbital: is often used in the management of acute seizure episodes that do not respond to the previous drugs and in recurrent seizure activity (Morresey, 2009). It can cause significant CNS depression when first administered and its half-life can be up to 100 hours in the foal, so special attention must be paid when monitoring neurologic function after administration of this drug (Wilkins, 2004a; McKenzie III, 2018). The therapeutic range is 5-30 µg/mL and serum levels must be monitored to make sure the range is respected (McKenzie III, 2018). If phenobarbital shows no effect in seizure control, then phenytoin therapy should be used (Wilkins, 2004a).

Pentobarbital: only indicated in foals with *status epilepticus* that failed to be controlled with other drugs. It is associated with high risk because it causes profound respiratory and cardiovascular depression, hypotension, and low cardiac output (Morresey, 2009; McKenzie III, 2018).

Phenytoin: not commonly used and has unpredictable kinetics, however, its use has been reported as an anticonvulsant in foals (Morresey, 2009; McKenzie III, 2018).

Potassium bromide: less side effects than phenobarbital and is well tolerated by foals. It should be used in long-term maintenance in foals with epilepsy. Although the therapeutic range of this drug has not been described for foals, in other species it is 70-240 mg/dL (McKenzie III, 2018).

Besides the use of the anticonvulsants, other measures need to be taken during a seizure, like protect the foal from injury and clean its airways, in order to prevent the onset of negative pressure pulmonary edema (Wilkins, 2004a). The combination of xylazine and ketamine should be avoided in foals with NE because of their association with increase of the intracranial pressure (Wilkins, 2004a).

6. Pharmacological approaches to neuroprotection

Dimethyl sulfoxide (DMSO): has been one of the most widely used drugs for decades. It has anti-inflammatory, osmotic and diuretic effects and alleged free radical scavenging properties, blocks sodium channel activation, suppresses calcium influx and prevents glutamate excitotoxic cell death. It's also easy to administrate and has a low cost. Despite

that, there is almost none scientific evidence regarding the use of DMSO in patients with NE and there's a lack of clinical consensus on its use as well (McKenzie III, 2018). That is probably why the use of dimethyl sulfoxide in neonates has decreased a lot in the past years, being rarely used, as no difference in outcome has been noted (Wilkins, 2004a).

Mannitol: is used to treat cerebral interstitial edema, however, it is minimally effective in treating cellular edema, which is present in the majority of the cases. Mannitol and dimethyl sulfoxide (DMSO) are only indicated when cellular necrosis and vasogenic edema are present, which is usually in the worst cases (Wilkins, 2004a). One study in human infants showed that the use of mannitol infusion decreased the intracranial pressure and improved cerebral perfusion pressure within 60 minutes (Whitelaw, 2000). However, evidence of efficacy is also lacking and is not frequently used (McKenzie III, 2018).

Magnesium sulfate: has a N-metyl-D-aspartate-receptor antagonist effects, can stabilize cell membranes, inhibit free radical production, and reduce secondary CNS inflammation and injury (McKenzie III, 2018). Early infusion of magnesium sulfate has been showed to be effective in improving outcome in neonates with severe NE. Its combination with hypothermia could be very beneficial (Bhat, 2009).

Pentoxifyline: has anti-inflammatory and immune-modulating effects and can improve local tissue perfusion. It is also thought to inhibit the Tumor Necrosis Factor alpha production in foals with NE (McKenzie III, 2018).

Antioxidants: may be a valuable approach to control CNS inflammation. Vitamins C, E and B1 (thiamine) have all been used in the treatment of foals with NE (McKenzie III, 2018). It has been described the use of thiamine supplementation in IV fluids to support metabolic processes, specially mitochondrial metabolism and membrane Na+, K+ ATPases involved in maintaining cellular fluid balance, however, it's still unproven in efficacy (Wilkins, 2004a). On the other hand, although allopurinol has shown some promising results in human neonates (McKenzie III, 2018) and good evidence from animal experiments (Whitelaw, 2000), it still remains preclinical in foals, as there are no reports of its use in this specie yet (McKenzie III, 2018).

7. Anti-inflammatories

Nonsteroidal anti-inflammatory drugs, such as flunixin meglumine, are frequently used (Hahn, 2008). Corticosteroids have no role in the treatment of NE (MacKay, 2005).

8. Nutritional support

Critically ill foals have minimal reserves in the form of glycogen and fat and a high metabolic rate relative to body mass. This makes the nutritional support of these animals extremely important, in order to meet their needs. Healthy foals should consume daily 23%-28% of their bodyweight as milk (120-150 kcal/kg bwt/day) during the first 2 to 3 weeks of life, in order to maintain a high metabolic rate and a rapid rate of growth (1-1.3 kg/day in a 50 kg foal). Jose-Cunilleras *et al.* (2012) stablished the nutritional requirements of critically ill foals, concluding that the resting energy requirements (REE) in these animals were 50 kcal/kg bwt/day, which is much lower than the REE for healthy foals. Also, as critically ill foals recover, their REE increases to values similar with the healthy ones (65-70 kcal/kg bwt/day). So, sick foals that need enteral nutrition, should receive at least 10% of bodyweight in mare's milk over a 24-hour period, which has 500-570 kcal/L, in order to meet their REE (Jose-Cunilleras, 2012; Bernard, 2018a). This feedings should be made every 1-2 hours (Bernard, 2018a). If parenteral nutrition is required, 50 kcal/kg bwt/day is a reasonable goal of energy provision (Jose-Cunilleras, 2012).

9. Squeeze-induced somnolence technique

First described in 2012 by Toth *et al.* with promising results. This approach was based on the fact that normal foals may collapse and become flaccid in lateral recumbency during a particular type of restraint, with this phenomenon being called as the flopping reaction or reflex relaxation. All that is needed is a soft linen rope (6.1 m in length and 1.27 cm in diameter). First, a bow-line knot is used to secure the rope around the neck and under the shoulder, to prevent tightening of that segment (it could cause pressure in the trachea and jugular veins). Then, two half-hitch knots are used to loop the rope around the thorax and abdomen 5 to 25 cm from each other and perpendicular to the vertebral column. The half-hitch knots are positioned directly on the dorsal thoracolumbar area.

After that, one person should stand behind the foal and pull on the rope, resulting in a generalized squeezing of the foal, while another person holds the foal and assists as it lays down. Tension on the rope must be maintained during 20 minutes. After that, pressure is released and it has been reported that foals woke up without signs of NE (Aleman, 2017).

A survey showed that foals that received this procedure with or without medical therapy were 3.7 times more likely to have a faster recovery than the ones that did not receive it. Squeezed foals had faster and higher recovery rates at different time points and were 15.1 times more likely to recover in less than 1h than non-squeezed foals. In addition, foals receiving only the squeeze procedure had 17.5 times more chances to recover within the first 24h than foals treated only medically (Aleman, 2017). This technique showed no adverse effects, so it can be considered a safe procedure (Toth, 2012; Aleman, 2017).

10. Hypothermia

In human infants with PAS, the use of regional hypothermia is the actual major treatment modality. It appears to slow the metabolism down with an inhibition of inflammation and apoptosis (Douglas-Escobar, 2015; Sarkar, 2015; Gold, 2017) and decrease intracellular edema and neuronal death (Wilkins, 2004a). This implies cooling the patient to 33.5ºC - 35ºC either with whole body hypothermia or selective head-cooling approaches. This procedure has been confirmed to be beneficial in human neonates with NE, as it reduces mortality without increasing long-term neurologic damage in the survivors (Douglas-Escobar, 2015; Sarkar, 2015; McKenzie III, 2018). As any procedure, it is also associated with some side effects, such as sinus bradycardia, thrombocytopenia, overcooling, skin problems, altered drug metabolism, and an increased risk of seizure during the rewarming period (Sarkar, 2015; McKenzie III, 2018). However, as promising this new approach seem to be, there is still not much information regardless to the use of hypothermia in neonatal foals (Gold, 2017), so the logistics regarding the appropriate technique of cooling, patient selection, and duration of the cooling period needs to be defined before its clinical application in equine medicine (McKenzie III, 2018).

11. Other treatment modalities

There's a wide range of new therapies being investigated in human infants with NE with promising results, including inhaled xenon (anti-excitotoxic), melatonin (antioxidant), erythropoietin (growth factor), and stem cells therapy (McKenzie III, 2018).

8. PROGNOSIS

It is highly desirable to provide the owner a prognosis for both survival and athletic outcome of the foal as soon as possible (Wilkins, 2015b). The main reasons leading to euthanasia in foals are poor prognosis for survival, poor athletic outcome, and financial constraint (Dembek, 2014).

Prognosis of NE depends on the severity of injury (Hagberg, 2015; Bernard, 2018a) but is usually good to excellent in uncomplicated cases (Lyle-Dugas, 2017). It is expected that 60% to 80% of foals with this condition will recover fully without neurological sequelae (Magdesian, 2014) and with productive athletics outcomes (Wilkins, 2004a). Long-term neurologic disabilities are rare and may include inability to suck from the dam, recurrent seizures, prolonged visual impairment, residual spasticity, and unusual docility as adults (Vaala, 1994; Bernard, 1995; Vaala, 2003). It was observed that if the foal survives the first 5 days with neurologic improvement, the prognosis is good with no long-term neurologic disabilities (Bernard, 1995). In humans, it was stablished that infants who survive the first 72h of age, typically improve over the next days/weeks (Wong, 2011).

A recent study showed that foals with failure of passive transfer immunity or with at least one complication/comorbidity during hospitalization were less likely to survive. Foals with seizure activity within the first 24h of hospitalization were less likely to survive as well (Gold, 2016).

According to another study, the reasons associated with death or euthanasia were the presence of pneumonia, sepsis or sepsis-associated complications, primary neurological disease or multi-organ failure secondary to ischemia (Lyle-Dugas, 2017). Besides that, foals with high total calcium or low alkaline phosphatase at admission, in recumbency, treated with vasopressors/inotropes or that had multiple comorbidities had significantly less chance to survive (Lyle-Dugas, 2017). On the other hand, pH greater

than 7.35 has been associated with increase newborn foal survival (Corley, 2005; Cruz, 2015).

Total Magnesium

Abnormalities in the concentration of total magnesium are usually present in critically ill patients with NE, being associated with increased mortality rate. In fact, hypermagnesemia present in the umbilical cord was associated with the development of mild NE, whereas when developed from the second day of life in infants that suffered from asphyxia, was associated with severe NE and poor prognosis. So, routine determination of total magnesium could be helpful in predicting outcome (Mariella, 2016).

L-lactate concentration

Lactate concentration has not only diagnostic value, but also therapeutic and prognostic. It is considered a marker of global hypoxia, inadequate oxygen delivery, sepsis and disease severity and mortality in critically ill patients, either humans or animals (Shah, 2004; Borchers, 2013).

A study reported the mean lactate concentration in 26 healthy foals immediately after birth was 3.8 ± 1.9 mmol/L (median 3.4 mmol/L and range 1.7-10.2 mmol/L). These values decreased within the first 72h, but remained slightly higher than normal adult concentrations. In some apparently healthy foals, lactate concentrations also remained high at 72h (Castagnetti, 2010).The measurement of blood L-lactate concentration, either single or serial, is a reliable prognostic indicator in ill neonatal foals, as it correctly predicts the outcome (survival vs. non survival) in approximately 75%-80% of the cases. It has been shown that higher blood lactate concentrations at admission are usually associated with increased mortality rates in critically ill neonatal foals (Hendersen, 2008; Borchers, 2013; Tennent-Brown, 2014; Sheahan, 2016). Persistent hyperlactatemia is associated with dysmetabolism and poor oxygen utilization by tissues due to the poor oxygen delivery (Wilkins, 2015c). Serial lactate measurements are theoretically more reliable predictors of outcome than one single measurement at admission, since it evaluates the change in lactate concentrations over time and captures the duration and severity of hyperlactemia (Borchers, 2013; Tennent-Brown, 2014; Wilkins, 2015c;

Sheahan, 2016). According to a study, for each 1mmol/L increase in lactate concentration, the odds for survival decreased significantly, therefore the need of sequential monitoring (Borchers, 2013). In fact, several studies showed that survivors had significant decreases in lactate concentrations within the first 24h of hospitalization, while non-survivors showed no decrease over time (Hurcombe 2008; Castagnetti, 2010; Borcher, 2013; Sheahan, 2016).

It seems that the time frame during which this should be used as a prognostic indicator in sick neonatal foals is until the first 3 days of life (Sheahan, 2016). Nevertheless, it is important to take into consideration that in all studies there were surviving patients with high lactate concentrations and non-surviving with normal lactate concentrations (Tennent-Brown, 2014). Therefore, blood lactate concentrations should not be the only method of assessing neonatal vitality, but rather in association with other variables and clinical examination of the foal (Cruz, 2017).

Glucose

Hypoglycemia (<75 mg/dL) and extreme hypoglycemia (<50 mg/dL) at admission have been associated with non-survival in critically ill foals. In fact, a study reported that each 18 mg/dL increase in blood glucose increased more than 3-fold the odds of survival to hospital discharge. It was also associated with other comorbidities, like sepsis, systemic inflammatory response syndrome, and positive blood culture. Hyperglycemia, only as its extreme form (>189 mg/dL), was associated with poor prognosis too (Hollis, 2008).

Multivariable mathematical model

A model was created to help clinicians in the early prediction of probability of survival in hospitalized foals up to 7 days of life. This model studied retrospectively 910 hospitalized foals and prospectively 163. In order to provide a more precise estimate, it should be combined the clinician's initial prediction of the probability of survival with the model's result, rather than relying on the model alone (Rohrbach, 2006). Another scoring system was developed in 2014 in order to help clinicians predict likelihood of survival in equine neonates using clinical data obtained on admission (Table 2 and 3). Data from 339 hospitalized foals with less than 4 days of age was collected to develop

the model and then 283 hospitalized foals were studied prospectively to validate it. It showed to be more effective in ***predicting*** survival (91%) than in predicting non-survival (86%) (Dembek, 2014).These models should be used as a supplementary tool in helping to determine the likelihood of survival of hospitalized neonatal foals, despite its condition (Dembek, 2014).

Table 2 and 3 - **Survival Scoring System (Left) and Probability of Survival (Right).** Adapted from Dembek, K. A., 2014.

Variable	Value	Points	Value	Points	Score
Cold extremities	No	2	Yes	0	
Prematurity (<320 days)	No	1	Yes	0	
>2 infection/ inflammatory sites	No	1	Yes	0	
IgG (mg/dL)	<400	0	≥400	1	
Glucose (mg/dL)	<80	0	≥80	1	
White blood cells x 10^3/µL	≤4	0	>4	1	

Score	Probability of Survival
0	3 %
1	8 %
2	18 %
3	38 %
4	62 %
5	82 %
6	92 %
7	97 %

CHAPTER II – RETROSPECTIVE STUDY

1. OBJECTIVES

The purpose of this dissertation is to evaluate the influence of some factors in the outcome and to stablish prognostic indicators in a population of foals with Neonatal Encephalopathy.

2. MATERIALS AND METHODS

Inclusion Criteria

A population of 61 neonatal foals with the diagnosis of Neonatal Encephalopathy (or any of its synonyms) was studied retrospectively, relying on medical records between 1982 and 2018. The foals were assisted in the Equine Hospital of the University of Tennessee, College of Veterinary Medicine, in the United States of America and in the Hospital La Equina, in Málaga, Spain.

Variables included in the study

Information regarding animal identification, clinical history, clinical signs, and treatment was collected and organized the following way:

- Surviving rate (including number of survivors, number of non-survivors and time until dead of non-survivors);
- Animal identification: gender and breed;
- Predisposing factors of the mare (dichotomous (yes/no) variables): delivery deemed a dystocia, mare's ingestion of fescue, maiden mare, mare's disease during gestation, twinning pregnancy, and premature placental separation.
- Predisposing factors of the foal (dichotomous (yes/no) variables): large foal, prematurity, and dysmaturity.
- Physical exam and laboratory findings on admission:

- **Numerical variables**:
  - Heart rate. In order to study heart rate, and according with reference values from Stoneham (2004), the foals were distributed in 3 groups: normal heart rate (80-120 bpm), tachycardia (>120 bpm) and bradycardia (<80 bpm);
  - Respiratory rate. In order to study respiratory rate, and according with reference values from Bernard & Reimer (2018), the foals were distributed in 3 groups: normal respiratory rate (30-40 rpm), tachypnea (>40 rpm) and bradypnea (<30rpm);
  - Body temperature. In order to study body temperature, and according with reference values from Bernard & Reimer (2018) the foals were distributed in 3 groups: normal range (37.2-38.6ºC), hyperthermia (>38.6ºC) and hypothermia (<37.2ºC);
  - Blood glucose concentration. In order to study blood glucose concentration, and according with reference values from McAuliffe and Slovis (2008), the foals were distributed in 3 groups: normal range (93.8-185.6 mg/dL), hyperglycemia (>185.6 mg/dL) and hypoglycemia (<93.8 mg/dL);
  - Total protein concentration. In order to study total protein concentration, and according with reference values from Stoneham (2004), the foals were distributed in 3 groups: normal range (4-6.6 mg/dL), hyperproteinemia (>6.6 mg/dL) and hypoproteinemia (<4 mg/dL);
  - Creatinine concentration. In order to study creatinine concentration, and according with reference values from Stoneham (2004), the foals were distributed in 3 groups: normal range (<2 mg/dL), slightly higher than normal values (2-4 mg/dL) and significantly higher than normal values (>4 mg/dL);
  - BUN concentration. In order to study BUN concentration, and according with reference values from Stoneham (2004), the foals were distributed in 2 groups: normal concentrations (<22 mg/dL) and higher than normal levels (>22 mg/dL).
- **Dichotomous (yes/no) variables:** manifestation of clinical signs within the first 24h, recumbency, hypoxemia, dehydration, gastrointestinal problems (including reflux, meconium impaction, gastric ulcers, colic, and diarrhea), seizures, absence of suckle reflex, tongue protrusion, abnormal vocalization, nystagmus, emaciation, and opisthotonus.
- Concurrent diseases (dichotomous (yes/no) variables): failure of passive transfer immunity, ophthalmic problems (which included corneal ulcers, entropion, conjunctivitis, and blindness), pneumonia, limb abnormalities, umbilical problems

(including enlarged umbilical structures, patent urachus, and omphalitis), anemia and sepsis.

- Therapeutic interventions (dichotomous (yes/no) variables): administration of IV fluids, use of antibiotics (that included one or more of the following: amikacin, penicillin G, ampicillin, gentamicin, ceftiofur, trimethoprim-sulfa, and metronidazole), supplementation with dextrose, plasma administration (varying from 1L to 5L), oxygen therapy, DMSO administration, administration of non-steroidal anti-inflammatory drugs (flunixin meglumine and/or ketoprofen), administration of vitamins (that included one or more of the following: B complex, Vitamin A and D, Selenium + Vitamin E, and Vitamin C), use of corticosteroids (dexamethasone or prednisolone), caffeine administration and use of diuretics (furosemide and/or mannitol).

Data analysis

In order to stablish indicators of outcome in this population, the variables described before were compared to survivors and non-survivors (which included foals that died naturally or were subjected to euthanasia). Animals were subjected to euthanasia due to poor prognosis and not responding to the medical treatment.

To test the independence between variables, the Chi-Squared Pearson Test was used. To compare proportions, the Z Test was performed.

In numerical variables, tests of normality were performed. Those that were well-modeled by normal distribution were then tested with the ANOVA Test, while those that were not well-modeled by a normal distribution were tested with Non-parametrical Tests, such as the Mann-Whitney Test and the Median Test.

In order to identify the factors that influence the time of survival, in the factors with enough animals in each group, Logistic Regression Models were used. In these models, the dependent variable was "mortality" (0=survived, 1=died). Given the small sample, only univariate models were used (models with one independent variable). The type of influence of the independent variables in the time of survival was evaluated through the Odds Ratio (OR) estimate and its Confidence Intervals (CI) at 95%.

The calculations were done with the IBM SPSS program (Statistical Package for Social Sciences v. 22) and the level of $p<0.05$ was stablished in order to reject the null hypothesis.

3. RESULTS

3.1. Surviving rate

The overall surviving rate was 57.4% (n=35). Of the total of non-survivors (n=26; 42,6%), 24 were euthanized and 2 died. In none of the cases financial constraints seemed to be the primary reason for euthanasia. In fact, there were two cases in which financial constraints were present, so the owners decided to treat at home and both of the foals survived.

3.2. Identification

3.2.1. Gender

Among the 61 foals diagnosed with Neonatal Encephalopathy, 28 were colts (45.9%) and 32 were fillies (54.1%). One foal did not have its gender defined in the medical records. In the fillies group, 19 survived, while 13 died. In the colts group, 15 survived and 13 died, with no statistically significant differences between groups (p=0.651).

3.2.2. Breed

The most common breeds were American Quarter Horse (29.5%; n=18) and Tennessee Walking Horse, (19.7%; n=12). The other breeds were divided in Thoroughbred (9.8%; n=6), Andalusian (8.2%; n=5), Appaloosa (6.6%; n=4), Arab (6.6%; n=4), Mixed breed (4.9%; n=3), American Paint Horse (3.3%; n=2), American Saddle Horse (3.3%; n=2), Belgian (1.6%; n=1), Clydesdale (1.6%; n=1), Percheron (1.6%; n=1), Pony (1.6%; n=1), and unknown breed (1.6%; n=1) (Table 4).

The most common breeds among the survivors were American Quarter Horses and Tennessee Walking Horses. The breeds represent more often among the non-survivors were American Quarter Horses and Thoroughbred. When breed was compared to survival there were no statistically significant differences between groups (p=0.209).

Table 4 - Percentage of the breeds included in this study among survivors and non-survivors.

Breed	% Within Survivors	% Within Non-survivors
American Quarter Horse	28.6 %	30.8 %
Tennessee Walking Horse	28.6 %	7.7 %
Thoroughbred	2.9 %	19.2 %
Andalusian	5.7 %	11.5 %
Appaloosa	2.9 %	11.5 %
Arab	8.6 %	3.8 %
Mixed breed	2.9 %	7.7 %
American Paint Horse	5.7 %	0 %
American Saddle Horse	2.9 %	3.8 %
Belgian	2.9 %	0 %
Clydesdale	2.9 %	0 %
Percheron	2.9 %	0 %
Pony	2.9 %	0 %
Unknown breed	0 %	3.8 %

3.3. Predisposing factors of the mare

All the information regarding these variables (delivery deemed a dystocia, the presence of maiden mare, presence of disease during gestation, and fescue ingestion during gestation) and its comparison with survival are described in Table 5.

Regarding the presence of twinning pregnancy there were only 3 cases of twins in this population and none of them survived. In the non-twins group (n=58), 35 foals survived and 23 did not.

Premature placental separation occurred in only 3 mares, of which 2 of their foals survived and 1 did not. There were 58 mares that did not suffer from premature placental separation, of which 33 of their foals survived and 25 did not.

Table 5 - Comparison between several predisposing factors of the mare and survival, using the Pearson Chi-Squared Test.

			Survival			*p*
			No	Yes	Total	
Dystocia	No	Count	14	22	36	0.874
		% within Survival	67%	69%	68%	
	Yes	Count	7	10	17	
		% within Survival	33%	31%	32%	
Total		Count	21	32	53	
		% within Survival	100%	100%	100%	
Maiden mare	No	Count	6	7	13	0.063
		% within Survival	100%	58%	72%	
	Yes	Count	0	5	5	
		% within Survival	0%	42%	28%	
Total		Count	6	12	18	
		% within Survival	100%	100%	100%	
Disease during gestation	No	Count	23	33	56	0.412
		% within Survival	89%	94%	92%	
	Yes	Count	3	2	5	
		% within Survival	12%	6%	8%	
Total		Count	26	35	61	
		% within Survival	100%	100%	100%	
Fescue ingestion	No	Count	22	31	53	0.651
		% within Survival	85%	89%	87%	
	Yes	Count	4	4	8	
		% within Survival	15%	11%	13%	
Total		Count	26	35	61	
		% within Survival	100%	100%	100%	

In Table 6 are the results of the univariate logistic regression models that evaluates the influence of a delivery deemed a dystocia in the mortality of this population.

Table 6 - Univariate logistic regression models to study the influence of a delivery deemed a dystocia in the mortality of the population in study.

Independent variable	***Odds Ratio* (IC 95%)**	***p***
Dystocia	**0.92** (0.30 – 2.87)	0.887

3.4. Predisposing factors of the foal

The results regarding the presence of a large foal and prematurity, and its comparison with survival are included in Table 7. There were only 4 dysmature foals and all of them survived. Of the 57 non-dysmature foals, 31 survived and 26 died.

Table 7 - Comparison between predisposing factors of the foal and survival, using the Pearson Chi-Squared Test.

			Survival			*p*
			No	Yes	Total	
Large foal	No	Count	24	32	56	0.901
		% within Survival	92%	91%	92%	
	Yes	Count	2	3	5	
		% within Survival	8%	9%	8%	
Total		Count	26	35	61	
		% within Survival	100%	100%	100%	
Prematurity	No	Count	18	30	48	0.120
		% within Survival	69%	86%	79%	
	Yes	Count	8	5	13	
		% within Survival	31%	14%	21%	
Total		Count	26	35	61	
		% within Survival	100%	100%	100%	

The results of the univariate logistic regression models that evaluates the influence of prematurity in the mortality of this population are described in Table 8.

Table 8 - Univariate logistic regression model to study the influence of prematurity in the mortality of the population in study.

Independent variable	***Odds Ratio* (IC 95%)**	***p***
Prematurity	**2.67** (0.76 – 9.41)	0.127

3.5. Physical exam and laboratory findings on admission

3.5.1. Numerical variables

On admission, information regarding heart rate was available in 47 foals; about

the respiratory rate in 44 foals; body temperature in 49 foals; blood glucose in 46 foals; total protein concentration in 47 foals; creatinine concentrations in 23 foals; and the levels of BUN in 40 foals. The comparison of these values with survival are shown in Table 9.

Table 9 - Comparison between intervals of the numerical variables on admission and survival, using the Pearson Chi-Squared Test.

			Survival			*p*
			No	Yes	Total	
Heart rate (bpm)	80-120	Count (% within Survival)	16 (73%)	18 (72%)	34 (72%)	0.966
	>120	Count (% within Survival)	3 (15%)	3 (12%)	6 (13%)	
	<80	Count (% within Survival)	3 (14%)	4 (16%)	7 (15%)	
Total		Count (% within Survival)	22 (100%)	25 (100%)	47 (100%)	
Respiratory rate (bpm)	30-40	Count (% within Survival)	4 (21%)	7 (29%)	11 (26%)	0.688
	>40	Count (% within Survival)	5 (26%	4 (17%)	9 (21%)	
	<30	Count (% within Survival)	10 (53%)	13 (54%)	23 (54%)	
Total		Count (% within Survival)	19 (100%)	24 (100%)	43 (100%)	
Body Temp. (ºC)	37.2-38.6	Count (% within Survival)	7 (28%)	18 (64%)	25 (47%)	0.016
	>38.6	Count (% within Survival)	1 (4%)	2 (7%)	3 (6%)	
	<37.2	Count (% within Survival)	19 (68%)	8 (29%)	25 (47%)	
Total		Count (% within Survival)	25 (100%)	28 (100%)	53 (100%)	
Blood glucose (mg/dL)	93.8-185.6	Count (% within Survival)	3 (14%)	13 (54%)	16 (35%)	0.009
	>185.6	Count (% within Survival)	5 (23%)	1 (4%)	6 (13%)	
	<93.8	Count (% within Survival)	14 (64%)	10 (42%)	24 (52%)	
Total		Count (% within Survival)	22 (100%)	24 (100%)	46 (100%)	
Total Protein (g/dL)	4-6.6	Count (% within Survival)	18 (78%)	20 (83%)	38 (81%)	0.153
	>6.6	Count (% within Survival)	2 (9%)	4 (17%)	6 (13%)	
	<4	Count (% within Survival)	3 (13%)	0 (0%)	3 (6%)	
Total		Count (% within Survival)	23 (100%)	24 (100%)	47 (100%)	
Creatinine (mg/dL)	2-4	Count (% within Survival)	3 (34%)	12 (86%)	15 (65%)	0.019
	>4	Count (% within Survival)	3 (33%)	0 (0%)	3 (13%)	
	<2	Count (% within Survival)	3 (33%)	2 (14%)	5 (22%)	
Total		Count (% within Survival)	9 (100%)	14 (100%)	23 (100%)	
BUN (mg/dL)	<22	Count (% within Survival)	8 (44%)	7 (32%)	15 (38%)	0.412
	>22	Count (% within Survival)	10 (56%)	15 (68%)	25 (62%)	
Total		Count (% within Survival)	18 (100%)	22 (100%)	40 (100%)	

The evaluation of means and standard deviation of the numerical variables, between survivors and non-survivors, through the ANOVA test are described in Table 10.

Table 10 - Comparison of means and standard deviation of the numerical variables, between survivors and non-survivors, through the ANOVA test.

					95% Confidence Interval for Mean				
	Survival	N	Mean	Std. Deviation	Lower Bound	Upper Bound	Min	Max	Sig.
Heart rate on admission	No	22	95.09	29.94	81.82	108.36	30	180	0.286
	Yes	25	103.56	23.79	93.74	113.38	56	160	
	Total	47	99.60	26.88	91.70	107.49	30	180	
Respiratory rate on presentation	No	19	36.42	23.64	25.03	47.82	16	120	0.267
	Yes	24	30.54	8.86	26.80	34.28	16	48	
	Total	43	33.14	17.07	27.89	38.39	16	120	
Blood Glucose on presentation	No	22	97.41	95.80	54.93	139.88	5	289	0.764
	Yes	23	104.43	56.02	80.21	128.66	10	201	
	Total	45	101.00	77.22	77.80	124.20	5	289	
Total Protein on admission	No	23	5.34	1.20	4.82	5.85	3.5	8.3	0.186
	Yes	24	5.76	0.97	5.35	6.17	4.2	7.6	
	Total	47	5.55	1.10	5.23	5.88	3.5	8.3	
Creatinine on admission	No	9	3.83	2.30	2.06	5.60	1.4	7.6	0.080
	Yes	14	2.61	0.82	2.14	3.08	0.7	3.7	
	Total	23	3.09	1.64	2.38	3.79	0.7	7.6	
BUN on admission	No	18	27.72	14.83	20.35	35.10	8	64	0.875
	Yes	22	27.09	10.41	22.48	31.71	12	57	
	Total	40	27.38	12.42	23.40	31.35	8	64	

Body temperature was the only numerical variable that was not modeled by a normal distribution by the Shapiro-Wilk Test (p=0.04). The mean average of body temperature among survivors was 37.3ºC, while among non-survivors was 35.3ºC. This variable was compared to survival with the Median test and the Mann-Whitney U Test, with both indicating that there were statistically significant differences between groups, given by the P value less than 0.05 (p=0.031 in the Median Test and 0.007 in the Mann-Whitney U Test).

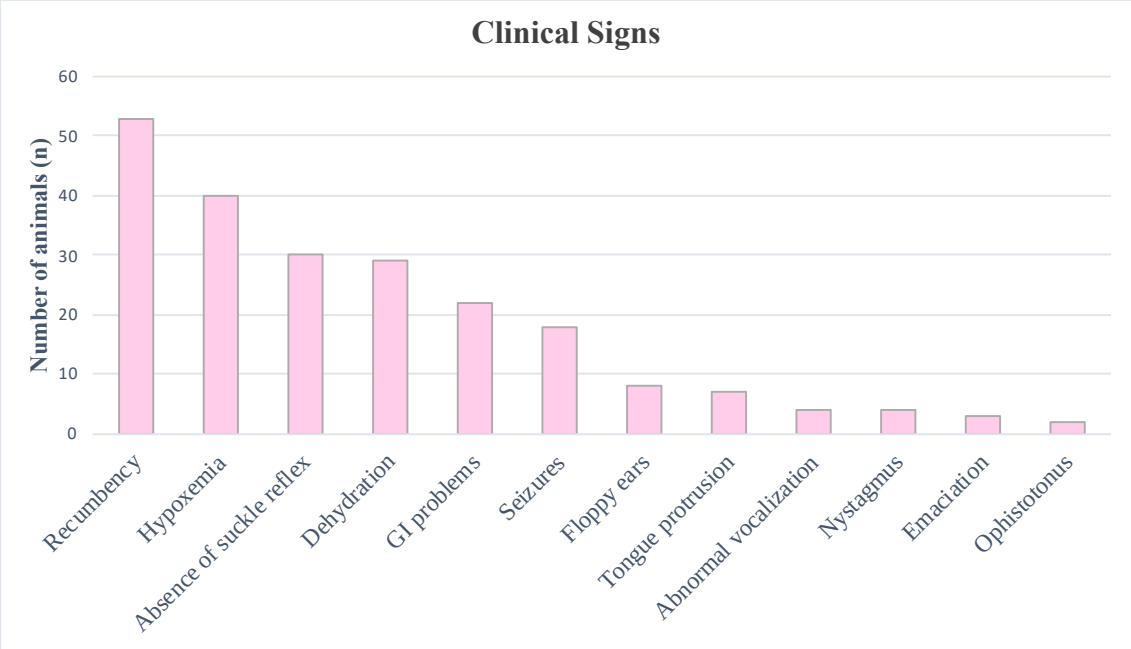
To evaluate the influence of the data collected in the clinical examination of the foals on admission in the mortality of this population, univariate logistic regression models were used (Table 11).

Table 11 - Univariate logistic regression models to study the numerical variables obtained during the physical examination. (a) – not possible to estimate OR due to cells with frequency zero.

Independent variable		***Odds Ratio* (IC 95%)**	***p***
Heart rate (bpm)	Normal heart rate (80-120)	Reference	
	Bradycardia (< 80)	**0.84** (0.16 – 4.36)	0.839
	Tachycardia (> 120)	**1.13** (0.20 – 6.39)	0.894
Respiratory rate (rpm)	Normal respiratory rate (30-40)	Reference	
	Bradypnea (< 30)	**1.54** (0.36 – 6.60)	0.562
	Tachypnea (> 40)	**3.33** (0.51 – 21.58)	0.206
Body temperature (ºC)	Normal body temperature (37.2-38.6)	Reference	
	Hypothermia (< 37.2)	**6.65** (1.79 – 24.73)	0.005
	Fever (> 38.6)	**1.19** (0.09 – 15.04)	0.894
Blood glucose (mg/dL)	Normal glycemia (93.8-185.6)	Reference	
	Hypoglycemia (< 93.8)	**6.07** (1.36 – 27.05)	0.018
	Hyperglycemia (> 185.6)	**21.67**(1.80–260.57)	0.015
Total protein (g/dL)	Normal total protein (4-6.6)	Reference	
	Hypoproteinemia (< 4)	(a)	
	Hyperproteinemia (> 6.6)	**0.56** (0.09 – 3.40)	0.525
Creatinine (mg/dL)	Normal creatinine (< 2)	Reference	
	Hypercreatinemia (2-4)	**0.17** (0.02 – 1.49)	0.109
	Marked hypercreatinemia (> 4)	(a)	
BUN (mg/dL)	Normal BUN (<22)	Reference	
	Increase of BUN (>22)	**0.58** (0.16 – 2.12)	0.414

3.5.2. Dichotomous variables (yes/no)

The most common clinical signs were recumbency (n=53), hypoxemia (n=40), absence of suckle reflex (n=30), dehydration (n=29), gastrointestinal problems (n=22), and seizures (n=18). Less common clinical signs included floppy ears (n=8), tongue protrusion (n=7), abnormal vocalization (n=4), nystagmus (n=4), emaciation (n=3), and opisthotonus (n=2). (Graphic 1).



Graphic 1 - Number of animals with each clinical sign found in the population in study.

The evaluation of the frequency of these clinical signs compared to survival is shown in Table 12, with the exception of abnormal vocalization, nystagmus, emaciation, and opisthotonus that, given the few number of cases, only a descriptive analysis was made.

Regarding abnormal vocalization, there were only 4 foals with this manifestation, of which 2 foals survived and the other 2 died. Among the 57 foals without abnormal vocalization, 33 survived and 24 did not. Of the 57 foals without nystagmus, 35 survived while 22 died, while all the 4 foals with nystagmus died. The 3 foals with emaciation died. Among the 58 foals without emaciation, 35 survived and 23 did not. Opisthotonus was present in 2 foals and all of them died. Of the 59 foals without opisthotonus, 35 survived and 24 died.

Table 12 - Comparison between the presence of several clinical signs and survival, using the Pearson Chi-Squared Test.

			Survival			*p*
			No	Yes	Total	
Clinical signs in the first 24h	No	Count (% within Survival)	1 (4%)	3 (9%)	4 (7%)	0.498
	Yes	Count (% within Survival)	22 (96%)	30 (91%)	52 (93%)	
Total		Count (% within Survival)	23 (100%)	33 (100%)	56 (100%)	
Recumbency	No	Count (% within Survival)	0 (0%)	8 (23%)	8 (13%)	0.009
	Yes	Count (% within Survival)	26 (100%)	27 (77%)	53 (87%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Hypoxemia	No	Count (% within Survival)	0 (0%)	2 (10%)	2 (5%)	0.129
	Yes	Count (% within Survival)	22 (100%)	18 (90%)	40 (95%)	
Total		Count (% within Survival)	22 (100%)	20 (100%)	42 (100%)	
Dehydration	No	Count (% within Survival)	14 (54%)	18 (51%)	32 (53%)	0.852
	Yes	Count (% within Survival)	12 (46%)	17 (49%)	29 (47%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
GI Problems	No	Count (% within Survival)	20 (77%)	19 (54%)	39 (64%)	0.069
	Yes	Count (% within Survival)	6 (23%)	16 (46%)	22 (36%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Seizures	No	Count (% within Survival)	16 (62%)	27 (77%)	43 (71%)	0.186
	Yes	Count (% within Survival)	10 (38%)	8 (23%)	18 (29%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Absence of suckle reflex	No	Count (% within Survival)	15 (71%)	18 (62%)	33 (66%)	0.490
	Yes	Count (% within Survival)	6 (29%)	11 (38%)	17 (34%)	
Total		Count (% within Survival)	21 (100%)	29 (100%)	50 (100%)	
Tongue protrusion	No	Count (% within Survival)	25 (96%)	29 (83%)	54 (89%)	0.107
	Yes	Count (% within Survival)	1 (4%)	6 (17%)	7 (11%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	

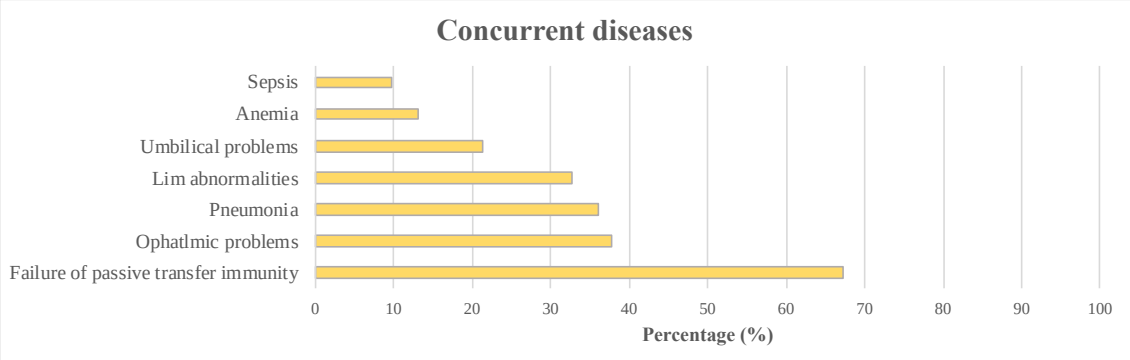
The results of the univariate logistic regression models to study the influence of dehydration, gastrointestinal problems, seizures, and absence of suckle reflex in the mortality are described in Table 13.

Table 13 - Univariate logistic regression models to study the influence of some clinical signs in the mortality of the population in study.

Independent variable	***Odds Ratio* (IC 95 %)**	***p***
Dehydration	**0.91** (0.33 – 2.51)	0.852
Gastrointestinal problems	**0.36** (0.12 – 1.10)	0.073
Seizures	**2.11** (0.69 – 6.44)	0.190
Absence of suckle reflex	**1.53** (0.46 – 5.11)	0.492

3.6. Concurrent diseases

In this study were included concurrent diseases that manifested during hospitalization. Those included failure of passive transfer immunity (n=41), ophthalmic problems (n=23), pneumonia (n=22), limb abnormalities (n=16), umbilical problems (n=13), anemia (n=8), and sepsis (n=6), as shown in Graphic 2.



Graphic 2 - Percentage of concurrent diseases in the population in study.

All the information regarding concurrent diseases comparison to survival is described in Table 14.

Table 14 - Comparison between the presence of several concurrent diseases and survival, using the Pearson Chi-Squared Test.

			Survival			*p*
			No	Yes	Total	
FPTI	No	Count (% within Survival)	9 (35%)	11 (31%)	20 (33%)	0.793
	Yes	Count (% within Survival)	17 (65%)	24 (69%)	41 (67%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Ophthalmic Problems	No	Count (% within Survival)	15 (58%)	23 (66%)	38 (62%)	0.523
	Yes	Count (% within Survival)	11 (42%)	12 (34%)	23 (38%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Pneumonia	No	Count (% within Survival)	11 (42%)	28 (80%)	39 (64%)	0.002
	Yes	Count (% within Survival)	15 (58%)	7 (20%)	22 (36%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Limb abnormalities	No	Count (% within Survival)	22 (85%)	23 (66%)	45 (74%)	0.097
	Yes	Count (% within Survival)	4 (15%)	12 (34%)	16 (26%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Umbilical Problems	No	Count (% within Survival)	19 (73%)	29 (83%)	48 (79%)	0.356
	Yes	Count (% within Survival)	7 (27%)	6 (17%)	13 (21%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Anemia	No	Count (% within Survival)	20 (77%)	33 (94%)	53 (87%)	0.047
	Yes	Count (% within Survival)	6 (23%)	2 (6%)	8 (13%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Sepsis	No	Count (% within Survival)	21 (81%)	34 (97%)	55 (90%)	0.034
	Yes	Count (% within Survival)	5 (19%)	1 (3%)	6 (10%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	

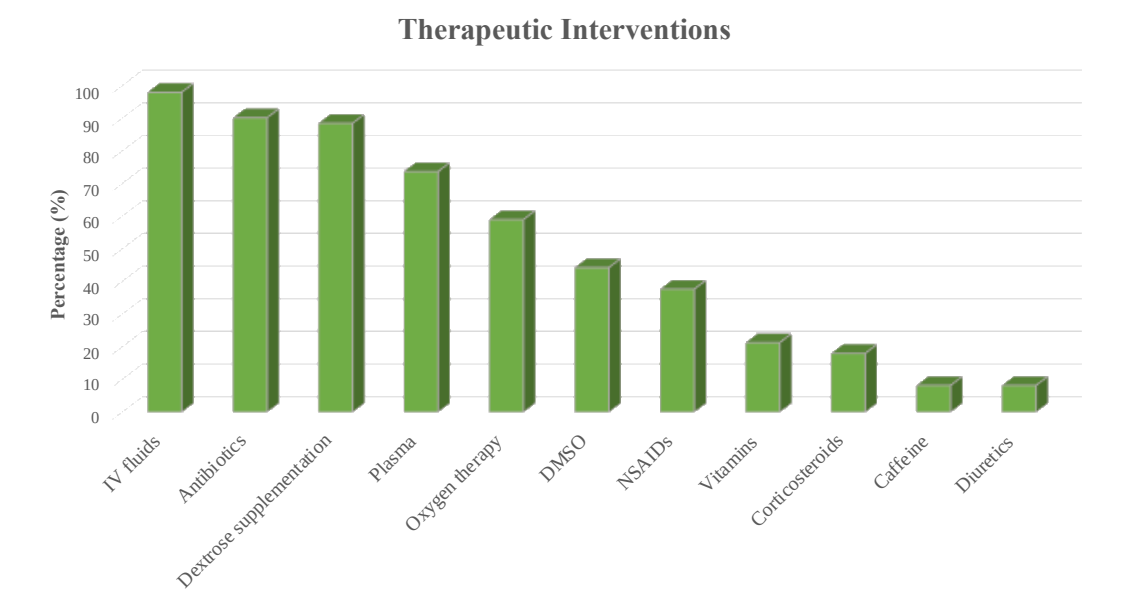
In Table 15 are shown the results of the univariate logistic regression models that evaluate the influence of the presence of ophthalmic problems, pneumonia and umbilical problems in the mortality of this population.

Table 15 - Univariate logistic regression models to study the influence of some concurrent diseases in the mortality of the population in study.

Independent variable	***Odds Ratio* (CI 95%)**	***p***
Ophthalmic problems	**1.41** (0.49 – 4.00)	0.523
Pneumonia	**1.94** (0.39 – 9.53)	0.415
Umbilical problems	**1.78** (0.52 – 6.12)	0.360

3.7. Therapeutic interventions

In Graphic 3 is shown the percental distribution of the most used treatments, which included intravenous fluid administration (n=60), antibiotics administration (n=55), dextrose supplementation (n=54), plasma administration (n=45), oxygen therapy (n=36), DMSO administration (n=27), non-steroidal anti-inflammatories administration (n=23), vitamins administration (n=13), corticosteroid therapy (n=11), use of caffeine (n=5), and diuretics administration (n=5).



Graphic 3 - Percentage of the therapeutic interventions performed in the population in study.

The different therapeutic interventions used in the population in study were compared to survival and its results are described in Table 16.

Table 16 - Comparison between therapeutic interventions and survival, using the Pearson Chi-Squared Test.

			Survival			*p*
			No	Yes	Total	
IV fluids	No	Count (% within Survival)	0 (0%)	1 (3%)	1 (2%)	0.385
	Yes	Count (% within Survival)	26 (100%)	34 (97%)	60 (98%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Dextrose	No	Count (% within Survival)	2 (8%)	5 (14%)	7 (12%)	0.424
	Yes	Count (% within Survival)	24 (92%)	30 (86%)	54 (88%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Antibiotics	No	Count (% within Survival)	5 (19%)	1 (3%)	6 (10%)	0.032
	Yes	Count (% within Survival)	21 (81%)	34 (97%)	55 (90%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Plasma	No	Count (% within Survival)	7 (27%)	9 (26%)	16 (26%)	0.915
	Yes	Count (% within Survival)	19 (73%)	26 (74%)	45 (74%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Oxygen therapy	No	Count (% within Survival)	8 (31%)	17 (49%)	25 (41%)	0.162
	Yes	Count (% within Survival)	18 (69%)	18 (51%)	36 (59%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
DMSO	No	Count (% within Survival)	14 (54%)	20 (57%)	34 (56%)	0.798
	Yes	Count (% within Survival)	12 (46%)	15 (43%)	27 (44%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
NSAIDs	No	Count (% within Survival)	21 (81%)	17 (49%)	38 (62%)	0.010
	Yes	Count (% within Survival)	5 (19%)	18 (51%)	23 (38%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Vitamins	No	Count (% within Survival)	17 (65%)	31 (89%)	48 (79%)	0.013
	Yes	Count (% within Survival)	9 (35%)	4 (11%)	13 (21%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Cortic therapy	No	Count (% within Survival)	21 (81%)	29 (83%)	50 (82%)	0.834
	Yes	Count (% within Survival)	5 (19%)	6 (17%)	11 (18%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Caffeine	No	Count (% within Survival)	25 (96%)	31 (89%)	56 (92%)	0.286
	Yes	Count (% within Survival)	1 (4%)	4 (11%)	5 (8%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	
Diuretics	No	Count (% within Survival)	23 (89%)	33 (94%)	56 (92%)	0.412
	Yes	Count (% within Survival)	3 (11%)	2 (6%)	5 (8%)	
Total		Count (% within Survival)	26 (100%)	35 (100%)	61 (100%)	

4. DISCUSSION

Even though NE is commonly reported, information regarding prognostic indicators specific to this disease are lacking. The present study provides an insight of the impact on survival of gender, breed, predisposing factors, physical examination, laboratory results and therapeutic interventions in foals with NE.

The overall surviving rate was 57,4%, which was lower than the 60 to 80% reported in recent literature (Wong, 2011; Magdesian, 2014; Tennent-Brown, 2015). Since this study relied on medical records within a large time frame (1982 to 2018) and older cases presented less surviving rates than the more recent ones, this may be the reason for the surviving rate being lower than expected. A lot of factors may have contributed to this situation, such as the fact that equine neonatal medicine was starting to evolve, more availability of some drugs in recent years, or an improve in the experience in neonatology of the clinicians team.

In the population in study, the number of fillies affected by NE was very similar to the number of colts. As seen in a previous report (Lyle-Dugas, 2017), no association between surviving rate and gender was observed.

Regarding breed, there was a higher number of American Quarter Horses and Tennessee Walking Horses, however, this was probably due to the geographical area from where the medical records were collected. It was also interesting to notice that besides Tennessee Walking Horses were the second most common breed both in the entire population and in the surviving group, among non-survivors it was fewer represented (7.7%), being replaced by Thoroughbreds in the second position with 19.2%. The surviving rate was not statistically associated with any breed included in this study, which is in accordance with recent studies (Gold, 2016; Lyle-Dugas, 2017).

The major predisposing factors for NE in this population were delivery deemed a dystocia, premature foal, and fescue consumption during gestation. When compared to survival, none of these factors seemed to have an influence in the foal's surviving rate. The presence of dystocia has previously been studied and compared to survival in foals with NE, but, as seen in the present study, no association was found (Gold, 2016; Lyle-Dugas, 2017). Although prematurity was not associated with the surviving rate in this population, recent reports demonstrated its association with non-survival in foals with NE (Gold, 2016; Lyle-Dugas, 2017). These differences in results may be due to the few cases of prematurity in the present population. Fescue toxicity is a known predisposing factor

for NE reported in the literature (McSloy, 2008) but, as far as we know, there is no impact reported in foal's survival.

Less common predisposing factors for NE included maiden mare, mare's disease during gestation, large foal, and dysmature foal. None of these factors were associated with survival in this population as well. Being dystocia an often reported predisposing factor for NE (Tennent-Brown, 2015) and it is more common in mares during their first foaling (McCue and Sitters, 2014), we decided to include the variable "maiden mare" in this study. However, since the presence of dystocia had no influence in the foal's survival, the presence of a maiden mare did not as well. A study in 2016 demonstrated that foals with NE born from mares that had experienced some disease during gestation had almost 8 times greater odds of non-surviving than the ones born from mares with a healthy pregnancy (Gold, 2016). Since in the population in study there were only 5 foals in this situation, this may limit its evaluation. Fetal oversize was included in this study since it is a reported predisposing factor for dystocia (Pynn, 2014), but no association with survival was found. The presence of dysmaturity is usually associated with placental insufficiency and can affect all body systems (Wilkins, 2004b). Given this multisystemic evolvement, its influence in the foal's surviving rate was evaluated, however, no association was discovered.

When evaluating heart rate on admission, it was demonstrated that neither a normal heart rate nor bradycardia nor tachycardia seemed to have an influence in survival (p=0.966). Non-surviving foals had a lower mean heart rate (95.09 bpm) than the surviving ones (103.56 bpm), with no statistically significant differences (p=0.286), similar to other retrospective report (Lyle-Dugas, 2017).

Evaluation of survival rate and respiratory rate showed that neither a normal nor an abnormal respiratory rate were associated with survival (p=0.688). As seen in another study (Lyle-Dugas, 2017), there were no statistically significant differences (p=0.267) between the mean respiratory rate of non-survivors (36.42 rpm) and survivors (30.54 rpm).

Regarding body temperature and survival, there were statistically significant differences between survivors and non-survivors (p=0.016), with higher mortality in hypothermic foals. This was an expected finding, since the presence of hypothermia in neonatal foals has been previously associated with non-survival in several studies (Furr, 1997; Giguère, 2017; Lyle-Dugas, 2017). Non-survivors had a lower mean body temperature (35.3ºC) than surviving foals (37.3ºC) with significant statistical differences

(p=0.031). Lyle-Dugas *et al.* (2017), also reported similar results, however, the mean averages were higher than the ones observed in this study (37.4ºC in non-survivors and 38.3ºC in survivors). The present study also demonstrated that foals with hypothermia were 6.65 times more likely to die (p=0.005) than the normothermic ones, suggesting that body temperature was a prognostic indicator in the population in study. This finding is very similar to the one reported by Giguère *et al.* (2017), that demonstrated that hypothermic neonatal foals were 6.24 times more likely to die than the normothermic ones, however, this study evaluated neonatal foals with a wide range of diseases, being unspecific for NE. Hypothermia in foals is of concern since it is not only associated with severe systemic compromise (McAuliffe, 2008) but is also a risk factor for infectious diseases (Barr, 2018). Shivering as a consequence of hypothermia also increases oxygen consumption, predisposing the foal to hypoxemia (Sinclair, 2015) which, in a foal with NE, is of extreme concern.

Even though the use of regional hypothermia is the actual major treatment modality in infants with PAS (Douglas-Escobar, 2015; Sarkar, 2015; Gold, 2017), in our population, the presence of hypothermia was an indicator of poor outcome. We believe there is a difference between intrinsic hypothermia and induced hypothermia. This means that if hypothermia is pathologically present, then a lot of deleterious events are already happening, while if it is induced, it can be a therapeutic measure by inhibiting inflammation and apoptosis. Also, therapeutic hypothermia usually relies on cooling just the head, while pathological hypothermia is not that selective. It often manifests concurrently with hypoglycemia, since the foal uses its limited glycogen stores (body fat percentages of 2-3%), to maintain body temperature within normal range. Therefore, glycemia measurements should be hastily assessed in hypothermic foals (McAuliffe, 2008).

Hypoglycemia is common in neonatal foals given their low glycogen stores, particularly if they suffered a significant stress at birth. If prolonged, severe hypoglycemia can cause cell damage and death (Bernard, 2018b). Hyperglycemia is also detrimental in foals with NE (Corley, 2005). Blood glucose concentration intervals were compared to survival, revealing that mortality was more frequent in foals with abnormal glycemia levels (p=0.009). Even without significant statistical differences, the mean glycemia of non-survivors (97.91 mg/dL) was inferior than the one among survivors (104.43 mg/dL). These results are in accordance with a recent report of Lyle-Dugas *et al.* (2017). Univariate logistic regression models revealed that hypoglycemic foals had a higher

probability to die (p=0.018) and hyperglycemic foals had an even higher probability to die (p=0.015), indicating that glycemia has an influence in the outcome of foals with NE. Blood glucose concentration has also been a frequent parameter when analyzing survival in newborn foals, being included in 3 multivariable models to predict outcome in neonatal foals (Rohrbach, 2006; Dembek, 2014; Giguère, 2017). Though, these models relied on populations of foals with a wide variety of diseases (including NE), but being unspecific for NE. Hypoglycemia and extreme hyperglycemia at admission have also been associated with non-survival in critically ill foals by Hollis *et al.* (2008).

Total protein intervals were evaluated and compared to survival and indicated that there were no statistically significant differences between survivors and non-survivors (P=0.153). Normal range total protein concentrations and hyperproteinemia had similar numbers of survivors and non-survivors, however, the 3 foals with hypoproteinemia died. Also, mean total protein concentrations had very similar values between survivors and non-survivors with no statistically significant differences (p=0.186), suggesting that this parameter did not seem to be associated with the surviving rate of this population. This is in accordance with previous studies, either specific for NE (Lyle-Dugas, 2017) or not (Hoffman, 1992; Furr, 1997; Giguère, 2017).

Hypercreatinemia in foals with NE can be suggestive of placental insufficiency (Bernard, 2018a; Corley & Barr, 2018). Foals that suffered a hypoxic insult during birth typically have some degree of renal insult too (Spraybarry, 2008). Even so, an increase in creatinine concentrations may also be a consequence of hypovolemia with subsequently decreased renal perfusion or a result of nephrotoxic drugs (Corley & Barr, 2018), which, if not solved promptly, may lead to death (SprayBarry, 2008). In the present study, there were statistically significant differences between survivors and non-survivors (p=0.019), in particular with extreme hypercreatinemia (>4 mg/dL), which was associated with increased mortality. Non-surviving foals had higher creatinine concentrations (3.83 mg/dL) than surviving foals (2.61 mg/dL) but with no statistical significant differences between groups (p=0.080), indicating that although creatinine levels at admission may have some influence in survival, the differences between survivors and non-survivors were not statistically significant. Lyle-Dugas *et al.* (2017) reported similar results in its population of 94 foals with NE, however, when populations of neonatal foals with several diseases were analyzed, increased serum creatinine concentration was associated with non-survival (Hoffman, 1992; Furr, 1997; Dembek, 2014; Giguère, 2017).

Regarding BUN intervals concentrations, there were no significant statistical differences between survivors and non-survivors (p=0.412). The descriptive analysis showed an almost identical mean BUN concentrations between survivors (27.72 mg/dL) and non-survivors (27.09 mg/dL), without significant statistical differences (p=0.875), suggesting that BUN concentrations on admission had no influence in the foal's survival. This non-relationship between BUN concentrations and survival has been previously reported, whether in NE-specific reports (Lyle-Dugas, 2017), whether in NE-unspecific reports (Hoffman, 1992; Furr, 1997; Giguère, 2017).

Almost all foals demonstrated clinical signs in the first 24h of life, with no influence in the prognosis when compared to survival (p=0.498). Recumbency was the most common clinical sign, being present in 53 of the 61 foals. It is often observed in foals with NE (MacKay, 2005; Tennent-Brown, 2015). This variable was compared to survival and very significant statistical differences were found between survivors and non-survivors (p=0.009), as reported in a recent study (Lyle-Dugas, 2017). Among the recumbent foals, the number of survivors and non-survivors was almost the same (27 and 26, respectively), however, all of the non-recumbent foals survived. This indicates that the absence of recumbency may be an indicator of good prognosis in the population in study. Recumbency has also been included in a mathematical multivariable model to predict foal's prognosis, but this study relied on a population of foals with a variety of diseases, not just NE (Rohrbach, 2006).

Blood gas measurements were only available in 42 foals, of which 40 had hypoxemia. This supports the role of a hypoxic-ischemic episode in the pathophysiology of NE in the population in study, as described in the literature (Wong, 2011; Tennent-Brown, 2015; Gold, 2017; McKenzie, 2018). No significant statistical differences between survivors and non-survivors were found (p=0.129), suggesting that the presence of hypoxemia did not seem to be associated with survival. A recent study evaluated the mean averages of PaO2 levels between survivors and non-survivors, but no statistical differences were described as well (Lyle-Dugas, 2017).

No significant statistical differences between survivors and non-survivors were found when dehydration was compared to survival (p=0.852). This means that dehydration did not seem to influence this population's surviving rate. This comparison has previously been analyzed by Hoffman *et al.* (1992), showing the same results. However, that study did not evaluated foals with NE exclusively.

Although while evaluating gastrointestinal problems and its association with survival showed no statistical differences, it is important to notice that most of these foals survived. Lyle-Dugas *et al.* (2017) reported similar findings. This is suggestive that, in spite of the gastrointestinal compromise present in these foals, they tend to survive.

According to the literature, foals with NE that have signs of seizure activity are associated with worse outcome than those that do not. However, with the right therapeutic interventions, the prognosis of these foals is good (Bernard, 2018a). In the present study, despite there were more foals dying with seizures than surviving, no statistical differences were found between survivors and non-survivors (p=0.186). This is in accordance with a recent study (Lyle-Dugas, 2017) but foals with seizures within the first 24h were more likely to die in another report (Gold, 2016). Since we did not make this differentiation of the onset of seizure activity due to a limitation of the number of animals, a comparison with the results from Gold *et al.* (2016) could not be made.

About the loss of suckle reflex and its comparison with survival, no significant statistical differences were found (p=0.490), suggesting that the absence of suckle reflex did not seem to be associated with the surviving rate. Although this parameter was previously included in a multivariable model to determine the outcome in foals (Rohrbach, 2006), a NE-specific report described no association between loss of suckle reflex and survival (Lyle-Dugas, 2017), similar to our study.

Regarding tongue protrusion, no statistical differences between survivors and non-survivors were found (p=0.107), suggesting that this variable had no influence in this population's survival. As far as we know, this association is not described in the literature.

The most common concurrent diseases found in this population were failure of passive transfer immunity, ophthalmic problems, pneumonia, limb abnormalities, and umbilical problems. Less common concurrent diseases included anemia and sepsis.

Failure of passive transfer immunity was not statistically associated with the surviving rate (p=0.793), indicating that it had no influence in the survival. This is in contrast with a recent study that showed that foals with NE that developed failure of passive transfer immunity were less likely to survive (Gold, 2016). However, opinions diverge when analyzing foals with disregard for disease. Giguère *et al.* (2017) reported that foals with FPTI were more likely to die, whereas Furr *et al.* (1997) stated that there was no association between FPTI and survival.

Ophthalmic problems were compared to survival, with no significant statistical differences found between survivors and non-survivors (p=0.523). The same results were

reported when Lyle-Dugas *et al.* (2017) analyzed the influence of abnormal pupils and corneal ulceration with survival.

Pneumonia in neonatal foals is usually associated with sepsis or milk/meconium aspiration. It is a well-known cause of morbidity and mortality in these animals (Slovis, 2008). As so, in the population in study it was statistically associated with mortality (*p*=0.002). It has been previously described as one of the main reasons for death or euthanasia in a population of 94 foals with NE (Lyle-Dugas, 2017). Gold *et al.* (2016) has also reported that foals with at least one complication during hospitalization (including pneumonia) had greater odds of non-survival. Also, Hoffman *et al.* (1992), associated respiratory compromise with non-survival in critically ill foals.

Although limb deficits are reportedly rare (Vaala, 2003), 26% of this population had some sort of limb abnormality. Even though its comparison to survival indicated that there were no significant statistical differences between survivors and non-survivors (*p*=0.097), almost all of these foals survived. This suggests that despite not being associated with the surviving rate, foals with limb abnormalities tended to survive. To the best of our knowledge, there is no literature associating foals with NE and limb abnormalities with survival, but a NE-unspecific study conducted by Furr *et al.* (1997) stated that joint disease had no influence with survival. However, the "limb abnormalities" group created in this study included much more than joint disease.

Umbilical problems had no statistical association with survival (*p*=0.356). This is in accordance with Furr *et al.* (1997), even though this study was not specific for NE.

Neonatal foals have poor adaptive mechanisms, so a hypovolemic shock may be easily installed when the capacity of the red blood cells to carry oxygen decreases (Bernard, 2018c). A low PCV can cause tissue hypoxia and consequent organ compromise (Corley, & Barr, 2018), hence the importance of quickly correct the anemia when in presence of a foal with NE. Significant statistical differences between survivors and non-survivors were found when anemia was compared to survival (*p*=0.04). In fact, most of the foals with anemia died, which indicates that the presence of anemia was an indicator of poor prognosis in the population in study. This is in disagreement with a recent report that evaluated mean PCV values between survivors and non-survivors, but no statistical differences were found (Lyle-Dugas, 2017).

Almost all foals with sepsis did not survive. When compared to survival, there were significant statistical differences between survivors and non-survivors (*p*=0.034), suggesting that the presence of sepsis is an indicator of poor outcome in this population.

This was an expected result, since sepsis is the main cause of mortality and morbidity in neonatal foals (Barr, 2018). According to Lyle-Dugas, *et al.* (2017), sepsis was one of the main reasons for death or euthanasia in foals with NE. Previous studies in critically ill foals also described a positive association between sepsis and non-survival (Dembek, 2014; Giguère, 2017).

The treatment was mainly supportive, as described in previous reviews (Wong, 2011; Tennent-Brown, 2015; Gold, 2017; McKenzie, 2018), with intravenous fluids with dextrose supplementation, use of antibiotics, plasma administration, and oxygen therapy as the main therapeutic interventions. The use of antibiotics was compared to survival, showing significant statistical differences between survivors and non-survivors (p=0.032). This suggests that antibiotics administration was positively associated with survival. The use of antibiotics is of major importance for a successful outcome, in order to prevent systemic infections (Barr, 2018). However, a similar study demonstrated no association between the use of antibiotics and the surviving rate (Lyle-Dugas, 2017).

Dextrose supplementation comparison with survival showed no statistical differences between survivors and non-survivors (p=0.424), indicating that dextrose supplementation did not seem to have an influence in survival.

As described in a previous study (Lyle-Dugas, 2017), there was no statistical association between plasma administration and survival (p=0.915). This makes sense, once there was no association with the presence of failure of passive transfer immunity as well.

Regarding oxygen therapy, there were no significant statistical differences between survivors and non-survivors (p=0.162), indicating that this variable did not seem to be associated with the surviving rate. Opposite results were described by Lyle-Dugas (2017), who evaluated the influence of mechanical ventilation in the surviving rate of foals with NE. This study showed that this therapeutic intervention was associated with mortality, but probably due to the fact that foals in need of respiratory support were already in a deteriorated and compromised state.

The comparison between the administration of DMSO and survival indicated no significant statistical differences between survivors and non-survivors (p=0.798). This is in accordance with a previous recent report (Lyle-Dugas, 2017).

The surviving rate was statistically associated with the administration of non-steroidal anti-inflammatory drugs (p=0.010). Foals that received NSAIDs tended to survive. It is reported in the literature that inflammatory mediators can lead to a cascade

of events ultimately causing neuronal brain injury (Shalak, 2002), so the use of anti-inflammatory drugs is often used in foals with NE (Hahn, 2008).

Most of the foals that received vitamins did not survive. When compared to survival, statistical differences between survivors and non-survivors were found (p=0.013). A possible explanation could be that foals that received vitamins were in bigger compromise than the others, so vitamins were administered as a last resource, rather than an first-choice therapy. Lyle-Dugas *et al.* (2017) evaluated the influence of the use of Vitamin E in the surviving rate of foals with NE, but no association was found.

Regarding the use of corticosteroid therapy, it is described in the literature that it has no role in the treatment of NE (MacKay, 2005), however, since it was used, we decided to include it in this study, but no association between surviving rate and the use of corticosteroids was observed (p=0.834).

Caffeine administration was evaluated and compared to survival, but there were no significant statistical differences between survivors and non-survivors (p=0.286), refuting its association with the surviving rate. However, most of the foals that received caffeine administration survived. The use of respiratory stimulants and its influence in survival has been previously reported, being associated with mortality (Lyle-Dugas, 2017), however, it is probable that these foals were already in a deteriorated condition when they required respiratory stimulants.

No association was found between survival and the use of diuretics (p=0.412). When the use of mannitol was tested for this association, no statistical differences were found as well (Lyle-Dugas, 2017). Still, we have also included furosemide in this group, but, to our knowledge, its influence on survival in foals with NE has not yet been documented.

As any retrospective study, there were some limitations in the current study. The fact that it relied on medical records made it dependent on completeness and accuracy of the information available. Since there is no definitive *ante-mortem* diagnostic test for NE, diagnosis was made based on history and clinical signs, which can be subjective. This can also lead to either under- or over-report of some subjective criteria. Another limitation in this study was the size of the population. 61 foals is a small sample, which led to some variables being contracted into larger groups for analytic reasons, for example, gastrointestinal problems and limb abnormalities.

5. CONCLUSIONS

In conclusion, the current study demonstrated the following:

- Some aspects regarding the physical exam and laboratory results can give the clinician useful information about possible survival or non-survival in foals with Neonatal Encephalopathy.
- Clinical signs associated with a good surviving rate were body temperature within normal range, normal glycemia levels, normal creatinine concentrations, and absence of recumbency. Clinical signs that seemed to negatively affect the survival were the presence of hypothermia, abnormal glycemia levels, and creatinine levels >4 mg/dL. Hypothermic, hypoglycemic or hyperglycemic foals had greater odds of non-survival.
- Concurrent diseases such as pneumonia, anemia and sepsis were associated with mortality.
- Among the different therapeutic interventions used to treat foals with Neonatal Encephalopathy, the use of antibiotics or non-steroidal anti-inflammatory drugs were positively associated with survival.

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APPENDIX I – Cases observed during the externships

Cases observed during the externships	Hospital La Equina	Ambulatory Equine Practice with Dr. Nuno Bernardes	Equine Hospital - UTCVM
Neonatology			
Prematurity	1		
Neonatal Encephalopathy			1
Colic			1
Diarrhea			1
Dysphagia			1
Aspiration pneumonia			2
Dermatology			
Dermatitis	2		2
Cellulitis			1
Habronemiasis (limbs)	1		
Orthopedics			
Arthrosis	1		
Arthritis/ Septic arthritis	1		2
Fracture of the 4th metatarsal bone	1		
Hoof abscess	1		2
Superficial digital flexor tendinitis	1		3
Proximal suspensory desmitis			1
Calcification of the deep digital flexor tendon			1
Laminitis	1		1
Exostosis of the radius	1		
Osteochondrosis	6		
Limb lacerations			2
Intra-articular medication (back and hocks)	5		4
Arthroscopy of the tarsal joint	1		
Palmar digital neurectomy	1		
Neurology			
West Nile Virus Infection	1		
Gastroenterology			
Esophageal obstruction			1
Esophageal ulcers			1
Gastritis (Gasterophilus spp)			1

Gastric ulcers			2
Colic - Enterolithiasis			1
Colic – unknown etiology			1
Colic – right dorsal displacement of the large colon			1
Colic - torsion of the ascending colon	1		
Diarrhea	3		
Oncology			
Keratoma	1		
Sarcoid			1
Squamous cells carcinoma			1
Chemotherapy			1
Respiratory			
Pneumonia			3
Strangles			1
Sinusitis	1		
Nebulization			2
Dentistry			
Routine dental examination		2	2
Fractured tooth			1
Tooth extraction			2
Diastema widening			1
Cardiovascular			
Supraventricular arrhythmia			1
Podiatry and Farriery			
Orthopedic shoeing		2	
Hoof trimming		10	
Hoof shoeing		12	
Reproductive			
Evaluation of the fetus			1
Paraphimosis	1		
Castration			5
Preventive Medicine			
Vaccination/Deworming		4	10
Trauma			
Ethmoid hematoma			1
Fractured mandible			1
Rehabilitation & Sports Medicine			
Acupuncture			4
Chiropractic			6
Hydrotherapy			5

Laser therapy			1
PRP Injection			2
Carrot Stretches			10
Use of an orthopedic boot			2
Others			
Pre-purchase exam		3	
Cachexia (rescued from situations of neglect)			3
Necropsy			2