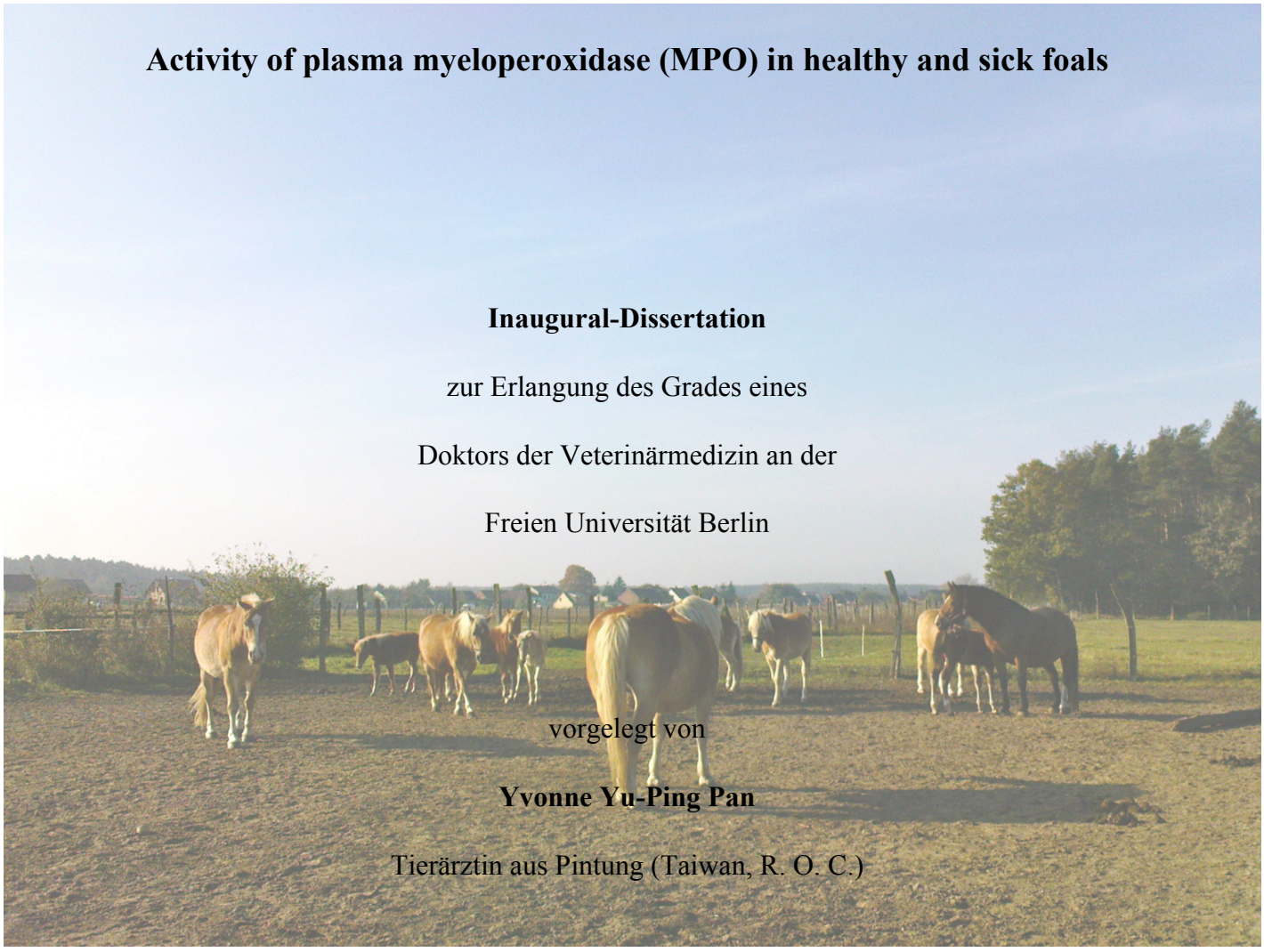
Aus der Klinik für Pferde, Allgemeine Chirurgie und Radiologie

des Fachbereichs Veterinärmedizin

der Freien Universität Berlin

Activity of plasma myeloperoxidase (MPO) in healthy and sick foals



Inaugural-Dissertation

zur Erlangung des Grades eines

Doktors der Veterinärmedizin an der

Freien Universität Berlin

vorgelegt von

Yvonne Yu-Ping Pan

Tierärztin aus Pintung (Taiwan, R. O. C.)

Berlin 2016

Journal-Nr.: 3907

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For the horses and the foals.

Content

1. Introduction

Foals are the precious future and hopes of the horse reproduction industry. It feels like they have accompanied us for a very long time; however, it is still so little we truly know about them. Similar to human babies and other infants, many physical and laboratory values change during growth (Harvey et al., 1984), and these young animals also tend to be more susceptible to many pathogens or more vulnerable than adults. Thus, knowing the foals' normal reference ranges of the parameters of clinical examination and the selection of indicators, which can reflect the severity of the illness or response to the treatment sensitively and rapidly, have become a key point in equine neonatal medicine nowadays.

Acute inflammatory diseases in foals still remain a main cause for losses in the horse reproduction industry (Cohen, 1994). Those diseases can be infectious or non-infectious, and the syndromes are not always identical for each foal. Frequently, foals would have more than just one lesion during the whole disease progress, and these young animals tend to exacerbate in a very short period if care was not taken properly. Many efforts have been put into developing diagnostic methods or parameters in order to support practitioners making more accurate diagnoses, choosing better therapies and providing more precise prognoses to help owners and animals. Besides the routine hematological examinations like CBC, there are some other parameters routinely used for recognizing acute inflammatory processes in equine neonatal medicine: fibrinogen (Koterba et al., 1984; Newquist and Baxter, 2009), glucose (Koterba et al., 1984), lactate (Castagnetti et al., 2010), and SAA (Hultén and Demmers, 2002a).

Myeloperoxidase (MPO) is a specific enzyme in the azurophic granules located in polymorphonuclear leucocytes (for example: neutrophils) (Klebanoff, 1999). An increased plasmatic MPO level has been reported as a marker of neutrophil activation in humans, and is assumed to be related to acute-inflammatory reaction (Buss et al., 2000). For over the last decade, many experiments were contributed to develop the techniques of purifying (Mathy-Hartert et al., 1998) and measuring the equine MPO level from samples like body fluid (Art et al., 2006; Grulke et al., 2008), plasma (Grulke et al., 2008) or even tissues (Riggs et al., 2007), in order to investigate the relationship between MPO level and the severity of diseases. Many studies have been done in adult horses with gastro-intestinal (Grulke et al., 1999 & 2008), respiratory (Art et al., 2006) or joint diseases (Fietz et al., 2008b; Wauters et al., 2012),

but such research in foals is lacking. In equine medicine, knowing the MPO level of a sick foal might aid more detail to the severity of the disease process or the response to the treatment; furthermore, it might help the practitioner to make more persuasive diagnoses or prognoses.

Since the determination of MPO activity from foals' plasma samples has not been evaluated in any previous study so far, it was our interest to investigate, whether changes of MPO activity are related to diseases in foals. We thus hypothesized that MPO levels are higher among foals suffering from acute inflammation compared to healthy foals.

Therefore, we established a standard MPO activity range from plasma samples of healthy foals with different ages; in the second part of this work, we collected blood samples from foals admitted to the clinic with any kinds of diseases, before and after the onset of treatment. All blood samples were analysed for MPO activity level and other blood parameters. There were three major points we wanted to address by the study: 1. Relationship between MPO activity levels and the age of the foals. 2. Comparison of MPO activities between healthy and sick foals at the same age. 3. Correlations between MPO activities and the severity of inflammatory status and responses to the treatments, respectively.

2. Review of the literature

2.1 Common diseases in neonatal foals

Pathological states and diseases that insult foals can arise during different periods: gestation (congenital defect, infection or asphyxia); parturition (trauma, acute asphyxia); or extrauterine life (FPT, infections, GI tract problem, trauma, etc.). Some foals suffering from these insults may appear normal at first, but then hindered to successfully adapt to extrauterine life later (Adams, 2005). Initial signs of disease in neonatal foals are usually faint; and it is often difficult to address the problem just to one specific organ system. Listed below are some conditions, which often occur in the foals included in the present study, they can often easily overlap with one with another.

2.1.1 Prematurity

The gestation period in mares has a very wide range based on breeds and other factors (Lester, 2005); therefore, the attempt to judge prematurity of a foal based only on its birth weight and crown-rump length tends to be inaccurate (Adams, 2005). However, premature foals often share some similar characteristics: low birth weight, small body size, short and silky hair coat, prominent rounded head, droopy ears, soft lips, lax flexor tendons, thin body condition, poor muscle development and/or ossification (Adams, 2005; Lester, 2005). Haematological evaluation usually reveals low total white blood cell and neutrophil counts, N:L ratio smaller than 1.0, even when foals are absent of sepsis (Jeffcott et al., 1982; Rossdale et al., 1984; Sanchez, 2005). The cortisol value over the first few hours after birth in premature individuals is low; the response to exogenous $ACTH_{1-24}$ is also poor (Silver et al., 1984). The impaired physical and physiological conditions can predispose premature foals to many systematic problem and complications, the treatments to them are usually challenging. The information of long-term outcomes from these patients is limited, but the problems at initial stage seem to be compromised as they grow (Lester, 2005).

2.1.2 Failure of passive transfer of immunity (FPTI)

Foals are born agammaglobulinaemic; globulins and antibodies are necessary for the neonatal period, which are mainly provided by the colostrum (Jeffcott, 1974; Koterba, 1989). The

intestinal absorption of colostral IgG is reported to be most efficient within the first twelve hours after parturition (Raidal et al., 2005), the highest serum IgG level can be detected after twelve to eighteen hours after colostrum intake (Jeffcott, 1974). When the colostral ingestion is delayed, the uptake of IgG by the neonatal intestine would be reduced due to the closure of the macromolecules absorption (Raidal et al., 2005).

Healthy and non-infected foals have normally IgG concentrations exceeding 800 mg/dL (Koterba et al., 1984, Koterba, 1990). Failure of transfer (FTP) is a status describing neonatal foals with inadequate amount of immunoglobulin after certain time after birth (eighteen to twenty-four hours) (LeBlanc et al., 1992; Raidal, 1996) it is also referred as hypogamma-globulinemia. This increases the susceptibility to illness and infection, and in some cases, also death (McGuire et al., 1975; McGuire et al., 1977; Robinson et al., 1993; Raidal, 1996). There have been many debates about the sufficient IgG concentration both in foals with well farm-management and in hospitalized foals that require intensive care. Baldwin et al. (1991) demonstrated in 132 Standardbred foals with well-managed environment, that the IgG levels didn't seem to correlate with the prevalence and severity of illness or survival rate neither in 21-day or 90-day postnatal period in this population. In neonatal foals, FTP is generally acknowledged nowadays having an IgG concentration < 400 mg/dL (LeBlanc et al., 1992), the incidence was observed between 1.9 to 13% in different studies (LeBlanc et al., 1992; Raidal, 1996; Nath et al., 2010). Although, foals with partial FTP (IgG concentration between 400-800 mg/dL) were recognised as "healthy" in a well-managed situation (Raidal et al., 2005), an IgG concentration more than 800 mg/dL is generally believed to be adequate and thus, providing a better survival chance both in farm and hospitalized foals (Koterba et al., 1984; Raidal, 1996).

2.1.3 Infections and sepsis

2.1.3.1 Infections

Neonates are under some unfavourable status – for example perinatal stress (including in utero hypoxia), prematurity and poor colostral intake – vulnerable to infections. Opportunistic bacteria, which normally are found in the genital tract, on the skin or in the environment, can cause infection. This may take place through placenta, during parturition and after birth (Koterba, 1989); the rate of infectious diseases increases from pre- to postnatal state (Thein and Essich, 1993). Similar to septic calves (Aldridge et al., 1993), gram-negative bacteria are the most predominate isolates in blood culture from bacteremic/septic foals in most of the investigations, in particular Escherichia coli (Koterba, 1989; Wilson and Madigan, 1989; Raisis et al., 1996; Russell et al., 2008; Sanchez et al., 2008, Hollis et al., 2008b).

Various clinical signs are associated with infection, but most of them are not conclusive to a specific organ or disease. Fever may or may not be present in the process, and the absence of fever should never be a reason to rule out any possibility of infection. High fibrinogen levels (10g/L) detected in new-born foals has been reported to be indicative for infection or exposition to inflammatory placental diseases during foetal stage; while mildly elevated fibrinogen levels (4 to 5 g/ L) are more likely to be associated with postnatal infections. However, when these postnatal acquired infections develop to more chronic stage, a dramatically increase in fibrinogen level may be seen (Koterba, 1989).

2.1.3.2 Sepsis

Sepsis is the reaction of the organism to an infection, which involves a non-specific systemic inflammatory response. The American College of Chest Physicians and the Society of Critical Care Medicine stated a set of definitions concerning sepsis in 1991 (Bone et al., 1992); these were also adopted for veterinary medicine use. According to the suggested definition, the term sepsis is appropriate when the systemic inflammatory response syndrome (SIRS) occurs in response to a confirmed infectious process. When sepsis is related to organ dysfunction, hypoperfusion or hypotension, it is then defined as severe sepsis.

For decades, sepsis has been reported to be the most common medical problem that affects young foals, and is the notorious cause for morbidity and mortality in neonates (Hoffman et al., 1992; Cohen, 1994). There are many factors that can predispose foals to infection. Maternal factors are dystocia, premature placental separation and placentitis. The most likely postnatal risk factor is the failure of passive transfer immunity (FPTI); other postnatal factors like prematurity, environmental or management conditions that might favour umbilical, gastrointestinal tract and respiratory tract infection (Adams, 2005; Sanchez, 2005).

Septic foals often show non-specific and indistinct clinical signs including depression, reduced intake, lethargy or increased recumbence. Commonly seen are tachycardia and tachypnea. Foals may have mildly increased rectal temperature, but it can also be within normal range. Besides the systemic signs, additional locations of infection such as diarrhea, uveitis, joint effusion, lameness alone or lameness associated with pain, respiratory disease, patent urachus and omphalitis should not be ignored (Sanchez, 2005).

Foals with acute sepsis tend to have leukopenia, characterized by neutropenia (Sanchez, 2005); some foals may have normal WBC count (Koterba, 1989). However, an increased number of band neutrophils (> 50 cells/ μL), degenerative left shift and evidence of toxic changes in the neutrophils are typical findings in these patients (Koterba, 1989; Sanchez, 2005; Barton, 2008). A very low WBC number with considerable toxicity was reported related to non-survival; nevertheless, a low WBC count should not be used as a definite rule of predicting death (Koterba, 1989). Other haematological findings like hypoglycaemia, acidaemia and elevated lactate value are also common (Koterba, 1989; Sanchez, 2005). Septic foals frequently have abnormal coagulation and fibrinolytic system, e.g. increased fibrinogen (Hultén and Demmers, 2002a; Sanchez, 2005; Newquist and Baxter, 2009).

Blood culture is still assumed to be the gold standard for diagnosis of systemic bacterial infection (Sanchez, 2005); most of the isolates from septic foals are gram-negative organisms (Koterba, 1989; Wilson and Madigan, 1989; Raisis et al., 1996; Barton et al., 1998; Russell et al., 2008; Sanchez et al., 2008, Hollis et al., 2008b). Despite its reliability as supposed, the results are not available within less than two days, and findings can be influenced by many factors (Sanchez, 2005); false-negative results were often reported (Wilson and Madigan, 1989). Therefore, some sepsis score systems were developed by experienced clinicians to identify foals with the need for urgent medical help within a shorter time. These systems

usually take historical, clinical and laboratory diagnostic parameters associated with sepsis into consideration (Brewer and Koterba, 1988). However, the scores tend to be inaccurate in case of some limitations, e.g. elder foals, different regions and different institutions (Sanchez, 2005).

Other pathological or laboratory parameters such as SAA (Stoneham et al., 2001; Hultén and Demmers, 2002a), blood lactate (Castagnetti et al., 2010) and blood glucose (Hollis et al., 2008a) were also reported to be valuable additions to diagnostic, predictive or prognostic indicators.

Systemic inflammatory response syndrome (SIRS)

The SIRS represents a systemic inflammatory response, despite the initiating causes. When at least two of the following symptoms are fulfilled, the presence of SIRS is most likely: fever, tachycardia, tachypnea or hyperventilation; leukocytosis, leukopenia or relative increase of circulating immature neutrophils (bands) (Bone et al., 1991).

Septic shock

In the case that sepsis progresses and induced hypotension cannot be compensated by appropriate fluid support causing signs of hypoperfusion and organ dysfunction, the stage of septic shock is reached (Bone et al., 1992).

2.1.4 Meconium impaction

Meconium is a combination of intestinal glandular secretions, swallowed amniotic fluid and other cellular debris stored in colon and rectum during foetal period. Foals normally pass this material within the first twenty-four to forty-eight hours after birth due to peristalsis stimulated by the ingestion of colostrum (Koterba, 1990; Wilson and Cudd, 1990). Meconium impaction is the most common cause for abdominal pain in newborn foals during the first day

of life (Koterba, 1990; Cudd 1990). Asphyxiation, impaired GI motility or some intestinal diseases could predispose foals to the failure of expelling meconium; in male foals, the narrower pelvic canal can also account for this problem. Clinically, the foals would show tenesmus and repeated posturing. With time abdominal distension and signs of colic become apparent (Wilson and Cudd, 1990). In most of the cases, the problem can be resolved with medical therapy (Koterba, 1990).

2.1.5 Neonatal maladjustment syndrome (NMS)

Neonatal maladjustment syndrome, also known as neonatal encephalopathy, or dummy foals, is a term describing a non-infectious central nervous system (CNS) disorder associated with behavioural abnormalities in new-born foals (Green and Mayhew, 1990). The exact cause(s) for this syndrome is (are) difficult to define, but it is generally believed that asphyxia/hypoxic-ischemic damage to CNS plays a crucial role in the pathogenesis of NMS. Any event that can lead to asphyxia may attribute to this injury, for example, dystocia, chronic placental insufficiency or acute placental separation (Green and Mayhew, 1990; Paradis, 2006). Usually the initial sign noted is the loss of suckle reflex, which can be followed by weakness, disorientation, wandering, loss of bonding to the dam, and recumbency. Not every foal goes through the entire process, some may just show loss of the suckle reflex. In more progressed cases, neurological syndromes like teeth grinding, hyperexcitability, extensor spasms of the limbs, neck and tail, convulsion or even semi-comatose unresponsiveness can also be observed (Green and Mayhew, 1990; Paradis, 2006). Diagnosis is usually made by exclusion of other conditions that can hinder foals from adapting to extra-uterine life (for example: sepsis, malformations) (Green and Mayhew, 1990).

2.2 Diagnostic approach to the sick neonatal foal

Physical examination and laboratory analyses are two essential components for making a presumptive diagnosis (Koterba, 1989; Knottenbelt et al., 2004b); information should be collected as much as possible during any evaluation.

2.2.1 Clinical examination

In contrast to adult horses, foals usually show vague signs at the initial stage of sickness; this is often left easily unrecognised and leads to irreversible devastation. Simple but thorough clinical examination should be assessed frequently for early recognition of any possible threat. The examination of the mare and the placenta, if possible, is always a part of the clinical examination of the foal. This can provide important information and, therefore, should not be left unnoticed (Koterba, 1989; Knottenbelt et al., 2004b). Sufficient clinical examination of foals should address the following points (Koterba, 1990; Knottenbelt et al., 2004b):

History

Previous disease, previous general and reproductive history of the dam, gestational age, parturition type and duration, time taken to rise and to nurse.

Behaviour

Maternal affinity, suckle enthusiasm and ability, any convulsions, ataxia, colic, or lethargy.

Vital signs

Body temperature, heart and pulse rate/quality, respiratory rate and character, mucous membrane colour, capillary refill time, petechial haemorrhage.

Cardiorespiratory system

Auscultation of the heart and lung.

Gastrointestinal tract

Passage of meconium, type and amount of faeces, auscultation of intestinal peristalsis, abdominal distension.

Urogenital system

Pattern and frequency of urination, size and moistness of umbilicus.

Musculoskeletal system

Contracture, laxity, angular limb deformities, any other malformations of the limbs, any heat swelling, oedema or pain around the joints and physes, any fractured ribs or trauma.

Eyes

Presence of entropion, ectropion, corneal erosions or ulcers, uveitis.

Neurological system

Convulsions, seizures, irregular breathing, random nystagmus and blinking, bruxism, chewing/ salivating, paddling of limbs and opisthotonus.

Congenital abnormalities

Any abnormal heart murmur, cleft palate, poor jaw conformation, umbilical and scrotal hernias.

Examination of the mare and placenta

The mare's udder and milk, any sign of mastitis, examination of the placenta, reproductive tract of the mare.

In case of emergency, cardiorespiratory/vital signs and neurological systems will be usually evaluated as main priorities (Knottenbelt et al., 2004b).

2.2.2 Laboratory parameters

Besides clinical examination, laboratory evaluation of selected parameters provides an extra view on the on-going disease process. Changes in these parameters in newborn foals often reflect the in utero environment and helps to identify foals in risk for developing clinical problems. However, the neonatal period is a transition phase, which foals have to go through from foetal to extra uterine life, the possible influence of age on the resulting values should be

considered. However, laboratory results should never be interpreted without considering the clinical examination findings (Axon and Palmer, 2008).

2.2.2.1 Haematology

Leukocytes counts

The normal total leukocyte count of foals varies in a relative wide range from birth to one year of age, which might be due to the decrease of neutrophils, the increase of lymphocyte count, the combination of these two and the interaction with parasites as well as other foreign antigens (Harvey et al., 1984). However, at the age of three to four months, foal leukocyte count values can be considered within adult reference intervals (Welles, 2010).

By the onset of acute septicaemia, the common hematologic finding is characterized by leukopenia (Sanchez, 2005), and then the white blood cell count would usually increase substantially in the following days (Koterba et al., 1984). In other diseases with severe infections, e.g. pneumonia or osteomyelitis, a prolonged leucocytosis is usually followed after the initial period of neutropenia (Koterba et al., 1984). In septic foals, when the age is considered as a factor, the younger (less than one week old) septic foals tended to have leukopenia, while the older ones showed leucocytosis at their present to the hospital (Barton et al., 1998).

Neutrophil-to-lymphocyte ratio (N:L-ratio)

The usefulness of N:L-ratio as a clinical index for evaluating the maturity of newborn foals has been reported (Jeffcott et al., 1982; Rossdale et al., 1982; Rossdale et al., 1984). Healthy newborn foals have a N:L ratio above two, while young foals with a N:L ratio lower than one are considered as premature (Jeffcott et al., 1982; Rossdale et al., 1982; Rossdale et al., 1984; Chavatte et al., 1991).

Differential blood count

Differential blood count provides relative percentages of each type of leukocyte. This examination still remains most reliable when performed manually. When examining a blood smear, leukocytes are classified as neutrophils, eosinophils, basophils, lymphocytes or monocytes. Neutrophils can be further subclassified as segmented neutrophils and band neutrophils. Metamyelocytes (juveniles), myelocytes, promyelocytes and myeloblasts (if present) may be subclassified within the neutrophil series if a severe or markedly degenerative left shift is present (Latimer and Rakich, 1992).

Neutrophils

Foetal neutrophil numbers increase slightly and correlate significantly with gestational age during the late gestational stage (280-305 days) and rise shortly before parturition (Chavatte et al., 1991). After birth, neutrophil counts vary with age but tend to decrease and reach a stable interval by four months of age (Harvey et al., 1984).

Neutrophil count after thirty-five hours *post partum* was used to predict survival or non-survival of premature foals (Chavatte et al., 1991). Foals with prematurity have lower neutrophil numbers and probably a smaller neutrophil storage pool, which together can be considered as a limitation in host defence and increase the susceptibility to diseases in prematurely delivered foals (Chavatte et al., 1991).

In many investigations neutropenia reveals a major haematological abnormality associated with equine neonatal sepsis (Raisis et al., 1996; Barton et al., 1998). It is in relation with acute sepsis (Sanchez, 2005) and often seen in newborn foals with virus infection (Chavatte et al., 1991). It served as a very useful parameter to predict sepsis in young foals (Koterba et al., 1984; Barton et al., 1998); in other studies it was even used for prediction of survival (Peek et al., 2006; Sanchez et al., 2008).

Neutrophilia is also a common haematological finding in foals with bacterial infections (Chavatte et al., 1991); septic foals older than one week showed a tendency to have neutrophilia rather than neutropenia at the time of admission (Barton et al., 1998).

Band neutrophils

Band neutrophils are not often seen in healthy horses, but when observed, they are usually thought to be associated with disease. Its presence usually indicates infection. In studies on septic foals, the increase in absolute numbers of band neutrophils was found to be one of the most typical haematological changes (Koterba et al., 1984; Raisis et al., 1996). In one long-term investigation with 423 Thoroughbred foals, band neutrophil numbers were negatively associated with survival (Sanchez et al., 2008).

Left shift

A left shift is referred to increasing numbers of non-segmented neutrophilic cells presenting in blood. This status can be associated with different types of disorders, but is usually seen during inflammation (Hearvey, 2001; Welles, 2010). Depending on the response to inflammatory conditions, left shifts can vary from slightly increased numbers of band neutrophils to severe left shifts with metamyelocytes, myelocytes and in rare cases promyelocytes. A left shift may occur with neutrophilia but also with neutropenia; the latter one indicates a more severe consumption of neutrophils by a more aggressive inflammatory lesion (Weiser, 2004).

Neutrophil toxic change

Toxic changes in neutrophils are ascribable to degenerative changes and can often be seen in blood smears from animals with left shift response (Weiss, 1984; Harvey, 2001a). This phrase derives from the early observation of the morphological alterations in cells associated with toxaemia in human patients (Weiser, 2004), and is reported to be related to severe bacterial infections or other inflammatory diseases (Gossett et al., 1985). The common described toxic changes seen in cytoplasm are basophilia, vacuolisation, Döhle bodies and sometimes, toxic granulation (Gossett et al., 1985; Harvey, 2001a; Weiser, 2004; Segev et al., 2006; Welles, 2010). These morphological abnormalities are responses to inflammatory stimuli acting on the bone marrow, and are formed before cells were released into the circulation. Under such circumstances, neutrophils are developed at an accelerated rate, and increased amounts of

certain organelles that are present during early development of neutrophils come along as a result (Harvey, 2001b; Weiser 2004).

The evaluation of toxic changes of neutrophils became one of the most useful haematological examinations in veterinary medicine when it comes to equine neonatal infections (Koterba et al., 1984; Barton, 2008; Thompson, 2008). It was also utilized as an item in a neonatal foal sepsis scoring system (Brewer and Koterba, 1988) and to predict septicaemia in calves (Aldridge et al., 1993; Lofstedt, 1999).

Among all the literature describing different types of toxic changes and their relation to the severity of different pathological status, a universal quantitative scoring or grading system has, according to our knowledge, not yet been developed. However, comparisons between different scoring systems are not possible, although some suggestions have been made (Weiss, 1984; Segev et al., 2006; Welles, 2010).

Basophilia of cytoplasm

Increased basophilia can be seen as different levels of increasing blue colour in the cytoplasm. It represents the increasing staining of RNA, and this is a result of the persistence of large amounts of rough endoplasmic reticulum and polyribosomes (Harvey, 2001b; Weiser, 2004). It is interpreted to be the principal evidence that neutrophils are produced at a faster rate (Weiser, 2004), and is the last abnormality to be resolved during the recovery (Latimer and Rakich, 1992).

Döhle bodies

Döhle bodies are composed of retained aggregates of rough endoplasmic reticulum (Weiser, 2004); they appear as gray-blue angular cytoplasmic inclusions at the periphery or near the nucleus (Harvey, 2001b; Thompson, 2008). This type of toxic change alone represents mild evidence of toxicity (Harvey, 2001b) and is reported to be the first sign among all toxic changes (Thompson, 2008).

Vacuolisation of cytoplasm

Different levels of cytoplasmic vacuolization can appear as loss of cytoplasm clarity and neutral-stained granules, occurrence of small cytoplasmic vacuoles and, observation of intense vacuolisation with greyish reticulation (Segev et al., 2006). The last two statuses are often seen in combination with basophilic cytoplasm and are referred as "foamy basophilia". When foamy basophilia is observed, it frequently means severe bacterial infections or in some cases toxaemia (Harvey, 2001b). Vacuolisation might happen as a consequence of using EDTA-anticoagulated blood for blood smears, but this artefact is rarely seen in freshly prepared samples and should not be classified as a toxic change (Harvey, 2001b; Segev et al., 2006).

Toxic granules

Toxic granules are the magenta-staining (pinkish-purple) granules present in cytoplasm. They are primary granules, which retain the staining intensity normally observed in promyelocyte stage in the bone marrow (Harvey, 2001b; Latimer and Rakich, 1992). This toxic change is rarely observed in equine blood smears, however, when present, it indicates severe systemic inflammation (Latimer and Rakich, 1992). This change should not be confused with other granules, which have no relationship to toxicity, and should be present along with other stages of toxicity (Harvey, 2001b; Thompson, 2008).

Erythrocytes

One common abnormality regarding erythrocytes in young foals is anaemia; haemolytic anaemia caused by neonatal isoerythrolysis happens most frequently. This can be diagnosed based on clinical signs and some laboratory tests for maternal alloantibodies on foal's RBCs. Haematological findings of these foals normally show decreases in RBC indices, neutrophilic leucocytosis and hyperbilirubinaemia (Axon and Palmer, 2008). Because horses reticulocytes seldom appear in response to anaemia, different sized erythrocytes (anisocytosis) are seen in stained blood films during regenerative anaemia instead (Harvey, 2001a).

Thrombocytes

Thrombocyte reference value was reported to be higher in newborn foals than in adults (Barton et al., 1995). Thrombocytopenia is often seen associated with disseminated intravascular coagulation (DIC), sepsis, viral infection, SIRS and other pathological statuses (Axon and Palmer, 2008). Among those, DIC is the most common acquired haemostatic problem in foals followed by diseases process such as sepsis and SIRS (Axon and Palmer, 2008). Although not significantly different, the numbers of thrombocytes in sepsis foals tended to be lower than in healthy ones; this together with other haemostatic indices could be helpful in recognising increased risk for coagulopathy in septic foals (Barton et al., 1998).

2.2.2.2 Immunoglobulin-G

Circulating IgG concentration in foals is usually estimated eighteen to twenty-four hours after birth (LeBlanc et al., 1992; Raidal, 1996). Failure of passive transfer of immunity (FTPI) is normally defined as IgG levels lower than 400 mg/dL (LeBlanc et al., 1992).

There are many assays used to evaluate IgG concentration in foals, for example, zinc sulphate turbidity, glutaraldehyde coagulation, quantitative/semiquantitative immunoassay, serum total protein concentration and radial immunodiffusion (RID) (David, 2005). Among those tests, the RID test represents the most quantitative and accurate results in horses; however, the prolonged waiting time (not less then eighteen hours) gives a major inconvenience (David, 2005; Axon and Palmer, 2008). In investigations evaluating various types of IgG assays, most of the assays mentioned above showed good sensitivity and were therefore suitable for initial screening tests for IgG concentration in hospitalized neonatal foals. Nevertheless, the specificity of these assays was relatively low, other disease status may also affect the result (e.g. bacteraemia); this means, there would be some foals with adequate IgG in fact, but might be tested as having a FTPI. This should be kept in mind when using those assays as a single definitive test (Davis and Giguère, 2005; Metzger et al., 2006). Serum total protein concentration proved to be an unreliable parameter in detecting FTPI in foals (Davis and Giguère, 2005; Metzger et al., 2006).

2.2.2.3 Venous blood gas, pH and base excess

Indicative blood gas evaluation is usually performed with arterial blood; however, higher venous pO_2 at admission was also reported to be useful in judging survival rate in foals (Hoffman et al., 1992). Generally, a higher blood pH (≥ 7.35), HCO_3, positive base excess value tend to promise a better outcome (Hoffman et al., 1992; Furr et al., 1997; Gayle et al., 1998), whereas abnormal findings such as academia and negative base excess are usually associated with mortality (Hoffman et al., 1992; Sanchez, 2005).

2.2.2.4 Metabolites: Glucose and lactate

Glucose

Hyperglycaemia is a metabolic alteration, which frequently occurs in critically ill adult and neonatal human patients (Bochicchio et al. 2005; Faustino, 2005). The duration of persistent hyperglycaemia is reported to be strongly associated with morbidity, mortality and the length of stay in an intensive care unit (Bochicchio et al., 2005; Faustino, 2005; Wintergerst et al., 2006). Hypoglycaemia on the other hand, although not often noticed, was also found related to worse prognosis in paediatric intensive care medicine (Wintergerst et al., 2006).

Hyperglycaemia seems to be a more frequent finding in adult horses with acute abdominal and GI track diseases and is most likely associated with mortality; while hypoglycaemia was rarely observed in patients with the same disease category (Hollis et al., 2007; Hassel et al., 2009). Unlike adult hoses, hypoglycaemia is consistently reported in young sick foals suffering from infection or other inflammatory situations and often indicates worse prognosis (Koterba, 1989; Lavoie et al., 1990; Koterba et al., 1984; Hollis et al., 2008a). A higher serum glucose concentration (> 120 mg/dL) at admission was reported to be a favourable finding (Gayle et al., 1998); however, extreme hyperglycemia (> 180 mg/dL) at admission was again associated with a worsened prognosis (Hollis et al., 2008a). Furthermore, any derangements of blood glucose during the first thirty-six hours of hospitalisation were also reported related to non-survival (Hollis et al., 2008a).

Lactate

Lactate is initially higher at birth and then decreases to a stable range within the first days of life (Lorenz et al., 1999; Castagnetti et al., 2010). Elevated concentration may be associated with increased metabolism seen in sepsis, SIRS, increased protein catabolism and muscle activity, and impaired hepatic clearance (Axon and Palmer, 2008).

Serial lactate measurements after initial septic shock in human patients have shown, not only the initial lactate level, but also the clearance rate was determinate for the prognosis; this includes the duration of a patient having hyperlactaemia, and the total concentration of lactate higher than reference value (Bakker et al., 1996). Increased lactate value is also a common finding in critically ill foals (Sanchez, 2005; Corley et al., 2005; Castagnetti et al., 2010). A cut off value of 5 mmol/L at admission was used as a predictive value for non-survival in a study carried out in an equine neonatal intensive care unit (Castagnetti et al., 2010). In the same study, lactate value of surviving foals returned to reference range within twenty-four hours of hospitalisation. This indicates, not only the lactate value at admission is important, but also the following measurements are valuable in monitoring critically ill foals (Corley et al., 2005; Castagnetti et al., 2010). Although it is not a specific diagnostic indicator, it is however, proved to be a strong prognostic parameter (Bakker et al., 1996; Corley et al., 2005; Castagnetti et al., 2010).

2.2.2.5 Anion gap

The anion gap is a calculated difference between the sum of specific anions (Na, K) and cations (Cl, HCO_3, TCO_2); the equation is shown as below:

$(Na + K) – (Cl + HCO_3)$

Since potassium concentration is low and fairly constant, it was sometimes not included into the equation; and HCO_3 would be replaced with TCO_2 (Oh and Carroli, 1977; Bristol, 1982; Gossett and French, 1983; Gossett et al., 1987). Measuring serum anion gap was used in critically ill human newborn babies to determine different types of metabolic acidosis (Lorenz et al., 1999). Despite different equations were applied, changes in anion gap were also reported in relation to metabolic imbalance in horses (Birstol, 1982). In studies on horses with

varying clinical disorders, the anion gap proved to be negatively correlated with survival rate, and is a suitable prognostic indicator (Bristol, 1982; Gossett et al., 1987).

Gossett and French (1983) found that age had a significant effect on anion gap values in foals, mainly because of the higher level of sodium. The highest level of anion gap was found in foals between two to three weeks of age comparable to adults reaching two years of age (Gossett and French, 1983). Investigations carried out in an equine neonatal intensive care unit showed that anion gap was a very strong predictor (Hoffman et al., 1992; Saulez et al., 2007); similar as in adult horse, a rise in anion gap in those foals was often associated with a worse outcome.

2.2.2.6 Acute-Phase Proteins (APPs)

Acute-phase proteins are proteins synthesized in liver and act as a pathophysiological reaction during acute-phase response (APR) (Jacobsen, 2007a). APPs are subdivided into three groups based on their pattern of response to stimulation (Jacobsen, 2007a). The major positive APPs have very few or undetectable concentrations in healthy individuals, however, their serum levels increase more than 100 times during the APR (for example: serum-amyloid A). In serum of healthy individuals, the moderate APPs (e.g., fibrinogen, haptoglobin) or minor APPs are normally detectable; during the APR, their concentrations will increase one to ten times. In equine and equine neonatal medicine, APP levels have been reported to be elevated during different kinds of inflammations and infections (Allen and Kold, 1988; Auer et al., 1989; Chavatte et al., 1991; Stoneham et al., 2001; Hultén and Demmers, 2002a; Jacobsen and Anderson, 2007b; Newquist and Baxter, 2009). In studies of cattle (Jacobsen et al., 2004), foals (Peek et al., 2006) and humans (Mozes et al., 1989), APPs' responses showed not only good correlations but also reflecting the severity of infection, tissue damage and inflammation at the same time. Therefore, they may be used as indicator of prognosis (Jacobsen, 2007a).

Fibrinogen

Fibrinogen is a positive moderate APP. Depending on the type of inflammation and methodologies applied, the increase of its concentration was reported from 16 to 72 hours

after initial onset of inflammation (e.g. tissue injury, infection), and the peak values remain around 72 to 144 hours in horses (Allen and Kold, 1988; Auer et al., 1989; Hultén et al., 2002b; Pollock et al., 2005; Miller et al., 2007). Elevated fibrinogen concentrations have also been reported in foals with sepsis (Koterba et al., 1984; Peek et al., 2006), infection (Hultén and Demmers, 2002a) and osteomyelitis (Newquist and Baxter, 2009). Taking this together, fibrinogen serves as an important clinical parameter for diagnosing and monitoring various inflammatory conditions in horses and foals in the last decades (Koterba et al., 1984; Jacobson, 2007a, Crisman et al., 2008). However, its reference range is relatively wide in healthy foals, and it takes a long period to show its response, which makes fibrinogen just a fair APP (Crisman et al., 2008).

Haptoglobin

Equine haptoglobin is referred to a moderate APP, which increases two to ten fold following inflammation, despite infectious or non-infectious stimulations (Hultén et al., 2002b; Lavoie-Lamoureux et al., 2012). Based on different research in adult horses, haptoglobin starts to rise from six to twenty-four hours after the earliest onset of inflammation (Taira et al., 1992; Hultén et al., 2002b); the peak value was reached and remained from the second to the fifth day (Kent and Goodall, 1991; Hultén et al., 2002b; Pollock et al., 2005), and it took one to two weeks to return to base line (Hultén et al., 2002b; Miller et al., 2007). Physiological haptoglobin value seemed to decrease with the age in foals, as well as in adult horses, despite the varying reference values reported (Taira et al., 1992; Pollock et al., 2005). Higher haptoglobin concentration has been reported in foals with different kinds of infections (Taira et al., 1992), adult horses after surgery (Kent and Goodall, 1991; Taira et al., 1992), induced non-infectious arthritis (Hultén et al., 2002b), lung disease (Lavoie-Lamoureux et al., 2012) and after equine influenza virus challenge (Kent and Goodall, 1991). Anaesthesia alone and commercially available influenza/tetanus vaccine however, did not induce an increased haptoglobin concentration (Kent and Goodall, 1991).

Serum haptoglobin levels may decrease during haemolysis, because it is haptoglobin's nature to bind haemoglobin to prevent iron loss during haemolysis (Allen and Archer, 1971); also, haptoglobin level might seem elevated in dehydrated individuals with an inflammatory induction less than twenty-four hours (Hultén et al., 2002b). One should notice this when

interpreting changes in haptoglobin level in sick animals. From these studies, one can say, haptoglobin provides useful indication for the detection of infection and/or inflammation, furthermore, also in haemolytic diseases (Kent and Goodall, 1991).

2.3 Myeloperoxidase (MPO)

2.3.1 Nature and function

An important content in primary cytoplasmatic granules of neutrophils is a lysosomal heme protein called myeloperoxidase (MPO). The synthesis occurs during the promyelocyte stage and terminates in the beginning of myelocyte stage during neutrophil development. It is then stored in azurophil (primary) granules in mature cells (Nabity and Ramaiah, 2010; Klebanoff, 1999). During phagocytosis, the cytoplasmic granules will be fused with phagosomes. Through lysing the connecting membranes of granules and phagosomes, MPO would be released to phagosomes. When an organism is too large to be ingested by neutrophils, or when it comes to a soluble stimulus or antibody/complement-coated surface, or during neutrophil lysis, MPO can also be leaked or secreted to extracellular space (Klebanoff, 1999).

Respiratory burst of neutrophils is an oxidative reaction driven by oxidase system when phagocytes respond to stimulation. MPO gains its microbicidal activity mainly by oxidizing a halide such as chloride (Cl) together with hydrogen peroxide (H_2O_2); this is believed to be the primary source of oxidants in phagocytes, and is powerful and effective against many different microorganisms. Hypochlorous acid (HOCl) is generally held to be the initial and primary products from the oxidation of chloride by MPO and H_2O_2 (Klebanoff, 1999; MacCallum et al., 2007); together with other primary products make it an extremely powerful oxidant. The chemical equation can be written as followed:

$$H_2O_2 + Cl^- \xrightarrow{\text{MPO}} HOCl + OH^-$$

The major role of MPO is thought to be serving as an important component in the phagosome during microbicidal events.

It has been proposed that tissue damage can be produced by infusion of MPO system components. In an *in vivo* research in rats where acute lung injury was simulated, MPO was proven to be associated with tissue pathology (Johnson et al., 1981). Based on its character of binding negatively charged easily on the surfaces, (e.g. microorganisms and tissue membranes) the MPO system is therefore, thought to be a friend and a foe for the self-defense system; because it helps to fight against infections, but at the same time work as a initiator of tissue damage, which leads to disease (Klebanoff, 1999; Klebanoff 2005).

2.3.2 Clinical application

Studies about MPO (Allen and Stephens Jr.; Pacheco-Yépez et al., 2011) in human medicine and its application as a parameter for clinical indication (Buss et al., 2000) have been carried out. Since the late twentieth century this topic also caught some equine medicine researching teams' attention. Early efforts have been made in purifying MPO from equine polymorphonuclear leucocytes (Mathy-Hartert et al., 1998), experiments designed for studying it's dynamic were seen in the work from Riggs et al. (2007) by giving horses black walnut extract and measuring neutrophil MPO in plasma, laminar tissue and skin afterwards; McConnico and her colleagues concentrated more on the MPO activity in large intestine during acute colitis (McConnico et al., 1999). The effects of ischemia of the small colon on MPO values in intestinal tissue were investigated by Faleiros and his colleagues (Faleiros et al., 2008). Based on the study design, specimens, the intensity of initial insult and detection methods, the plasma MPO concentration was significantly higher at one to eight hours after receiving black walnut extract; in laminar tissue and skin specimen it rose within three to twelve hours (Riggs et al., 2007). In the study of McConnico and her co-workers, the MPO activity increased gradually from six to twelve hours after application of castro oil; caecal mucosal biopsy MPO values decreased again within sixteen hours (McConnico et al., 1999). The MPO activity from serosa tissue was significantly higher by one and a half until twelve hours after decompression in an ischemic study (Faleiros et al., 2008).

Until today, many types of assays have been developed to measure MPO in different biological or in vitro samples, e.g. RIA (Deby-Dupont et al., 1998), ELISA (Franck et al., 2005), SIEFED (specific immunological extraction followed by enzymatic detection) method (Franck et al., 2006) and MPO photometric activity assay (Fietz et al., 2008b). Recently, the

work focused more on cell level, for example the flow cytometric detection (Wauters et al., 2011) or the MPXI (myeloperoxidase index) generated by an automated leucocyte differential counter as a parameter (Schwarz et al., 2011; Pivani 2011). Neutrophilic myeloperoxidase, either its activity or concentration, has been further explored in different diseases and physiological or pathological status in adult horses and seems to be a promising clinical parameter. Major topics are GI tract (McConnico et al., 1999; Grulke et al., 1999; Faleiros et al., 2008 ; Grulke et al., 2008) and joint diseases (Riggs et al., 2007; Fietz et al., 2008b). Other publications investigated the relationship of MPO to septic pathology (Deby-Dupont et al., 1998), lung system (Art et al., 2006) and systemic inflammation (Schwarz et al., 2011). To our knowledge, only one publication (Piviani et al., 2011) reported MPO values in foals; the authors could not detect differences between healthy and sick foals.

3. Material and methods

3.1 Study animals

In order to establish MPO reference values in foals, we compared two groups of foals. One group included healthy foals, and another group contained ill hospitalized foals. Foals up to six months of age and not weaned were included in the study.

3.1.1 Healthy foals

During the reproduction season in 2011 and 2012, these foals were either presented to the Clinic for Horses in the Faculty of Veterinary Medicine, Free University Berlin, accompanying their dam, or were born in the clinic. The birth of these foals was unassisted and they went through a normal adaptive period. The foals were clinically healthy at the time of examination, and based on the requests of their owners, blood was taken for routine examinations.

During the reproduction season 2012 we also collected blood samples from foals belonging to a private farm in the federal state Brandenburg in Germany. According to the information given from the owner of this farm, all the foals were born without medical assistance and had a normal adaptive period. Foals from this group were clinically healthy during entire sampling period. This procedure was approved by the Ministry of Environment, Health and Consumer protection in federal state of Brandenburg (V3-2347-A-3-1-2012).

3.1.2 Hospitalized ill foals

Foals referred to the Clinic for Horses because of acute or chronic medical complaints during 2011, 2012 and in the early breeding season 2013 were included in this group. In the reproduction season in 2012, two additional foals were born from two hospitalized mares; one with red bag and the other premature. These two foals were also included in this group. Foals in this group were sampled at least once at the time of admission and repeatedly after receiving therapy for monitoring further development.

3.2 Clinical data

3.2.1 Signalment and clinical history of the foals

Each foal included in this study was given either a clinical number or/and a laboratory number. Other signalments from foals were also included: Time and date of birth; time and date of admission; age by the time of admission; breed; colour of fur and weight. Reason of being transferred to the clinic and the clinical history were either provided from the local veterinarian or from the animal owner or farm staff. In case of having transportation time longer than three hours, it was noted on the protocol. (Appendix II: Signalment and clinical history chart. A: Signalment and clinical history).

3.2.2. Birth information

According to the owner or the person who attended the birth process, the following data were collected: type of birth; time of sternal recumbency; time and method of tearing the navel; time of first standing attempt; was the foal assisted by standing up or not; time of first stand-up, first udder-searching, first urination, first nurse and method of it. Volume of colostrum or milk was also documented if the foal was initially fed with a feeding bottle or through a nasogastric tube. Date, time and amount of meconium passage; was the navel been treated and if yes, what kind of agent was applied; did the foal receive any antibiotics shortly after birth as a prophylactic therapy; at what time placenta passed; was the placenta examined and if yes, were there any morphological or pathological alterations. The chart that was used is shown in Appendix II: Signalment and clinical history chart. B: Birth information.

3.2.3 Information on the dam

The following data collected from the mares included in this study were based on the information documented on the horse passports, or were given by owners (Appendix II: Signalment and clinical history chart. C: Information on the dam): Date of birth or age; breed; latest dates of vaccination against EHV, tetanus and equine influenza before parturition. Information concerning the pregnancy resulting in the present foal: date of last service

(included AI) or length of gestation (days); any milk leakage or diseases during pregnancy; vaginal discharge before parturition. If time point, duration or amount of milk leakage and/or vaginal discharge was known, it would be also noted.

3.2.4 Clinical examination of the foals

Clinical examination was performed before each blood sampling. During the examination, foals were restrained with a proper fixation and were kept in the sight of their own dams. All the clinicians who performed the examination or clinical staffs who assisted were wearing fresh gloves during the whole process. The following items were included in the examination: general behaviour; suckle reflex; heart rate; respiratory rate; auscultation of the heart, auscultation of lungs; rectal temperature; eyes; sclera; membrane colour of eye conjunctiva and muzzle; colour of tongue; capillary refill time; petechiae; mandibular lymph nodes; auscultation of intestinal peristalsis; dehydration; distensibility of both jugular veins; cough; size of internal umbilicus; moistness of external umbilicus; umbilical management; joints and lameness; pattern of faeces; congenital deformities; any wound or trauma; any other abnormality that should be noted. Detailed descriptions of all examination items and results are listed in Appendix III: Clinical examination items. For documentation of clinical examination, we used a foal clinical examination chart (Appendix IV: Foal clinical examination chart).

3.2.5 Diagnosis, length of hospitalization and outcome

The diagnosis of each foal or, if more diagnoses were obtained, were also documented. Length of hospitalization (days) and outcome (discharged, euthanized or died) were also noted. In this study, being discharged from the hospital, was defined as survived; foals that were euthanized or died in the hospital were classified as non-survived.

3.3 Blood sampling

3.3.1 Sampling time

Blood samples were taken once from the foals belonging to group 1 (control group), which were accompanying their dam to the clinic in reproductive season in 2011. For the healthy foals that were born in the clinic in 2012, one blood sample was taken around 24 hours *post natum*. Foals that belonged to the private farm in Frankendorf in the federal state of Brandenburg in Germany were sampled four times during their first six months of life. The time points of sampling were: approximately one day, seven days, four weeks and sixteen weeks *post natum*, respectively. All the samples were collected based on the requests and/or agreements of owners.

For hospitalized ill foals in this study, we collected blood samples according to the following four standard sampling time points: 0 hour (at admission and before treatment), 12 hours, 24 and 48 hours after admission and onset of treatments. All blood samples were collected for clinical use. Any additional specimens taken outside of the standard sampling time points were also analysed, processed and preserved properly for further use based on the type of sample. All the results were documented.

3.3.2 Sampling technique

Blood sampling was performed shortly after the clinical examination. Between these two procedures, the clinician changed gloves in order to reduce contamination to the sample and the chance of infection through puncture site. Foals were then kept either in standing position or in lateral recumbency with a firmer restraint; the contact between foals and their dams was available through the whole time.

3.3.2.1 Blood sampling through venepuncture

All the foals in control group (group 1) and some of the hospitalized ill foals from group 2 were sampled through venepuncture. Jugular veins were chosen for this method, cephalic vein

was also used in one case. Aseptic precaution was performed with a sterile spirit-soaked[A] swab by wiping toward one direction over the vein at the chosen site. The vein was raised by placing a finger at it´s proximal part and not retouching the cleansed site. A 18[B]- or 20[C]-gauge needle was quickly but smoothly inserted, once the needle hit the vein and blood was observed at the needle hub, a 20 mL syringe[D] (with a maximum volume of 24 mL) was then connected to the needle and 22 to 24 mL blood was gently aspirated.

3.3.2.2 Blood sampling through a jugular vein catheter

Most of the hospitalized ill foals were placed a catheter[E] in the jugular vein for therapeutic purpose; blood samples were taken from those foals through this device. The vein catheter together with an extension set[F] was placed with standard procedure (e.g. clip the hair around the area where the catheter and extension would be set, cleanse and disinfect the area thoroughly with spirit-soaked[A] swab, the clinician who operated this procedure would wear sterile gloves with cleaned hands).

When taking the blood sample through the catheter, we slowly injected 5 mL physiological infusion solution[I] through this device to make sure that the cannulation was without obstruction. Then we took another new syringe[J] and connected it to the extension; a volume of at least 5 mL from the mixture of physiological infusion solution[I] left in the cannulation and blood was then aspirated and discarded. After that, a new syringe[D] was used to take a 22 mL blood sample. Finally, another five to ten millilitre physiological saline[I] was injected into the catheter through the extension to make sure that no blood was left in the cannulation system.

The catheter and the extension were protected by a bandage, which was made by covering a swab over the connection between catheter and extension, and two other elastic bandages[G, H] were then wrapped without tension around the neck one after another. A small opening on the outer bandage was left, so one could reach the extension when it was needed. Twice daily the insertion site, the vein, the catheter and extension set were inspected inclusive the swab and bandage. Maintenance was done two times a day by flushing physiological infusion solution[I] from the extension through the catheter, and the protection bandage was changed every day. Once the catheter was obstructed, or the insertion site or/and the vein developed signs of heat,

swelling, pain, exudate or purulence, the whole catheter device was removed right away. Further blood sampling was then performed by venepuncture; and the vein where the catheter device set was not used for this purpose.

3.3.3 Sample processing

As soon as the syringe was disconnected from the needle or the extension set, blood was then gently introduced into open anticoagulant tubes along the containers' wall. All the anticoagulant tubes were filled up to the marked level and slowly swivelled 180 degrees back and forth for a couple of times, so that the anticoagulant and the blood would be mixed well. All samples were transferred to the laboratory within 15 minutes after collection. EDTA anticoagulated blood samples from the private farm in Frankendorf were kept at room temperature, while all other samples were brought into a cooling box within 15 minutes after blood collection. The inner environment of this cooling box was kept around 5° C by some frozen ice packs. The transportation from Frankendorf to the laboratory in Clinic for Horses in the Faculty of Veterinary Medicine in Free University Berlin took no longer than two hours.

3.3.3.1 Anticoagulant tubes

The collected blood specimens were distributed into the following anticoagulant tubes with the amount indicated by the manufacturer: 4 mL each in 2 tubes filled with EDTA anticoagulant[K], 2 mL in a tube contained citrate anticoagulant[L]. Another 2 mL was distributed in a lithium-heparin filled syringe[M] and mixed well; the air in the syringe was kept as few as possible; the remaining 10 mL in a serum tube with clot activator particles[N]. Extra 2 mL blood was drawn from the foals in the private farm in Frankendorf, it was then distributed into a florid anticoagulant tube[O]. All the further analytic procedures will be described in the rest of this chapter.

3.3.3.2 Centrifugation, portioning and storage of serum and plasma

Serum specimens were allowed to clot at room temperature around 30 minutes after blood was transported into the lab, they were then centrifugated with 3500 rpm (4871 rcf)[P] at room temperature for ten minutes. After all the analyses were done with EDTA blood samples, which were then put into a programmed centrifuge[Q] (2500 rpm, 2562 rcf) and centrifuged for ten minutes at room temperature. The obtained serum and plasma were pipetted in small amount (500 µL) in aliquots[R]. Afterwards, all the aliquots were brought to a refrigerator and kept at -20° C until further use.

3.4 Haematology analysis

EDTA anticoagulated blood samples were used for analysing packed cell volume (PCV), total protein, complete blood count and evaluations of differential blood count and neutrophil toxic change. Before any haematological analysis was begun, EDTA blood was slowly swivelled 180 degrees back and forth for at least ten times to make sure sample was well mixed, so the results could be representative.

3.4.1 Packed cell volume (PCV) and total protein

Two micro-capillary tubes[S] were filled with EDTA blood and then sealed with some haemato-crit sealing compound[M]. They were then put in a microcentrifuge[N] and centrifuged with 12000 rpm (1581 rcf) for three minutes. Capillary tubes were then taken out and PCV value was read with help from a haematocrit card, which was provided from the manufacture of this centrifuge.

A hand-held refractometer[O] was used to evaluate total protein. After PCV was determined, capillary tubes were broken and some drops of the plasma were placed on the glass plate of the refractometer. The lid was closed firmly over the plate, and the result could be read by viewing through the eyepiece. The scale for total plasma protein in g/dL was used.

3.4.2 Complete blood count (CBC)

After the sample amount for PCV and total protein evaluation was taken, the EDTA tube was put to analyse for complete blood count by a fully automated haematology analyzer[P]. This bench top haematology cell counter determines 22 parameters described as followed: WBC, LYM, MON, NEU, EOS, BAS, LYM%, MON%, NEU%, EOS%, BAS%, HGB, RBC, HCT, MCV, MCH, MCHC, RDWc, PLT, PCT, MPV and PDWc.

3.4.3 Differential leukocyte count and neutrophil toxic change evaluation

3.4.3.1 Blood smear preparation

One to three blood smears were prepared from each blood sample no longer than 30 minutes after sample was taken. Blood smears from the samples that were taken in the Clinic for Horses in the Faculty of Veterinary Medicine in Free University Berlin were made in the clinic´s laboratory. Blood smears of the foals belonging to the private farm in Frankendorf were made at the farm directly after blood samples were taken, further processing was done in clinic´s laboratory.

The blood smear preparation by wedge method was describe elsewhere (Latimer and Rakich, 1992; Harvey, 2001a). Briefly, blood smear was made by using 2 clean glass slides[Q]. A small drop of blood (about 5 µL) was placed near the frosted end of one slide by using a pipette (When slide was made at the farm in Frankendorf, a micro capillary tube was used instead). The second slide (spreader slide) was held at an angle about 30 degrees and drawn slightly backward into the small drop of blood. As soon as the blood flowed along the rear side of the spreader slide, it was than moved rapidly and smoothly forward, carrying the blood with it. The slide was then air dried, labelled on the frosted end and kept for staining.

3.4.3.2 Blood smear staining

Pappenheim method is the routine staining method for blood smears made for differential blood count in the laboratory of the Clinic for Horses in the Faculty of Veterinary Medicine in

Free University Berlin. This method is carried out by combining May-Grünwald[S] and Giemsa solutions[T]. Firstly, the blood smear was stained in May-Grünwald solution for five minutes. The second step was to rinse the blood smear with distilled water until the color of the water from the slide turned clean. The slide was then placed into Giemsa solution for at least 20 minutes for the second staining. After that, the slide was rinsed gently with tap water until no more staining solution or colour particles were visible. In the end, the slide was air-dried. All the blood smears were stained no later than 24 hours after they were made.

3.4.3.3 Blood smear evaluation

A differential leukocyte count was routinely evaluated with a clinical microscope[U] using 1000X magnification (10 X oculars and 100 X magnification, with immersion oil[V]) by identifying 100 consecutive leukocytes in the laboratory of the Clinic for Horses. The area in the front half of the smear and just behind the feathered edge, where the well-stained monolayer of cells appeared, was evaluated as described (Harvey, 2001a). Evaluation was done by examining cells in a pattern that one edge and the centre of the smear was evaluated. An electronic memory programmed counter[W] was used to facilitate counting. If a smear contained very few cells, and the routine-100 cells evaluation could not be fulfilled therefore, 50 cells were evaluated instead; in very few cases 25 cells. After the count was completed, the percentage (%) of each leukocyte type presented was than generated by the programmed counter.

For the neutrophil toxic change evaluation, the same area as differential leukocyte count was chosen and the same pattern was applied to evaluate, 1000 X magnification of the same microscope was used. A total of consecutive 50 intact neutrophils were identified and classified based on different degrees of morphological change in cytoplasm. Categories and criteria used to evaluate neutrophil toxic change in this study were summarized in Appendix VI: Neutrophil toxic change categories and criteria. Basically, toxic change of cytoplasmic basophilia, Döhle bodies and cytoplasmic vacuolization were the three main morphological changes been recognised, they were further categorised in three different levels based on severity: mild, moderate and marked. If very few neutrophils were available for the evaluation, then 25 cells would be evaluated instead of 50. The result of each type of toxic change was documented in numbers as well as in percentages (%).

3.4.3.4 Toxic change scoring system

In order to put toxic changes in a comparable level, we developed our own toxic change scoring system. We defined that each type of mild toxic change has 1 point; moderate ones 2 points and marked ones 3 points. If there were more than one toxic changes observed in a neutrophil, its toxic change points would be the sum of all kinds of toxic changes seen in this cell. For example, a neutrophil had toxic changes such as mild cytoplasmic vacuolization, moderate Döhle bodies and marked basophilia; it then had 1 + 2 + 3 = 6 points. We summarized point(s) of each type of toxic change multiplied the amount of the neutrophils concerned and then divided it through the total number of neutrophils, which were evaluated on one slide. This result is the toxic change score that one slide showed. A band neutrophil was seen as a neutrophil in this evaluation. The following table explains the scoring system.

Table 3.4: Toxic Change scoring system

	Toxic change(s) in a neutrophil			
	mild	moderate	marked	multiple
Toxic change point(s) (*p*)	1	2	3	p_x
Amount of concerned neutrophil(s) (*n*)	n_1	n_2	n_3	n_x
Sum of toxic change points	$\Sigma (p \times n)$			
Total number of evaluated neutrophils on this slide	*N*			
Toxic change score of this slide	$\Sigma (p \times n) / N$			

3.5 Immunoglobulin-G (IgG)

In order to detect the immunoglobulin-G blood values in foals, a commercial foal IgG test kit[X] was applied. This kit uses ELISA technology and provides semi-quantitative detection of IgG values. EDTA anticoagulated whole blood was utilized and the test was carried out following the test procedure provided from the manufacturer.

3.6 Blood glucose, blood lactate, blood gas, acid/base status and electrolytes analysis

Lithium-heparinised venous blood samples were used for blood glucose, blood lactate, blood gas, acid/base status and electrolytes determination. There were in total two multi-parameter electrolyte analysers applied for the analysis through the entire study; GEM® Premier™ 3000 was used as the main analyser, Cobas b 123 POC system was included in the study in the later year of 2011 for anion gap calculation. From the foals in the private Farm in Frankendorf, an extra florid anticoagulant blood sample was obtained for blood glucose measurement by Reflotron® System.

3.6.1 GEM® Premier™ 3000 analyzer

Blood sample was swivelled again shortly before it was introduced to the electrolyte analyzer; the latest rectal temperature of foal was given to the computerized system for temperature correction of pH and blood gas. If the temperature of foal was unknown, a standard system built-in temperature of 37.0°C was given instead. The following parameters could be measured from this analyzer: pH, pCO_2, pO_2, Na^+, K^+, Ca^{++}, glucose, lactate and haematocrit. Calculated parameters of this analyzer were: HCO_3-, HCO^3std, BE(ecf), BE(B), sO_2c and cHbc.

3.6.2 Cobas b 123 POC system

The measure procedure was basically the same as GEM® Premier™ 3000 analyser. The cobas b 123 POC parameter measured in our clinic includes pH, pCO_2, pO_2, Na^+, K^+, Ca^{++}, Cl^-, haematocrit, lactate, glucose, tHb, SO_2 O_2Hb COHb. Calculated parameters were cHCO3-, FO_2Hb, BE(act), BE(ecf), ctO_2, and $AaDO_2$.

3.7 Acute-Phase protein analysis

Haptoglobin

Blood haptoglobin concentrations were detected with equine TECO® haptoglobin ELISA kits purchased from TECOmedical[e]. The whole process was carried out according to the instruction provided by the manufacturer. Briefly, all reagents from ELISA kit were allowed to stand at room temperature for at least thirty minutes. Frozen plasma samples were placed on the ice bath at room temperature and allowed to thaw slowly. Wash buffer, dilution buffer and standards were prepared according to the instruction. After the samples were thawed, they were diluted in 1:10000 factors with diluted dilution buffer in two dilution steps. The test began with washing the equine haptoglobin antibody coated microwell plate three times with diluted wash buffer. Assay buffer was added to each well, standards and diluted samples were allocated into the corresponding wells as planed. The plate was incubated at room temperature for one hour on a shaker (500 rpm). After incubation, the fluid was discarded by inverting the plate. The plate was then washed three times with diluted wash buffer, the excess wash buffer was removed by tapping the plate on a dry absorbent surface several times. HRP antibody was added into each well, the plate was incubated in the dark at room temperature on the shaker (500 rpm) for thirty minutes. After incubation, the fluid was discarded by inverting the plate. The plate was then washed three times with diluted wash buffer, the excess wash buffer was removes by tapping the plate on a dry absorbent surface for couple of times. TMB substrate solution was pipetted in each well, the plate was incubated in the dark at room temperature on a shaker (500 rpm) for twenty minutes. The reaction was stopped by adding stop solution into each well, the plate was read within 10 minutes at 450 nm with a 630 nm reference filter by a computer-assisted microplate reader[f]. Results were analysed by logistic 4PL regression assisted by a microplate software[g].

According to the instructions manual of our haptoglobin ELISA kit provider TECOmedical, plasma, serum or cell culture samples could be applied for haptoglobin determination. In order to find out which type of sample was the suitable one, three plasma and three serum samples from three sampling time points of a single ill hospitalized foal were used. The evaluation was carried out on the same ELISA kit. The measurements from plasma samples turned out to be a more suitable candidate. A plasma sample of a healthy foal was used to evaluate inter- and intra-assay difference. This sample was measured three times repeatedly one plate and twice on another two plates separately. The differences were determined to be minimal. All the plasma samples used for haptoglobin evaluation had not more than three freeze/thaw cycles; most of them were thaw for the first time. All standards and most of the samples were measured in duplicate; twenty-four samples were done in single measurement.

3.8 Myeloperoxidase (MPO) activity analysis

3.8.1 MPO photometric activity assay

The extracellular myeloperoxidase activity of foals was detected using an in-house method established by the Institute of Veterinary Biochemistry (Fietz et al., 2008b). MPO activity was measured using MPO photometric activity assay, this method is MPO enzyme specific and it function is based on conversion of o-Dianisidine. Briefly, oxidation reaction was triggered by aiding H_2O_2 into sample mixtures containing o-Dianisidine, the kinetic study was followed by observing the increase in absorbance due to oxidation of o-Dianisidine. Triplicate measurements from each sample were read on a microtiter plate reader[f] every five minutes until the experiment was terminated after twenty-five minutes.

3.8.2 Chemicals

Citrate buffer[h] (pH 5.5) was prepared and stored in a stock solution at -40°C, which was thawed at room temperature before use. H_2O_2 (30%) purchased from Sigma[i] was stored at 4°C; a stock of diluted H_2O_2 solution (1:100) was freshly prepared daily with double distilled water shortly before analyse and kept away from light in room temperature. O-Dianisidine[j]

was stored at room temperature, a stock solution of o-Dianisidine would be prepared with DMSO[k] each day before analyse and kept driven from light.

Citrate buffer mixture consisted following compounds: 0.1% Triton X-100[l], which was normally stored at room temperature; 0.08 M citrate buffer and 0,65 mM O-Dianisidine solution. Purchased pure MPO inhibitor[m] was stored at -40° C. For the experiments, a stock of 2 mM MPO inhibitor solution was diluted with DMSO[k] and stored in aliquots at -40° C; small aliquots were taken out and thawed at 4°C on ice each time when it was needed, so each aliquot would not go through too many frozen-thawed-cycle.

3.8.3 Sample processing

The frozen plasma sample aliquots were thawed slowly on ice (4° C) for about one hour. Before each sample was diluted, it was taken out from ice and stood in room temperature for three to five minutes, so that plasma would accommodate to ambient temperature. Each plasma sample was diluted with citrate buffer. After dilution, the original (undiluted) sample was placed on the ice right away; the diluted plasma sample remained at room temperature.

3.8.4. Dilution factor

A preliminary serial dilution experiment was carried out in testing 1.25-fold, 5-fold and 10-fold dilution factors, to find out the most suitable dilution factor for foal plasma samples run by the kinetic test. The adequate dilution factor was then determined as an optimal of 1.25-fold dilution; the kinetic from those samples remained optimal when diluted with 5-fold or 10- fold. Some plasma samples from hospitalized foals had to be further diluted in 1:5 or 1:10 ratio in order to have an optimal kinetic curve.

3.8.5 MPO activity assay

Test assay from each plasma sample contained 260 µL citrate buffer mixture, 30 µL of diluted plasma and 10 µL citrate buffer in a separated reaction vessel. Each plasma sample was measured in triplicate. There was a specific MPO inhibitor assay run additionally parallel to each plasma sample. The inhibitor assay contained similar compounds; just the 10 µL citrated buffer was replaced by 10 µL (30 µM) MPO inhibitor. The reaction was triggered by adding 0.43 mM H_2O_2 into each test assay, each reaction vessel was then well mixed and a final volume of 250 µL liquid mixture was transferred into a well on a 96-well microtiter plate[n] as quickly as possible. The measurements were carried out at 450 nm wavelengths by a computer-assisted microplate reader[f].

3.8.6 MPO kinetic study

Absorbance was read at 0 minute (1st measurement) and at every following five minutes until the test was terminated at twenty-five minutes (6th measurement). Graphs were made by plotting absorbance against time by a microplate software[g]. The kinetic study was performed on each sample, only when the correlation coefficient was as close as to 1.0, would the MPO activity value enter statistical analysis.

3.8.7 Calculation of MPO activity

Volume enzyme activities (mU/µL) were calculated based on the following equation:

$$\frac{\Delta E \times V}{t \times \varepsilon \times d \times v} \times 1000 \times \mathrm{D}$$

ΔE	OD value at 25 minutes – OD value at 0 minute
V	final total liquid volume in the well (µL)
t	duration (minute)
ε	molar absorption coefficient for o-dianisidine at 450 nm ($mM^{-1}cm^{-1}$)
d	light path (cm)
v	plasma sample volume added in the reaction vessel (µL)
D	dilution factor

According to the report from De Mendez et al. in 1999, an increase in absorbance of 11.3/ minute/µL of sample at 460 nm and corresponds to the amount of enzyme catalysing the oxidation of 1 mM o-Dianisidine is defined as one unit of MPO; in other words, an absorption coefficient for o-Dinisidine at 460 nm is 11.3 $mM^{-1}cm^{-1}$. Considering the fact that we used 450 nm to carry out our measurements, the molar absorption coefficient in our case constituted 11.48 $mM^{-1}cm^{-1}$ (Spellmeyer, 2004).

Peroxidase activity of each measurement was noted and the mean of each sample was calculated. The mean value subtracted from that obtained from the parallel MPO inhibitor assay was the resulting MPO activity. Seven MPO activity values from three foals were calculated from five minutes (2^{nd} measurement) to twenty-five minutes (6^{th} measurement) based on kinetic study. The effect of calculating a shorter time period on the result was proved to be minimal.

3.9 Statistical analysis

Since all tested parameters were not normally distributed (Shapiro-Wilk test), data were presented as median, minimum and maximum values. Further, non-parametric tests such as Wilcoxon-signed-rank test and Kruskal-Wallis test were used for statistical analysis. Results were considered significant at $P \leq 0.05$.

4. Results

4.1 Sample size

4.1.1 Sample size according to age-group-category

There were in total 13 healthy foals and 30 hospitalized ill foals included in this study; among those foals we had 38 and 30 sample size of healthy and hospitalized foals, respectively.

The detailed sample size of healthy and hospitalized ill foals is shown in Table 4.1-1.

Table 4.1-1: Sample size of healthy and hospitalized ill foals based on age-group-category.

Age group	**Sample size**		
	healthy foals (n)	**ill foals (n)**	**Total (n)**
1-3 days	11	11	22
4-19 days	9	10	19
20-59 days	9	4	13
60-130 days	9	5	14
Total	38	30	68

4.1.2 Sample size according to sampling time point (time after admission) of hospitalized ill foals

Table 4.1-2 demonstrates the sample size of each sampling time point (time after admission) in hospitalized ill foals.

Table 4.1-2: Sample size of each sampling time point (time after admission) in hospitalized ill foals.

Time after admission	**Sample size allocated in each age group (n)**				
	total	1-3 days	4-19 days	20-59 days	60- 130 days
0 hour	30	11	10	4	5
12 hours	22	9	7	1	5
24 hours	22	5	10	2	5
48 hours	19	1	10	3	5
Total	93	26	37	10	20

4.2 History of the dam

Information concerning mares and course of pregnancy are presented in Table 4.2.

Table 4.2: Age, breed, vaccination status, diseases during pregnancy, milk leakage and vaginal discharge before parturition in mares with healthy and ill foal

Parameter		**Mares with**		**n**	
		healthy foals	**ill foals**	**healthy foals**	**ill foals**
Age (years; mean, median, min-max)		10.38 (10, 4-22)	11.63 (12, 3-21)	13	30
Breeds (n, %)	Haflinger	2 (15)	-	13	30
	Draft horse	2 (15)	4 (13)		
	Shetland pony	1 (8)	2 (6)		
	Standard bred	2 (15)	2 (6)		
	Warm blood	1 (8)	16 (53)		
	Crossbred	4 (30)	-		
	Others	1 (8)	6 (22)		
EHV-Vaccination (n, %)	yes	5 (45)	11 (92)	11	12
	no	6 (55)	1 (8)		
Tetanus-Vaccination (n, %)	yes	9 (69)	9 (64)	13	14
	no	3 (23)	4 (29)		
	not sure	1 (8)	1 (25)		
Influenza-Vaccination (n, %)	yes	3 (27)	10 (71)	11	14
	no	7 (63)	3 (21)		
	not sure	1 (9)	1 (7)		
Disease during pregnancy (n, %)	yes	2 (15)	4 (25)	13	16
	no	11 (85)	10 (63)		
	not sure	0 (0)	2 (13)		
Milk leakage (n, %)	yes	3 (23)	5 (31)	13	16
	no	9 (69)	11 (69)		
	not sure	1 (7)	0 (0)		
Vaginal discharge (n, %)	yes	0 (0)	1 (6)	13	16
	no	12 (92)	13 (81)		
	not sure	1 (8)	2 (13)		

4.3 Pregnancy and parturition

The following table shows the duration of pregnancy and the type of parturition of all foals included in this study (Table 4.3).

Table 4.3: Duration of pregnancy, type of parturition

Parameter		**Mares of healthy foals**		**Mares of ill foals**	
		n		n	
Duration of pregnancy	days; mean/median (min-max)	8	340.63/340 (327-363)	8	341.25/334.5 (325-365)
Type of parturition	spontaneous birth (n, %) manual traction (n, %) manual correction (n, %) C-section (n, %)	13	11 (85) 1 (8) 1 (8) 0 (0)	22	18 (82) 1 (5) 2 (9) 1 (5)

4.4 Signalment and clinical history of the foals

4.4.1 Age, breed and gender

Information concerning age according to the allocation of four age groups from healthy and ill foals is presented in Table 4.4-1; breed and gender of the healthy and ill foals in Table 4.4-2. None of the healthy foals received any medical treatment before the initial sampling. Three of 13 healthy foals were accompanying their mother to the hospital; the transportation time was less than three hours.

Table 4.4-1: Age of healthy and ill foals

Age group	Age of foals (days; mean, min-max, median)					
	healthy foals		ill foals		total	
	n		n		n	
1-3 days	11	2.18 (2-3, 2)	11	2.09 (1-3, 2)	22	2.14 (1-3, 2)
4-19 days	9	9.67 (7-13, 10)	10	7.30 (4-13, 5.5)	19	8.42 (4-13, 8)
20-59 days	9	30.56 (21-37, 29)	4	30.25 (24-46, 25.5)	13	30.46 (21-46, 28)
60- 130 days	9	93.78 (62-114, 97)	5	98.60 (61-124, 112)	14	95.5 (61-124, 100)
Total	38		30		68	

Table 4.4-2: Breed and gender of healthy and ill foals.

Parameter		healthy foals	ill foals
Breed (n, %)	Haflinger	2 (15)	-
	Draft horse	-	4 (13)
	Shetland pony	1 (8)	2 (6)
	Standardbred	2 (15)	2 (6)
	Warmblood	1 (8)	16 (53)
	Crossbred	4 (30)	-
	Others	3 (23)	6 (22)
Gender (n, %)	Male	10 (77)	16 (53)
	Female	3 (23)	14 (47)
Total		13 (100)	30 (100)

4.4.2 Prior treatment and transportation time

Table 4.4-3 shows the information about prior treatment and transportation time of hospitalized ill foals by the time of admission according to different age-group-category.

Table 4.4-3: Prior treatment and transportation time of hospitalized ill foals according to age-group-category.

Parameter		Age groups (days)				Total
		1 – 3	4 – 19	20 – 59	60 – 130	
Prior treatment (n, %)	yes	4 (36.3)	5 (50)	3 (75)	3 (60)	15 (50)
	no	7 (63.7)	5 (50)	1 (25)	2 (40)	15 (50)
Transportation time (n, %)	< 3 hrs	5 (17)	5 (17)	2 (7)	4 (13)	16 (53)
	not specified	6 (20)	5 (17)	2 (7)	1 (3)	14 (47)

4.5 Diagnoses, length of hospitalization and outcome of hospitalized ill foals

Diagnoses of all hospitalized ill foals are listed in Table 4.5-1. When a foal had various diagnoses, the major one was taken into statistic analysis based on the suggestion of the physician.

Table 4.5-1: Diagnoses of hospitalized ill foals according to age-group-category.

Diagnoses (n)	**Age groups (days)** 1 – 3	4 – 19	20 – 59	60 – 130	**Total**
Maladaptation	2				2
Flexural deformity	1				1
Colon rupture	1				1
Meconium obstipation	3				3
Obstipation	1				1
Subepiglottic cyst	1				1
Suckling weakness	1				1
Immaturity	1	1			2
Joint injury		1			1
Healthy		1			1
Hyperthermia		1			1
Hypogammaglobulinaemia		2			2
Omphalitis		1	1		2
Umbilical fistula		1			1
Septicaemia		1			1
Injury		1		1	2
Diarrhoea			1		1
Luxation of patella			1		1
Oesophageal obstruction			1		1
Fracture				1	1
Umbilical hernia				1	1
Osteomyelitis				1	1
Toxicopathy				1	1
Total (n)	11	10	4	5	30

Information about length of hospitalization and outcome of hospitalized ill foals according to age-group-category is presented in Table 4.5-2 and 4.5-3.

Table 4.5-2: Length of hospitalization of hospitalized ill foals according to age-group-category.

Duration of hospitalisation	**Age groups (days)** 1 – 3	4 – 19	20 – 59	60 – 130
Days (mean; min-max, median)	5.18 (1-13, 4)	14.2 (3-34, 11)	10.5 (7-15, 10)	20.6 (7-41, 20)
n	11	10	4	5

The total length of hospitalization from all ill foals differed from one to 41 days.

Table 4.5-3: Outcome of hospitalization of hospitalized ill foals according to age-group-category.

Outcome (n, %)	**Age groups (days old)** **1 – 3**	**4 – 19**	**20 – 59**	**60 – 130**	**Total n**
discharged	8 (72)	10 (100)	4 (100)	4 (80)	26 (87)
euthanized	2 (18)			1 (20)	3 (10)
died	1 (1)				1 (3)
Total	11	10	4	5	30

4.6 Results of clinical examinations

4.6.1 General behaviour

All the healthy foals appeared to be bright and alert at the time of each clinical examination. The results of general behaviour of hospitalized ill foals are shown separated by age groups and the results were categorized according to the time after admission in Table 4.6-1.

Table 4.6: General behaviour of hospitalized ill foals.

Time after admission	**Findings**	**Age groups (days)** **1 – 3**	**4 – 19**	**20 – 59**	**60 – 130**	Total **(n)**
0 hrs	bright, alert (n, %)	6 (54)	6 (60)	3 (75)	4 (80)	19
	faint (n, %)	2 (18)	2 (20)			4
	faint hypotonic (n, %)	1 (9)				1
	hypotonic (n,%)	1 (9)				1
	colic (n,%)	1 (9)				1
	increased reactive (n, %)		1 (10)			1
	stupor (n, %)		1 (10)	1 (25)		2
	seizures, convulsion (n, %)				1 (20)	1
Total	n	11	10	4	5	30
12 hrs	bright, alert (n, %)	6 (67)	5 (71)	1 (100)	4 (80)	16
	faint (n, %)	2 (22)	2 (29)		1 (20)	5
	faint, hypotonic (n,%)	1 (11)				1
Total	n	9	7	1	5	22
24 hrs	bright, alert (n, %)	2 (40)	8 (89)	3 (100)	5 (100)	18
	faint (n, %)	1 (20)	1 (11)			2
	faint, hypotonic (n,%)	2 (40)				2
Total	n	5	9	3	5	22

Table 4.6 Continued:

Time after admission	Findings	Age groups (days) 1 – 3	4 – 19	20 – 59	60 – 130	Total (n)
48 hrs	bright, alert (n, %)		8 (80)	3 (100)	5 (100)	16
	faint (n, %)		1 (10)			1
	faint, hypotonic (n,%)	1 (100)				1
	seizures/convulsion (n, %)		1 (10)			1
Total	n	1	10	3	5	19

4.6.2 Rectal temperature

Rectal temperatures of healthy and ill foals are demonstrated in Fig. 4.6-1 and Fig. 4.6-2. There were no differences among age groups in healthy foals.

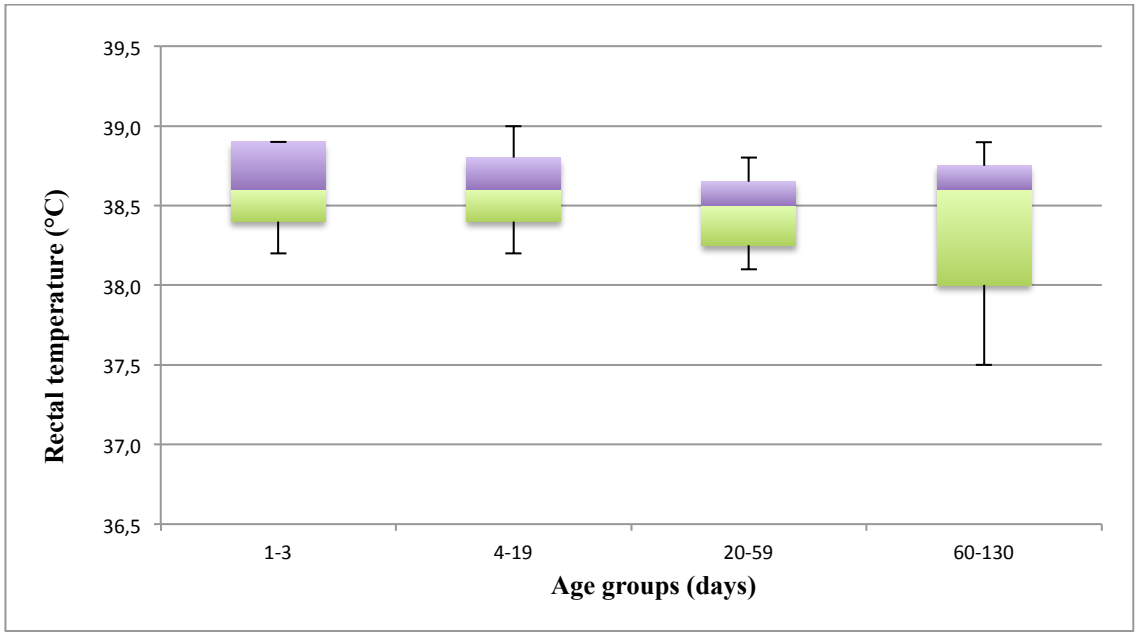
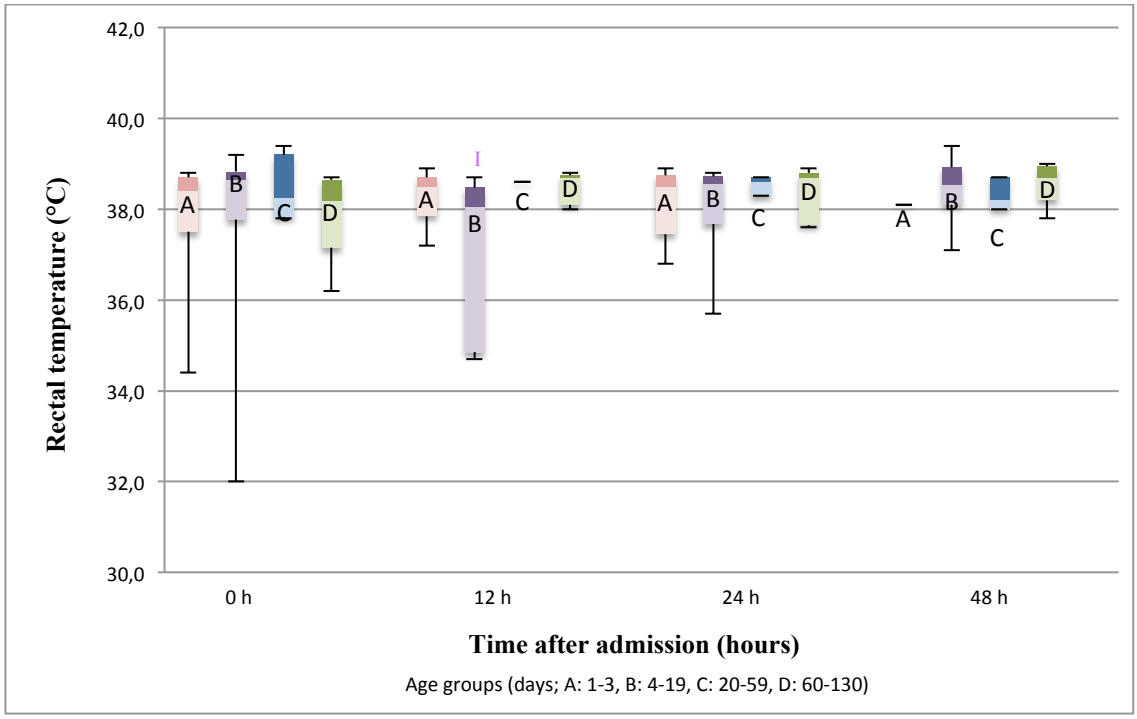


Fig. 4.6-1: Rectal temperature of healthy foals



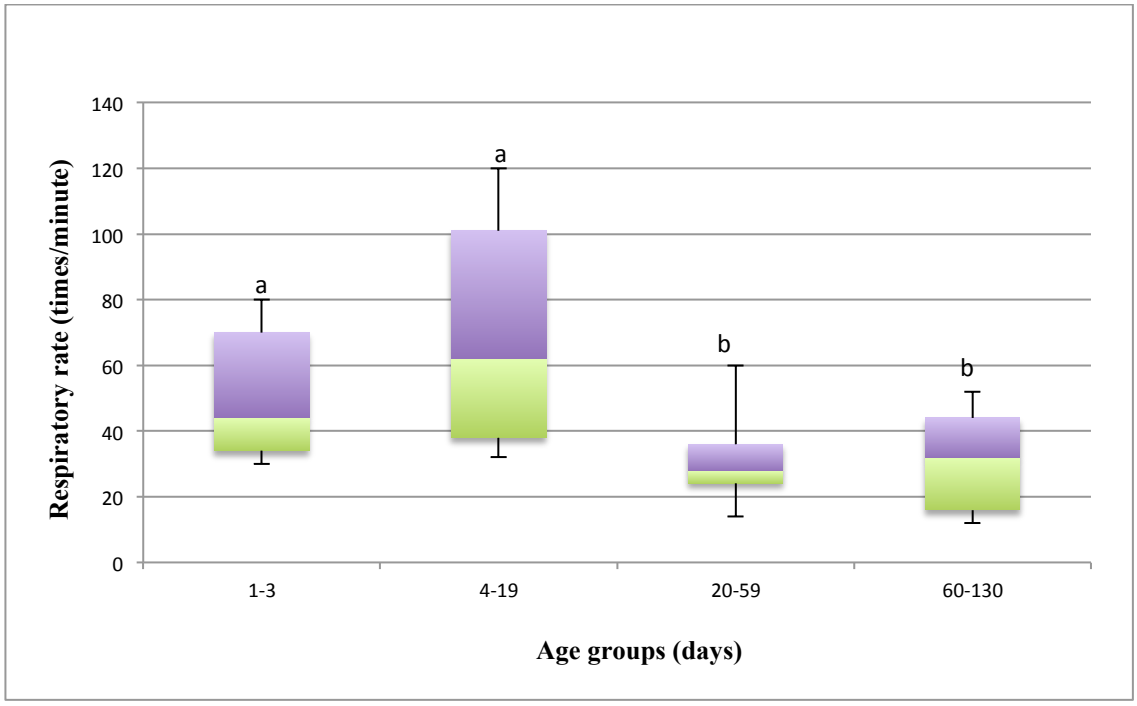
I: Values of time point 0 and 12 hours after admission were significantly different ($p<0.05$) within the same age group.

Fig. 4.6-2: Rectal temperature in ill hospitalized foals

The rectal temperature values did not differ between healthy and ill, hospitalized foals in all age groups at time point 0 (at admission). Comparisons of rectal temperatures among the four time points within age groups in ill hospitalized foals did not show differences. The values of all four age groups did not differ significantly ($p>0.05$) within any of the four time points.

4.6.3 Respiratory rate

There was no significant difference between respiratory rates of healthy foals (Fig. 4.6-3) and the values of ill hospitalized foals (Fig. 4.6-4) at the time of admission within the same age groups ($p>0,05$). The respiratory rate also did not differ between the four time points within a same age group among ill hospitalized foals.



a, b: Age groups with different superscripts differ significantly ($p<0.05$)

Fig. 4.6-3: Respiratory rate of healthy foals

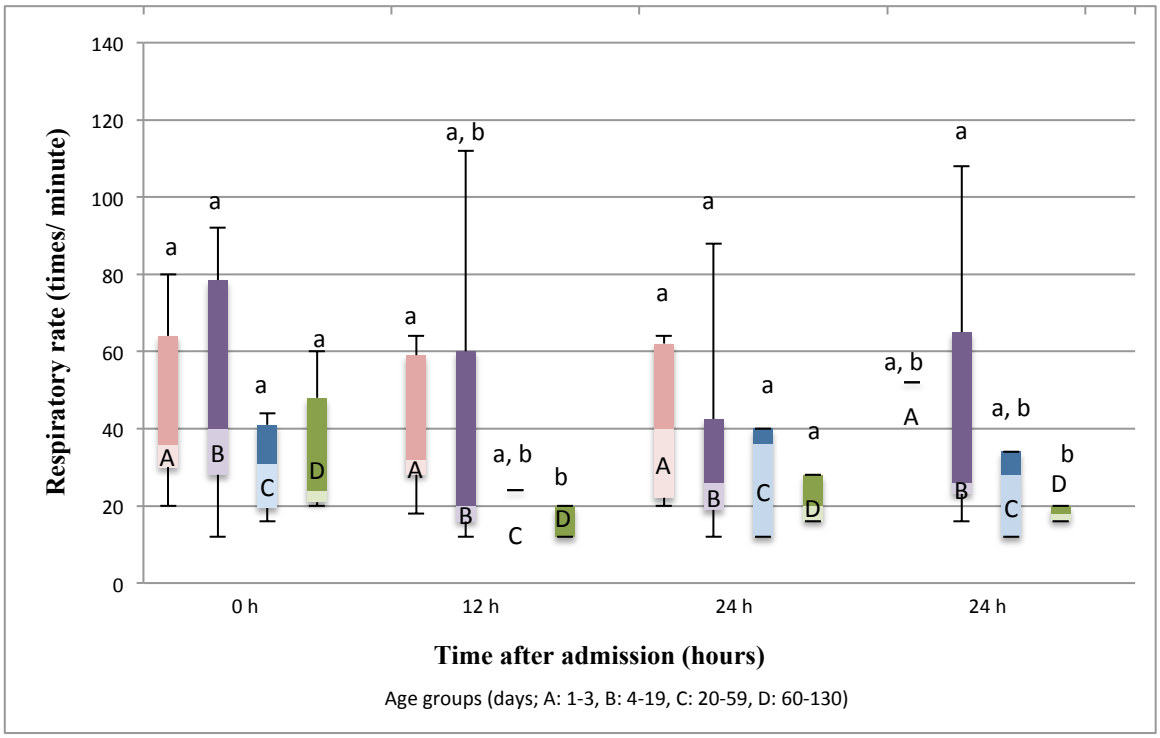
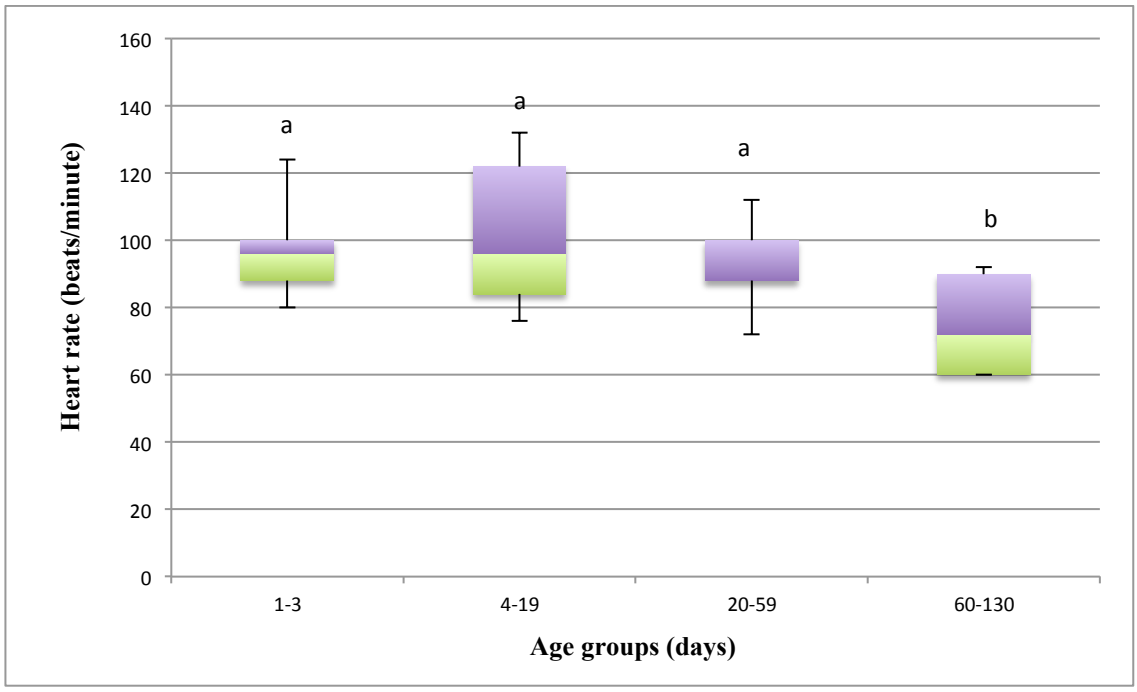


Fig. 4.6-4: Respiratory rate of ill hospitalized foals

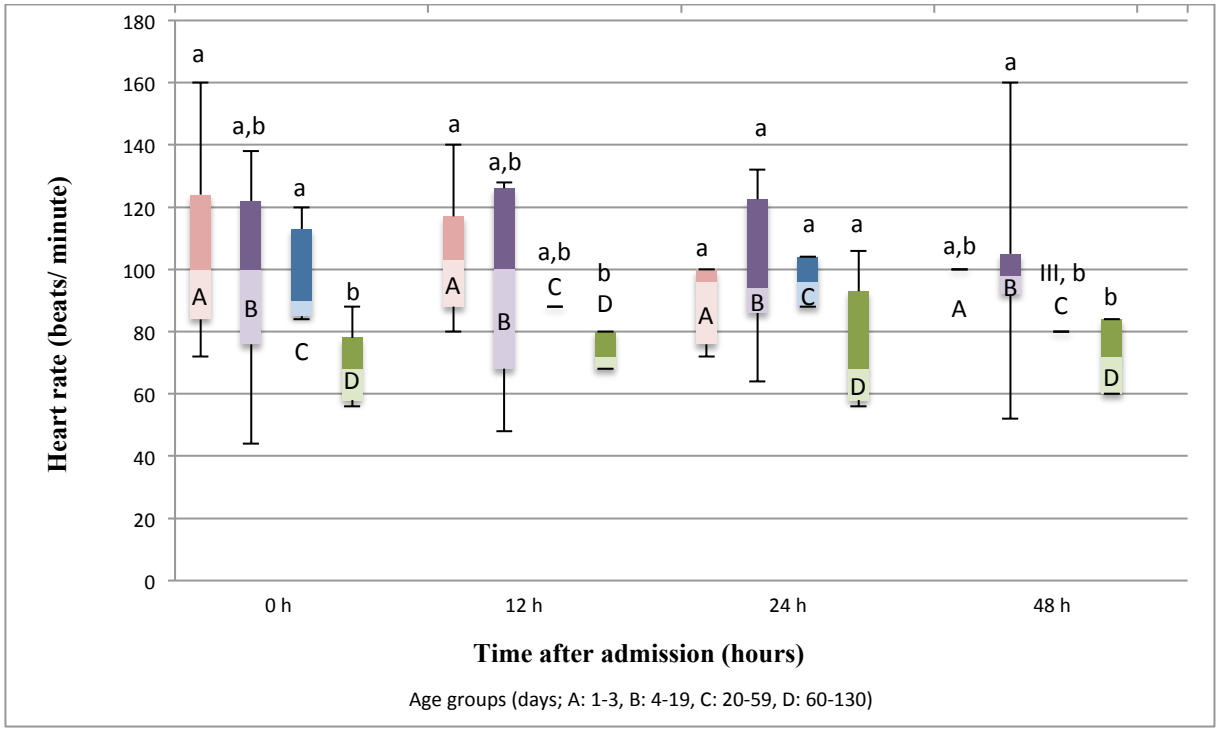
4.6.4 Heart rate

Heart rates of healthy and ill foals are given in Fig. 4.6-5 and Fig. 4.6-6.



a, b: Age groups with different superscripts differ significantly ($p<0.05$)

Fig. 4.6-5: Heart rates of healthy foals



III: Values of time point 0 and 48 hours after admission were significantly different ($p<0.05$) within the same age group.
a, b: Age groups with different superscripts differ significantly within time points ($p<0.05$).

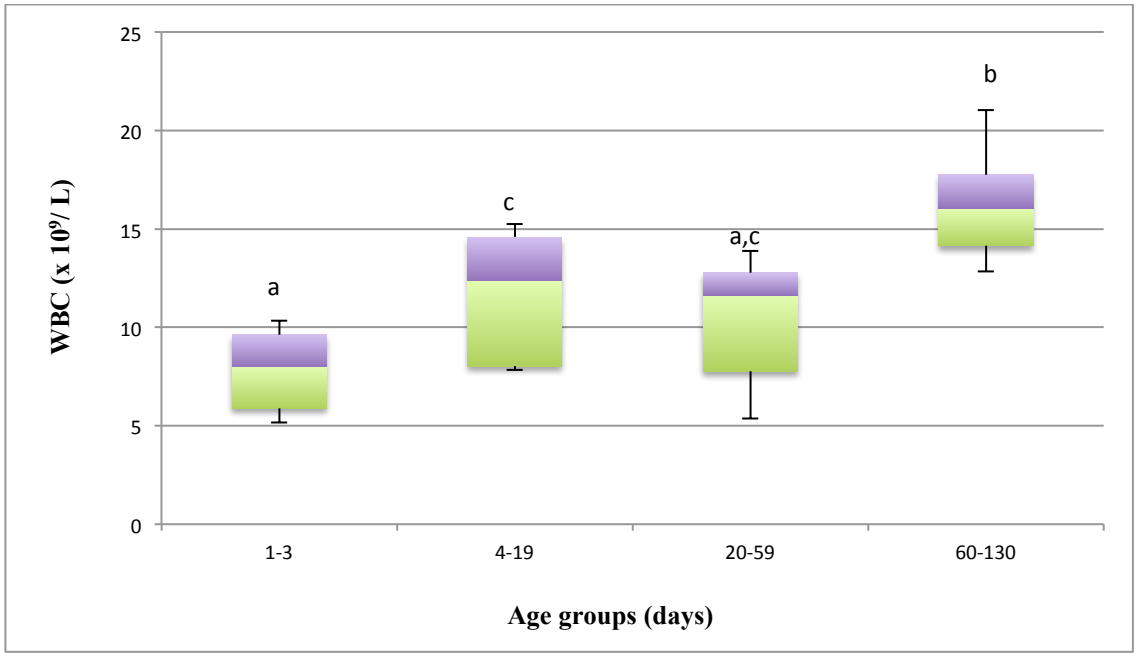
Fig. 4.6-6: Heart rates of ill hospitalized foals

No statistical differences were seen in the heart rate values between healthy and ill hospitalized foals at admission when compared within age groups ($p>0.05$). Other comparisons of heart rates among the four time points within same age groups in ill hospitalized foals also did not differ.

4.7 Results of CBC

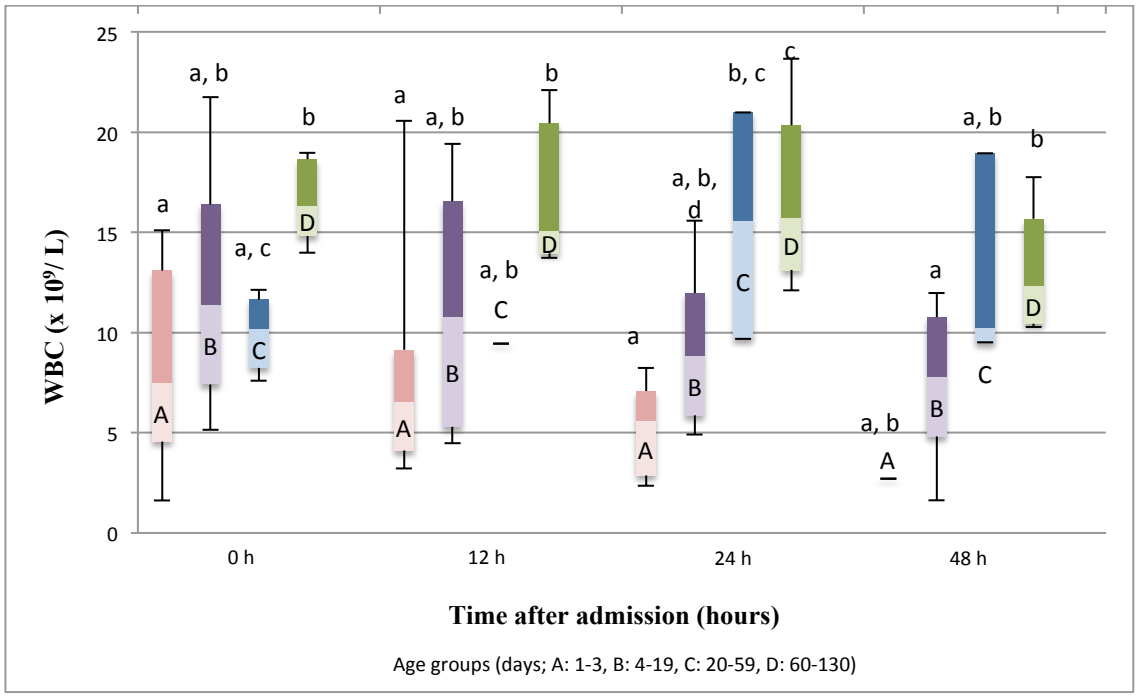
4.7.1 WBC

The WBC counts of healthy and ill foals are presented in Fig. 4.7-1 and Fig. 4.7-2. Values did not differ between healthy and ill hospitalized foals at admission in all age groups; they also did not differ among the four time points within age groups in ill hospitalized foals.



a, b, c: Age groups with different superscripts differ significantly ($p<0.05$).

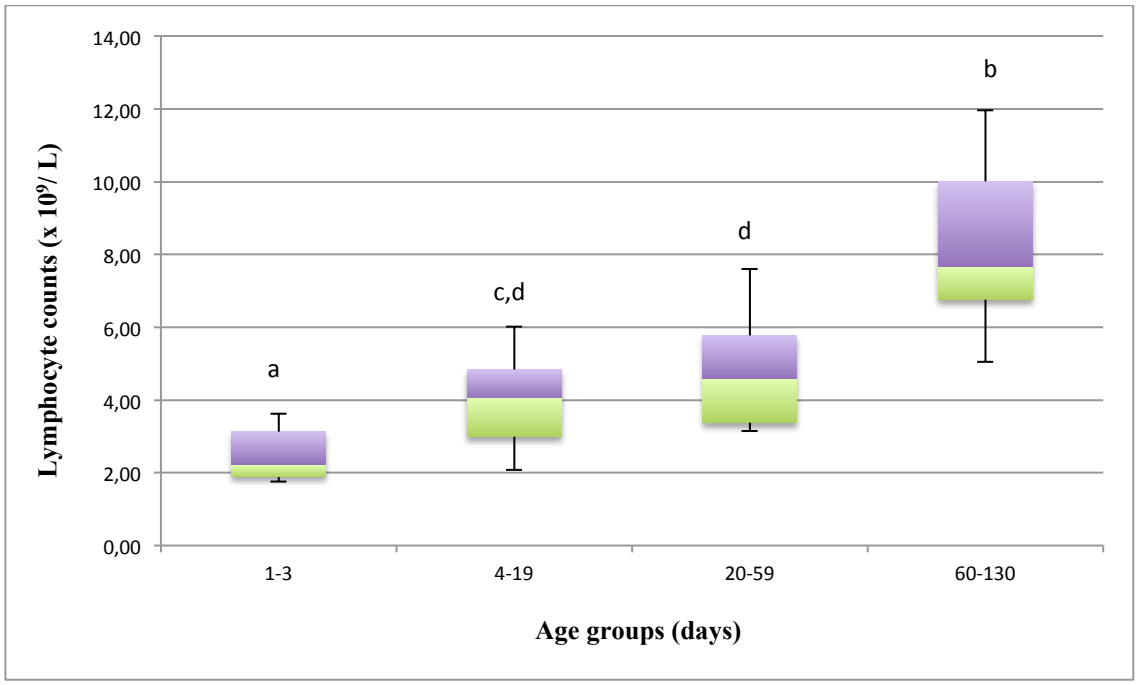
Fig. 4.7-1: WBC of healthy foals



a, b, c, d: Age groups with different superscripts differed significantly within time points ($p<0.05$).

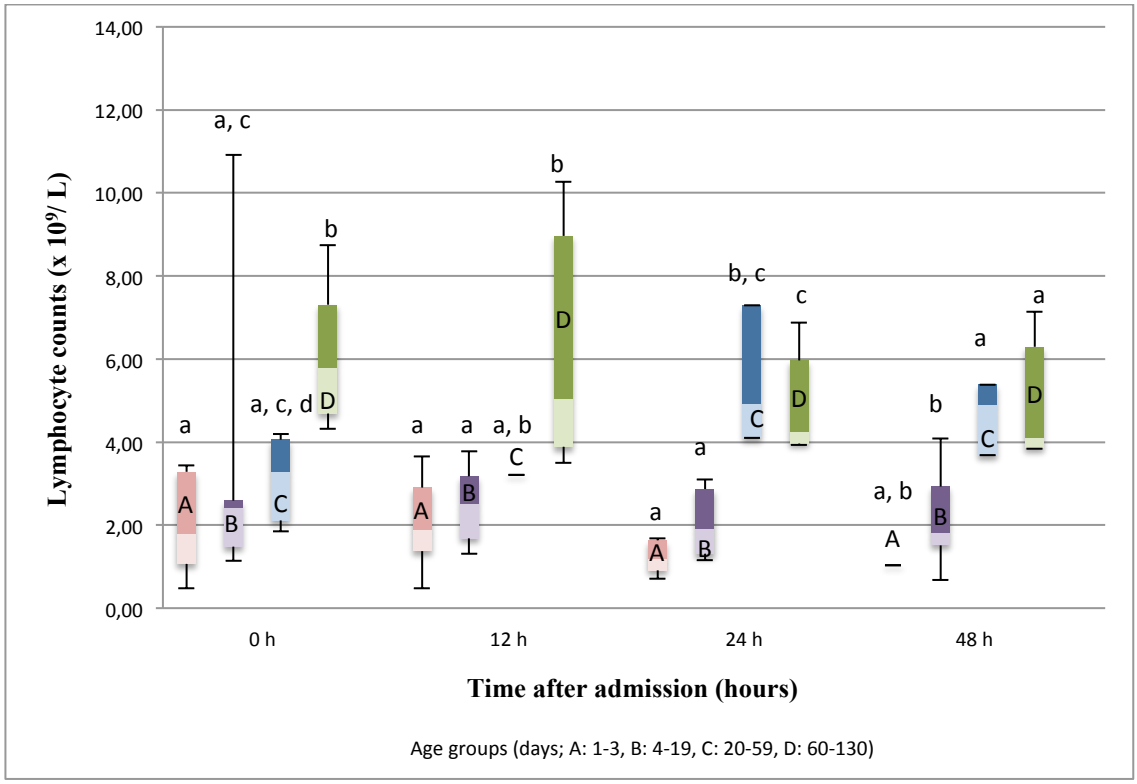
Fig. 4.7-2: WBC of ill hospitalized foals

4.7.2 Lymphocyte counts



a, b, c, d: Age groups with different superscripts differed significantly ($p<0.05$)

Fig. 4.7-3: Lymphocyte counts in healthy foal



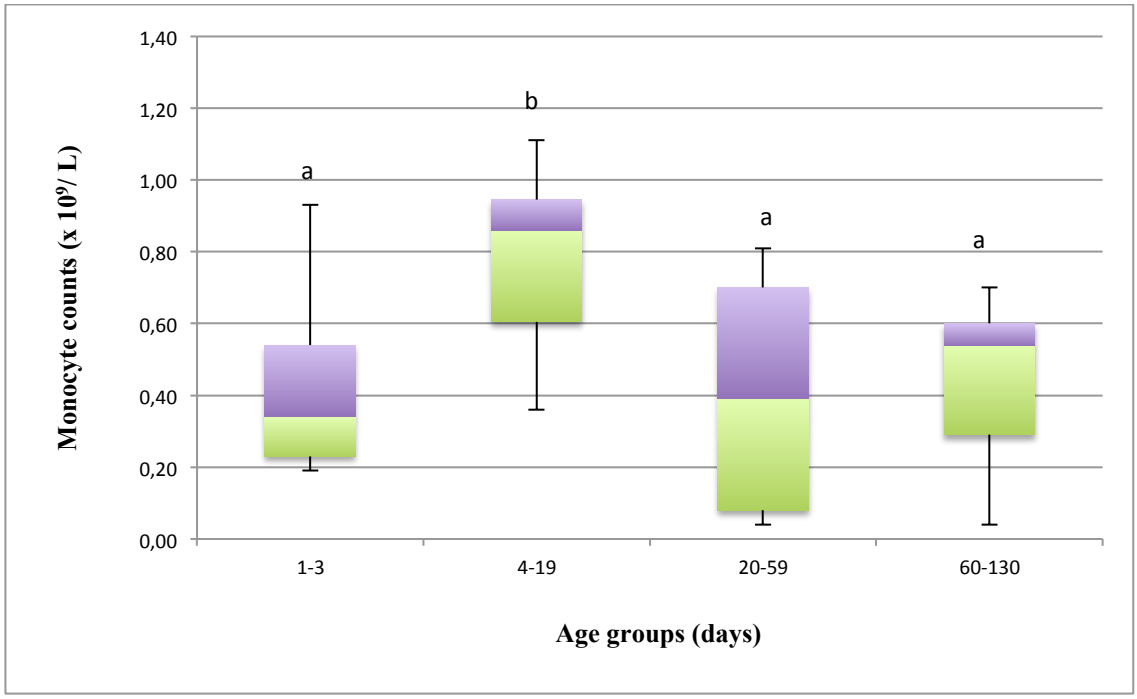
a, b, c, d: Age groups with different superscripts differed significantly within time points ($p<0.05$).

Fig. 4.7-4: Lymphocyte counts in ill hospitalized foals

Lymphocyte values at admission were significantly lower in 4-19 and 60-130 days old hospitalized foals compared with the healthy ones. The other two age groups showed no differences. No differences of lymphocyte counts were found among the four time points within the same age groups in ill hospitalized foals.

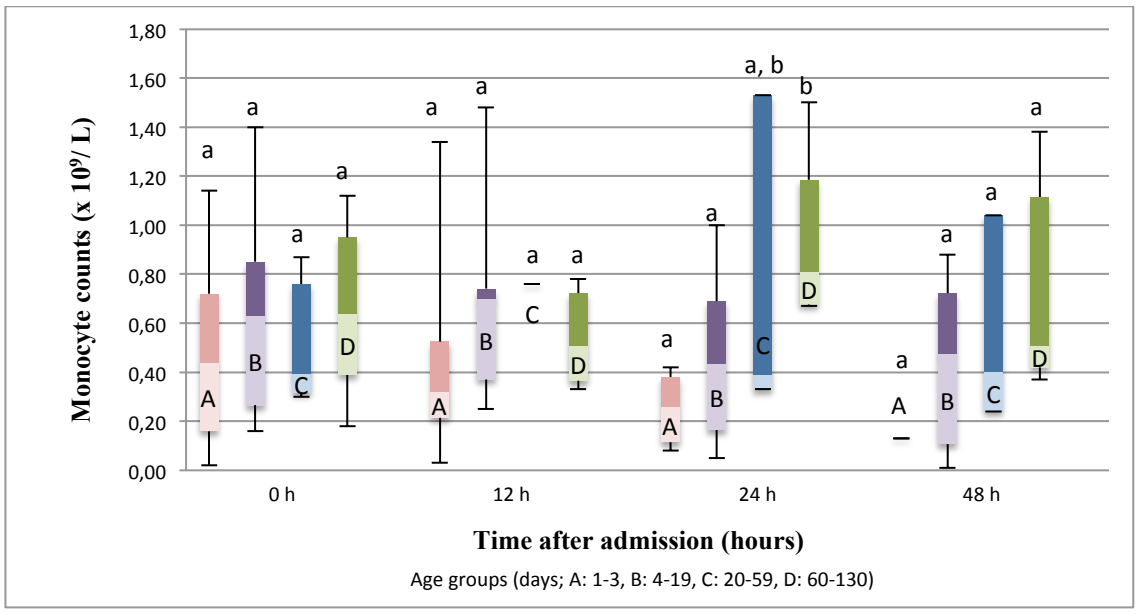
4.7.3 Monocyte counts

No differences were seen in monocyte counts between healthy (Fig. 4.7-5) and ill hospitalized foals at admission in all age groups. When the same parameter was compared among the four time points within the same age groups in ill hospitalized foals, also no differences were found (Fig. 4.7-6).



a, b: Age groups with different superscripts differ significantly ($p<0.05$)

Fig. 4.7-5: Monocyte counts in healthy foals

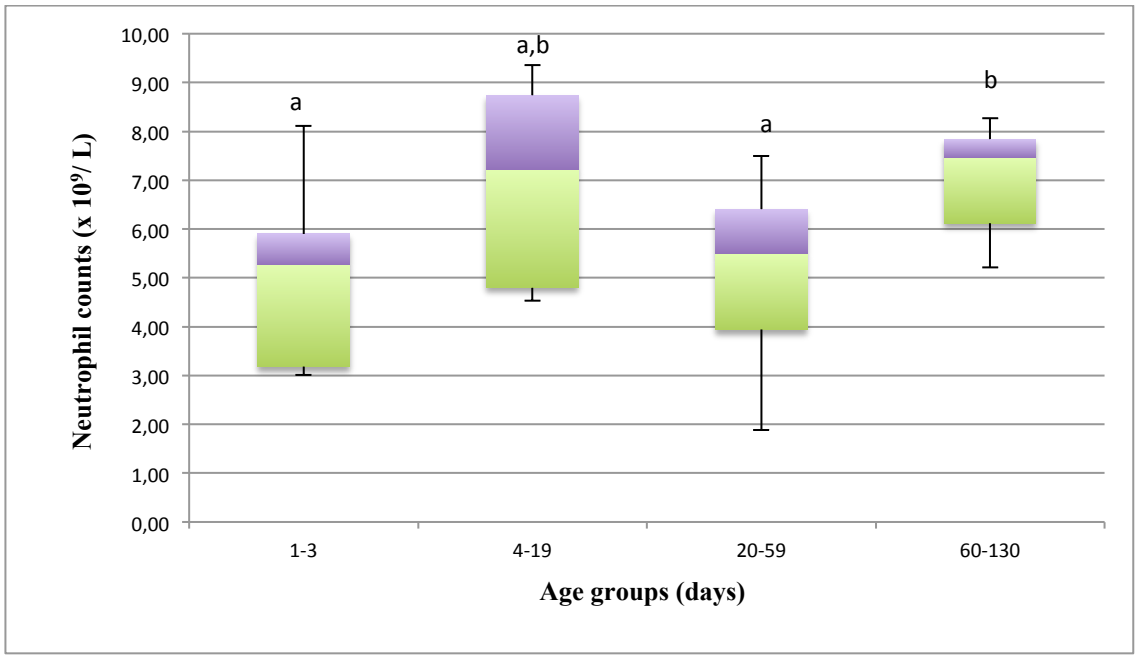


a, b: Age groups with different superscripts differed significantly within time points ($p<0.05$).

Fig. 4.7-6: Monocyte counts in ill hospitalized foals

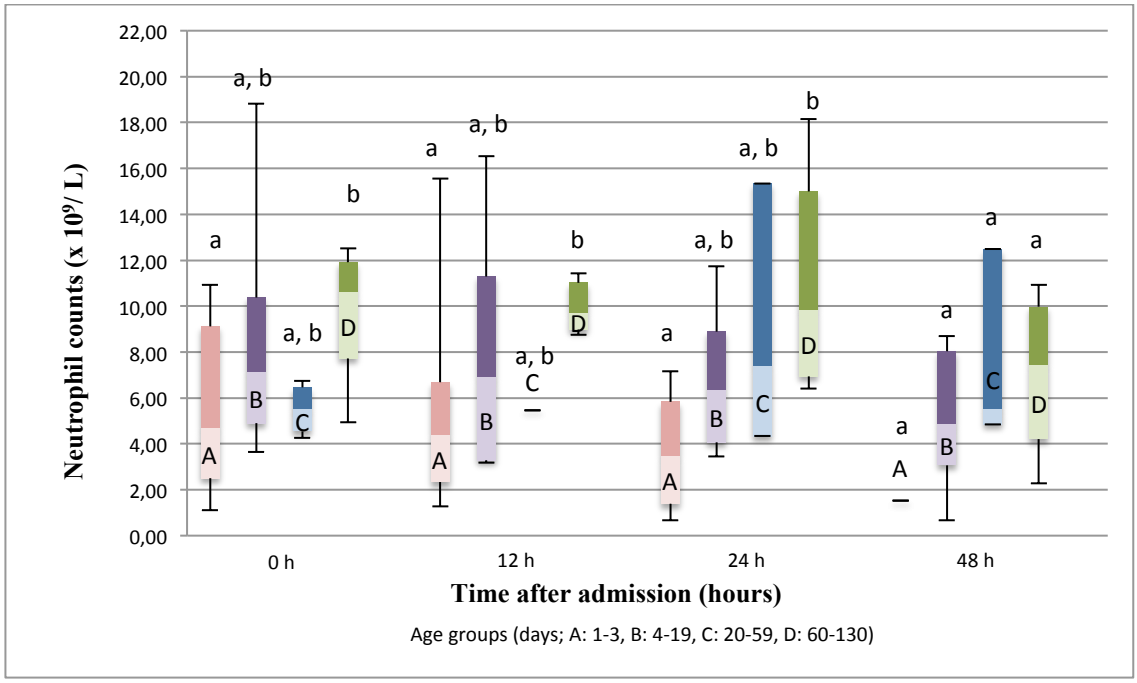
4.7.4 Neutrophil counts

The neutrophil counts of ill hospitalized foals at time point 0 did not differ from those of their healthy counterparts. There also were no differences among the four time points within the same age groups in ill hospitalized foals.



a, b: Age groups with different superscripts differed significantly ($p<0.05$)

Fig. 4.7-7: Neutrophil counts in healthy foals

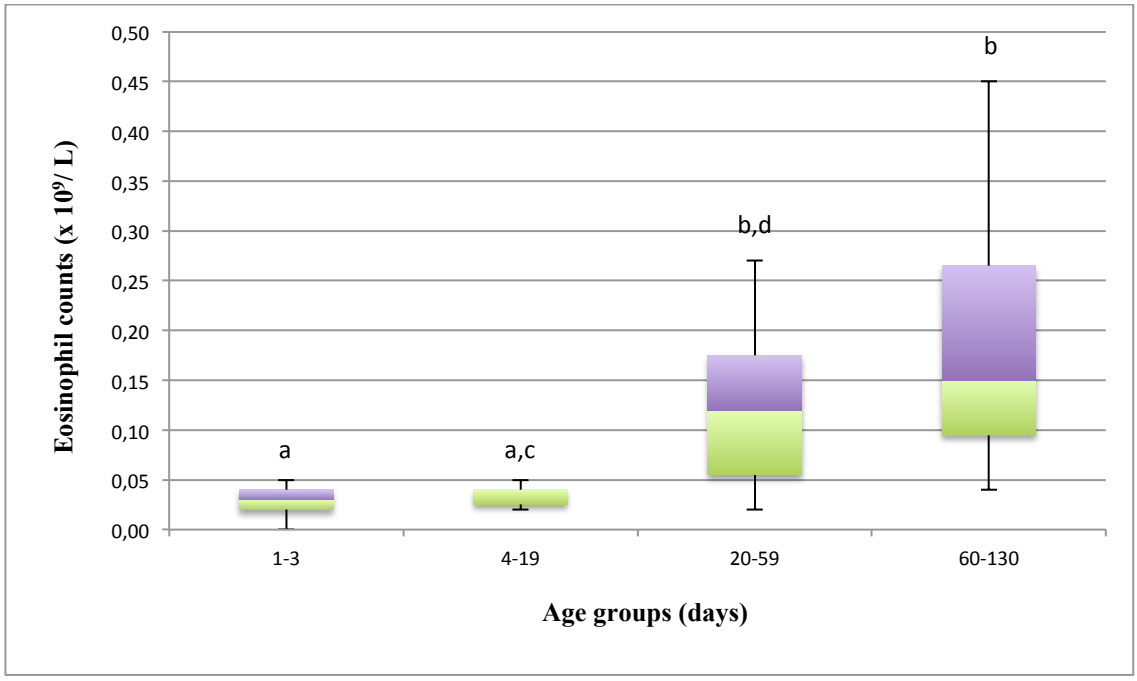


a, b: Age groups with different superscripts differed significantly within time points ($p<0.05$).

Fig. 4.7-8: Neutrophil counts in ill hospitalized foals

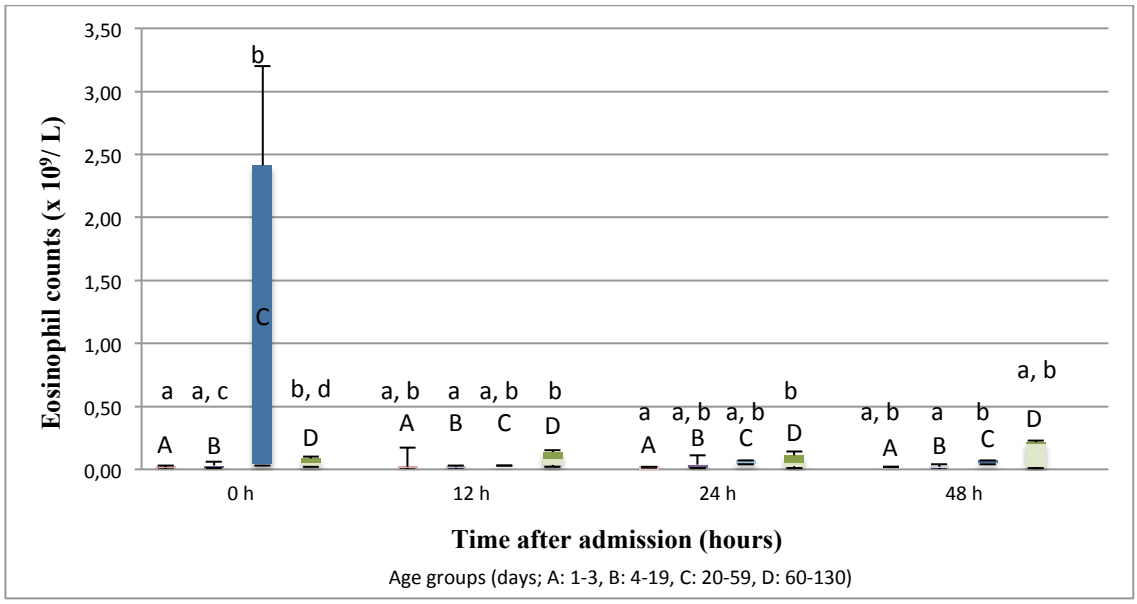
4.7.5 Eosinophil counts

Eosinophil counts in healthy and ill foals are presented in Fig. 4.7-9 and Fig. 4.7-10.



a, b, c, d: Age groups with different superscripts differed significantly ($p<0.05$)

Fig. 4.7-9: Eosinophil counts in healthy foals



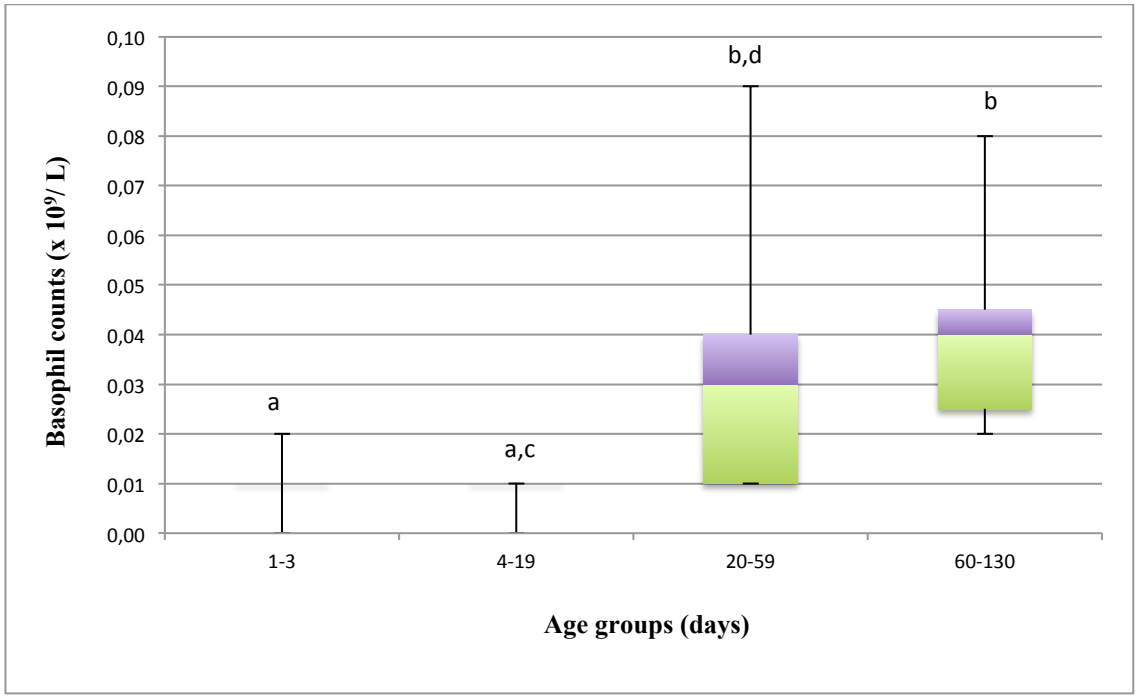
a, b, c, d: Age groups with different superscripts differ significantly within time points ($p<0.05$).

Fig. 4.7-10: Eosinophil counts in ill hospitalized foals

Eosinophil counts at admission were lower in 1-3 and 60-130 days old ill hospitalized foals ($p<0.05$); the 4-9 days old ill hospitalized foals had higher eosinophil counts when compared with the healthy ones at the same age ($p<0.05$). The other groups had similar values. No differences were obtained among the four time points within the same group of ill hospitalized foals.

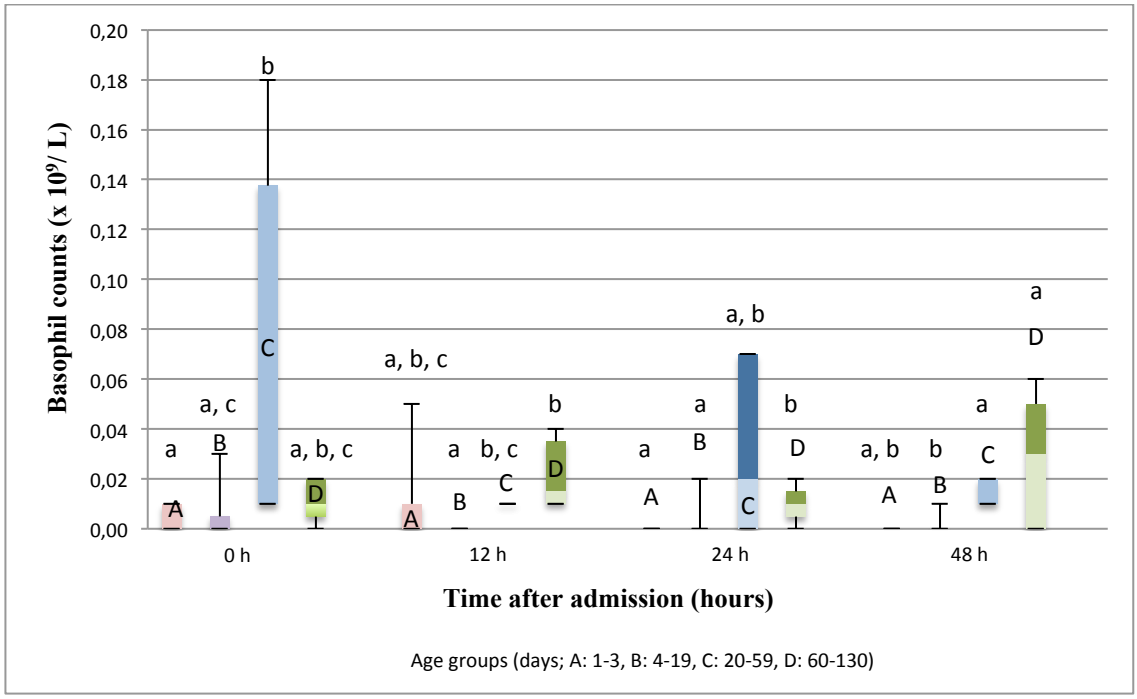
4.7.6 Basophil counts

At admission, ill hospitalized foals from the age groups 1-3 and 60-130 years had lower basophil counts ($p<0.05$). In contrast to the 20-59 days groups, the 4-9 days old ill hospitalized foals showed higher values compared with the healthy ones ($p<0.05$). No differences of basophil counts were found among four time points within the age groups in ill hospitalized foals.



a, b, c, d: Age groups with different superscripts differed significantly ($p<0.05$)

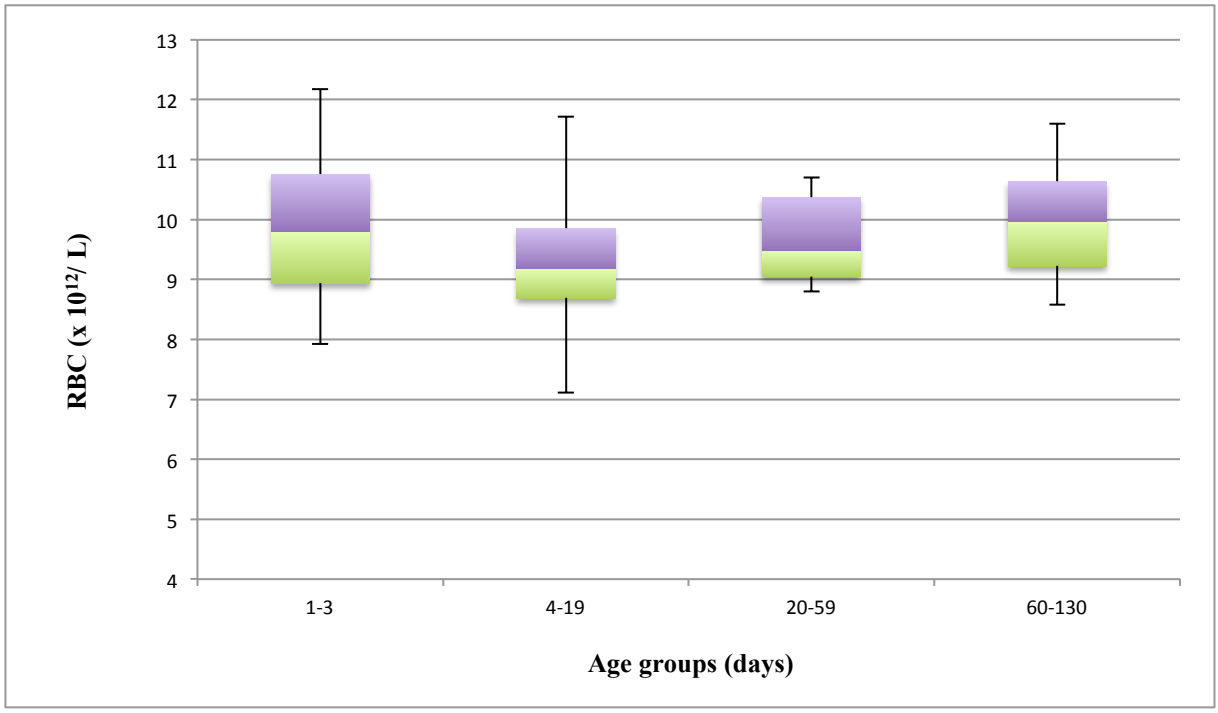
Fig. 4.7-11: Basophil counts in healthy foals



a, b, c: Age groups with different superscripts differed significantly within time points ($p<0.05$).

Fig. 4.7-12: Basophil counts in ill hospitalized foals

4.7.7 RBC



RBC value did not vary between age groups within healthy foals.

Fig. 4.7-13: RBC of healthy foals

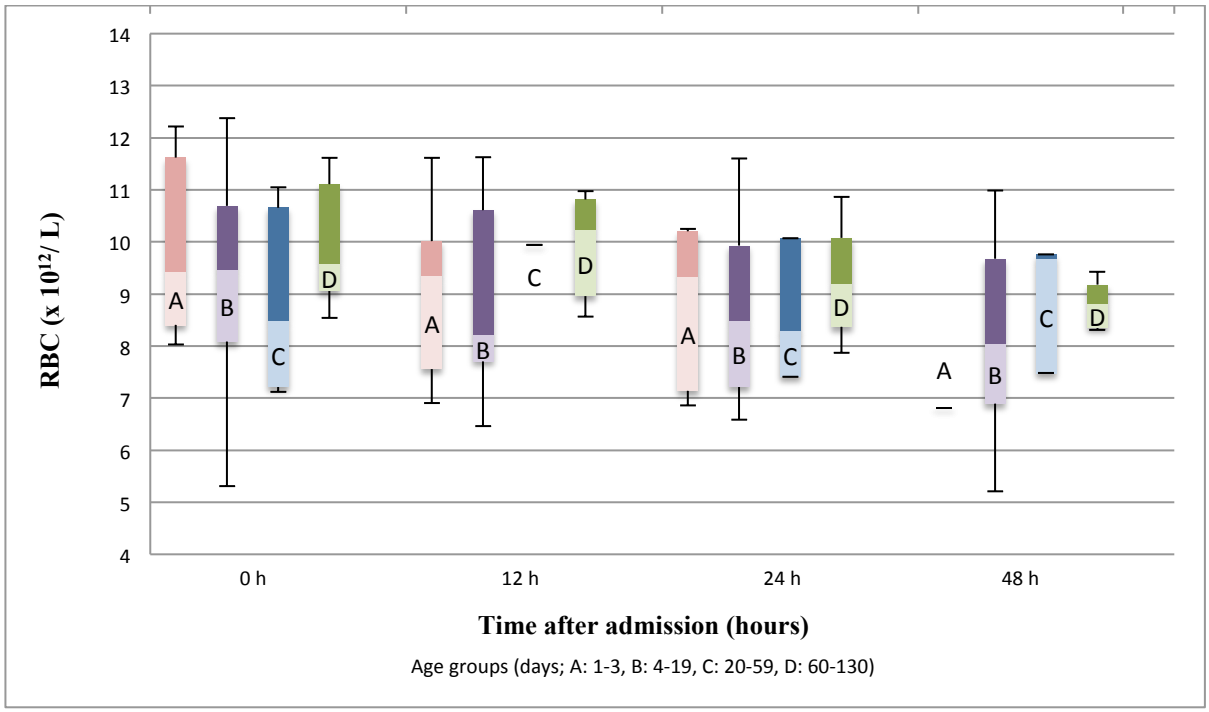
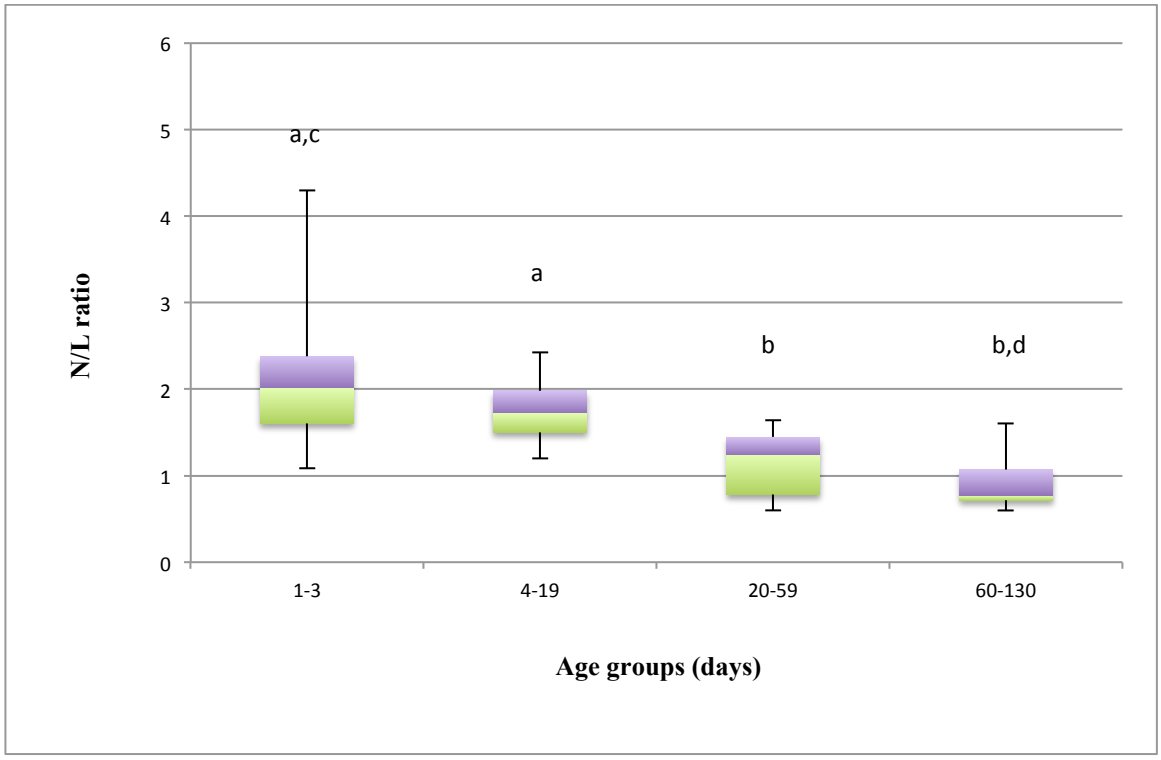


Fig. 4.7-14: RBC of ill hospitalized foals

RBC values did not differ between healthy (Fig. 4.7-13) and ill hospitalized (Fig. 4.7-14) foals in all age groups at time point 0 ($p>0.05$). They also did not vary among the four time points within the same age groups in ill hospitalized foals. No RBC values of any age groups differed significantly ($p>0.05$) within any of the four time points.

4.7.8 N/L ratio



a, b, c, d: Age groups with different superscripts differ significantly ($p<0.05$)

Fig. 4.7-15: N/L ratio of healthy foals

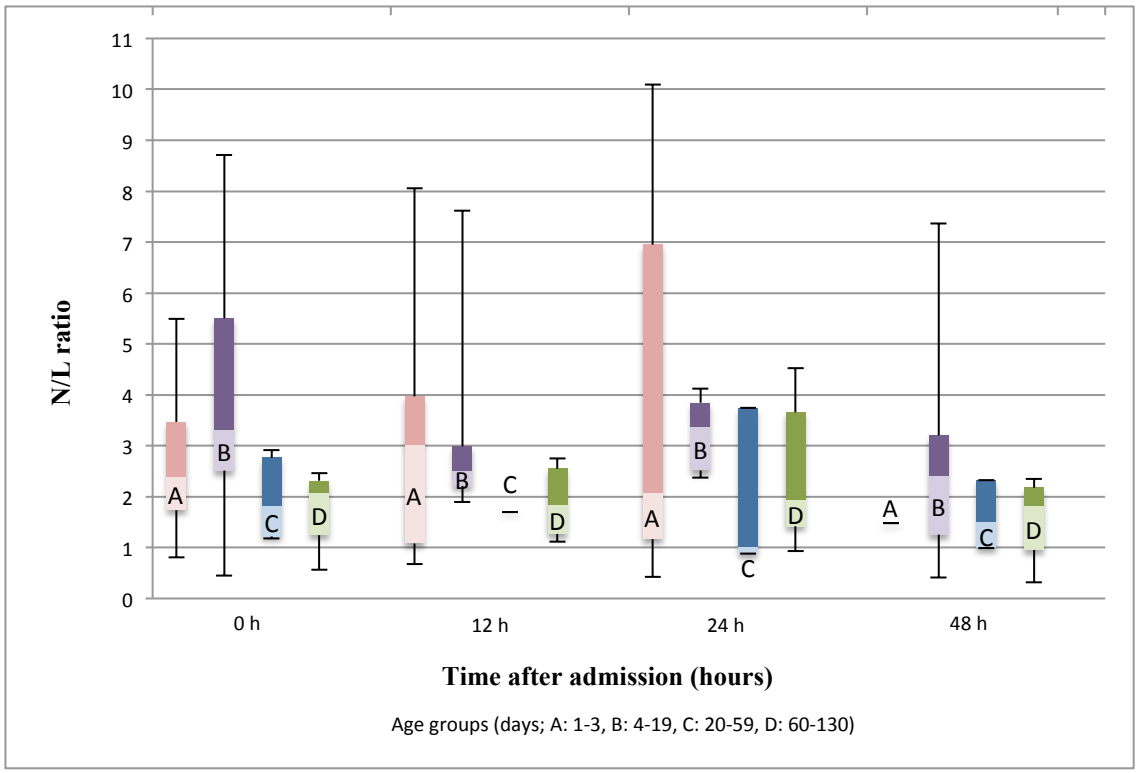


Fig. 4.7-16: N/L ratio of ill hospitalized foals

Values of N/L-ratio revealed significant lower values in healthy 4-19 days old foals (Fig. 4.7-15) compared to ill hospitalized foals (Fig. 4.7-16) at admission. The other groups had similar values. Comparison made between the four time points within the same age group among ill hospitalized foals did not show variation. Also none of the age groups differed significantly ($p<0.05$) within any of the four time points.

4.7.9 PCV

The PVC values of healthy foals did not differ between age groups (Fig. 4.7-17). The PCV values at time point 0 did not show differences between ill hospitalized and healthy foals in all age groups; they also did not vary among the four time points within the same age group in ill hospitalized foals. None of the age groups had significantly different values ($p<0.05$) when compared with other age groups within the same time point (Fig. 4.7-18).

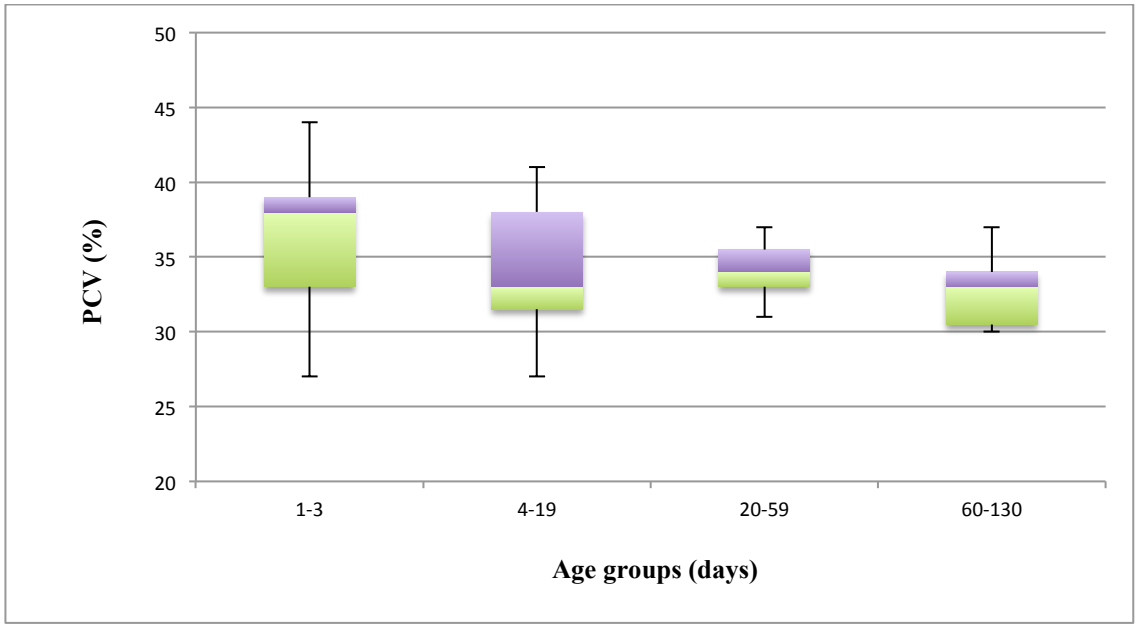


Fig. 4.7-17: PCV value of healthy foals

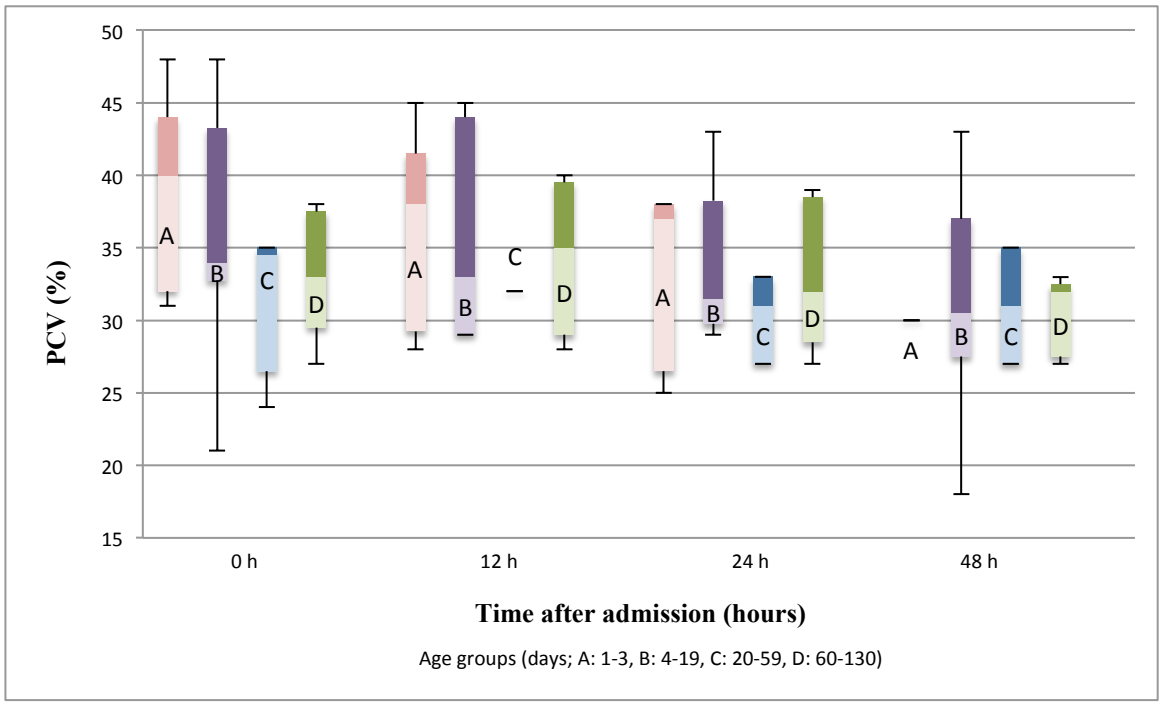


Fig. 4.7-18: PCV value of ill hospitalized foals

4.7.10 Total protein

Total protein values did no vary among different age groups in healthy foals (Fig. 4.7-19). The youngest ill hospitalized foals (1-3 days old) had lower total protein values at admission (Fig. 4.7-20) compared to their healthy counterparts. The total protein values did not differ in other age groups between healthy and ill hospitalized foals. There were no differences found among the four time points within age groups in ill hospitalized foals.

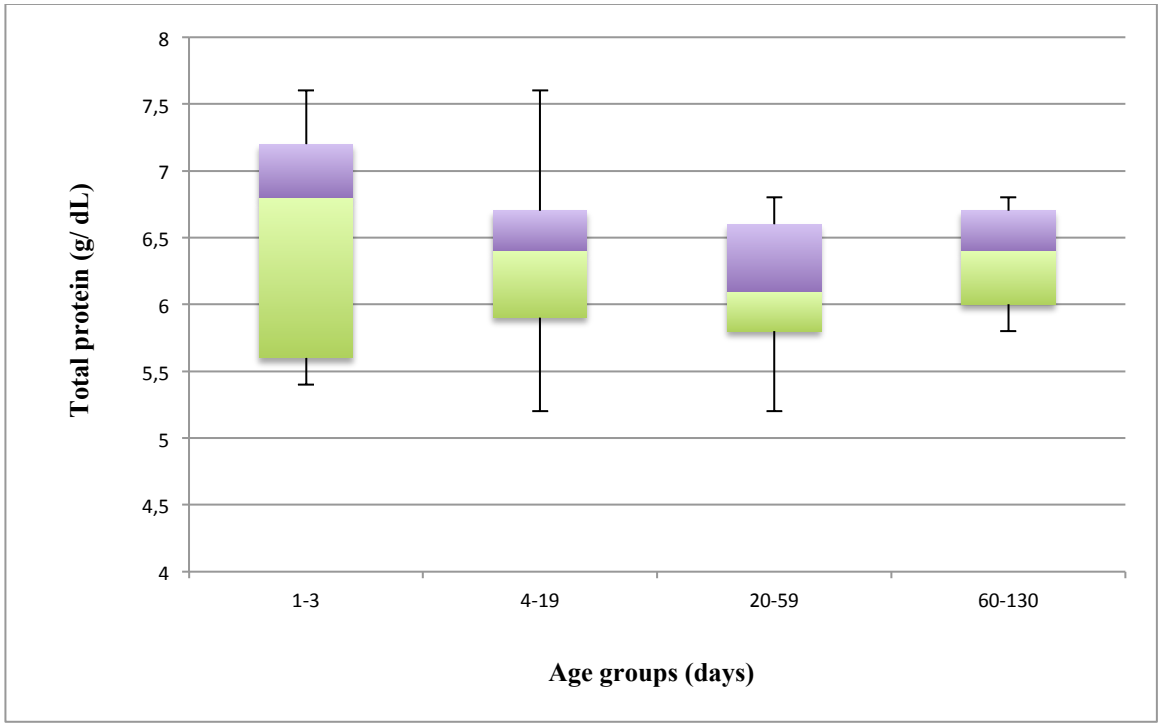
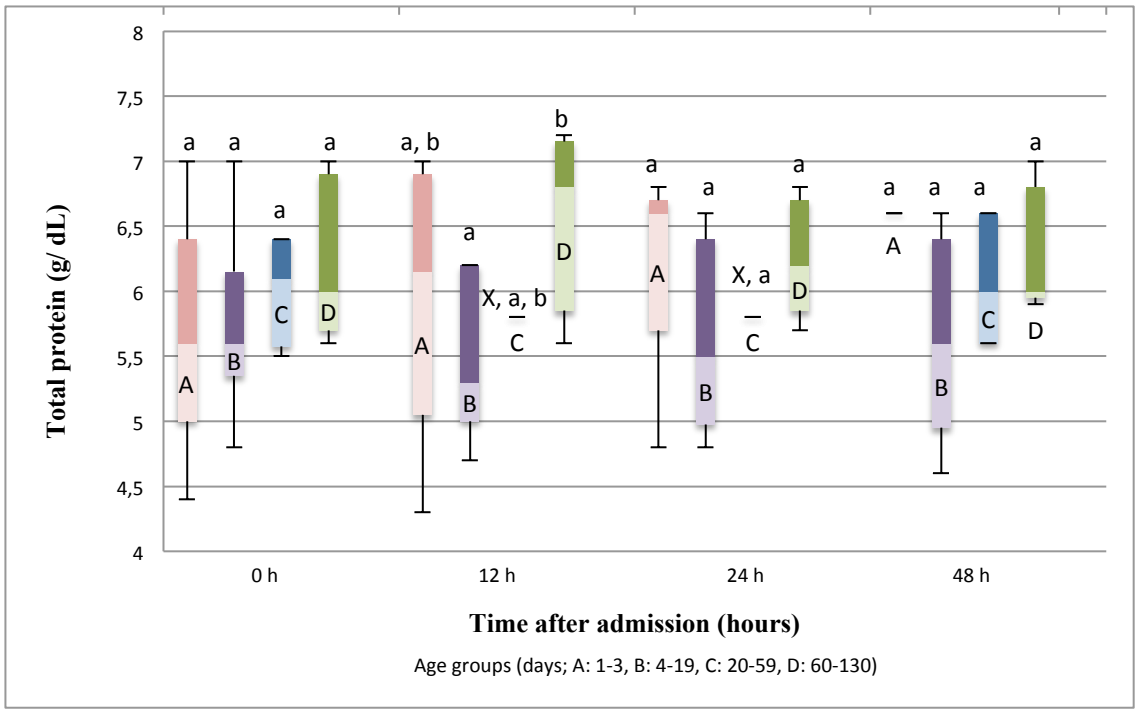


Fig. 4.7-19: Total protein of healthy foals

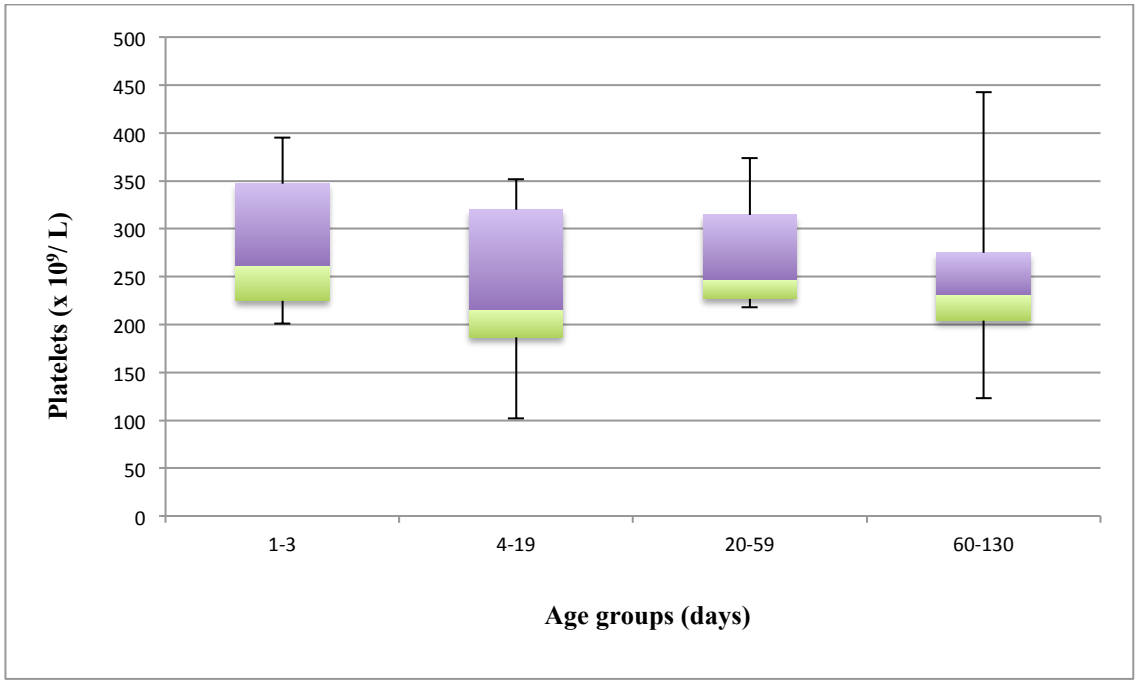


X: The values within the same age group at different time points could not be compared due to too less data.
a, b: Age groups with different superscripts differ significantly within time points ($p<0.05$).

Fig. 4.7-20: Total protein of ill hospitalized foals

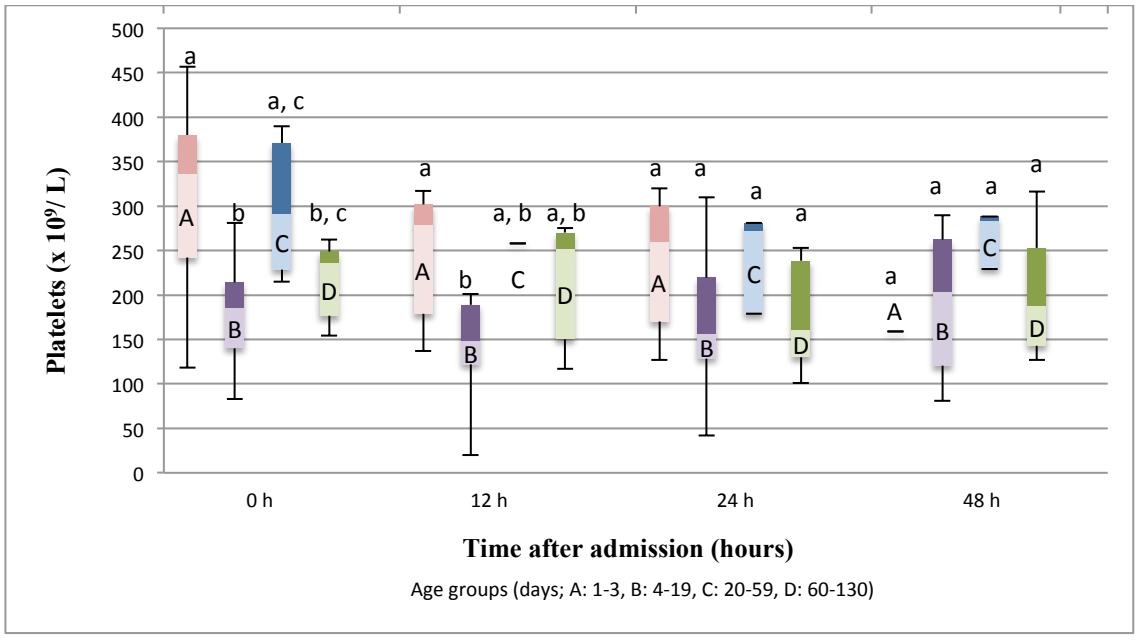
4.7.11 Platelets

No differences of platelet values were observed between healthy (Fig. 4.7-21) and ill hospitalized (Fig. 4.7-21) foals at admission in any age groups. We found also no differences among the four time points within age groups in ill hospitalized foals.



Platelets values of healthy foals did not show differences among age groups.

Fig. 4.7-21: Platelets of healthy foals



a, b, c: Age groups with different superscripts differed significantly within time points ($p<0.05$)

Fig. 4.7-22: Platelet of ill hospitalized foals

4.8 Results of differential leukocyte counts and neutrophil toxic change evaluation

4.8.1 Differential leukocyte counts

Table 4.8-1: Differential leukocyte counts of healthy foals

Parameter		**Age groups (days)**			
		1 – 3	**4 – 19**	**20 – 59**	**60 – 130**
Lymphocytes	(%; median, min-max)	28 [a] (17 - 40)	29 [a, d] (26 - 49)	45 [c] (35 - 49)	52 [b, c] (33 - 62)
Neutrophils	(%; median, min-max)	70 [a] (57 - 79)	67 [a, d] (49 - 69)	48 [c] (34 - 62)	40 [b, c] (36 - 60)
Band neutrophils	(%; median, min-max)	0 [a, o] (0 - 0)	0 [a, o] (0 - 0)	0 [a, o] (0 - 0)	0 [a, o] (0 - 12)
Juvenile neutrophils	(%; median, min-max)	0 [o] (0 - 0)	0 [o] (0 - 0)	0 [o] (0 - 0)	0 [o] (0 - 0)
Eosinophils	(%; median, min-max)	0 [a] (0 - 1)	0 [a] (0 - 1)	0 [a] (0 - 6)	0 [a] (0 - 3)
Basophils	(%; median, min-max)	0 [a] (0 - 0)	1 [b] (0 - 2)	0 [a, b] (0 - 1)	0 [a, b] (0 - 1)
Monocytes	(%; median, min-max)	2 [a] (0 - 6)	3 [a] (0 - 6)	2 [a] (1 - 6)	2 [a] (0 - 6)

a, b, c, d: Age groups with different superscripts differed significantly ($p<0.05$)
o: Age group(s) contained too less or no data, which is why no statistical analysis was carried out.

In healthy foals differential counts of lymphocytes, neutrophils and basophils differed among age groups (Table 4.8-1; $p<0.05$). At time of admission, the lymphocyte counts were lower in 4-19 and 20-59 days old ill hospitalized foals; these groups of ill hospitalized foals had also higher values of neutrophil counts (Table 4.8-1). A higher band-neutrophil value was seen in 1-3 days old hospitalized young foals, but there were no juvenile neutrophils observed in any of the foals at any time point. Eosinophil, basophil and monocyte counts didn't differ between healthy and ill hospitalized foals in all age groups at time point 0.

Table 4.8-2: Differential leukocyte count of ill hospitalized foals

Time after admission	Age groups (days)	Parameter					
		Lymphocytes	**Neutrophils**	**Bands**	**Eosinophils**	**Basophils**	**Monocytes**
		(%; median, min-max)	(%; median, min-max)	(%; median, min-max)	(%; median, min-max)	(%; median, min-max)	(%; median, min-max)
0 h	**1 – 3**	21.5 [a] (6 - 53)	75.5 [a] (28 - 90)	2.5 [a] (0 - 11)	0 [X, **a, o**] (0 - 0)	0 [a] (0 - 4)	3.5 [a] (0 - 12)
	4 – 19	17 [a] (5 - 30)	81 [a] (57 - 89)	0 [a] (0 - 11)	0 [a] (0 - 2)	0 [a] (0 - 1)	2 [a] (0 - 5)
	20 – 59	28 [a] (27 - 29)	69 [a] (67 - 69)	0 [X, a, o] (0 - 0)	0 [X, **a, o**] (0 - 0)	0 [X, a] (0 - 0)	4 [a] (2 - 5)
	60 – 130	27.5 [a] (19 - 65)	69.5 [a] (33 - 79)	0 [X, a, o] (0 - 0)	0 [**a, o**] (0 - 0)	0 [a] (0 - 1)	2.5 [a] (1- 3)
12 h	**1 – 3**	20 [a] (16 - 48)	72 [a] (48 - 78)	2 [a] (0 - 11)	0 [X, a, o] (0 - 0)	0 [X, a, o] (0 - 0)	2 [a] (0 - 9)
	4 – 19	25 [a] (17 - 46)	71 [I ,a] (50 - 80)	1 [a] (0 - 4)	0 [X, b, o] (0 - 0)	0 [a, b] (0 - 1)	5 [I, a] (1 - 8)
	20 – 59	34 [a] (34 - 34)	61 [a] (61 - 61)	0 [X, a, o] (0 - 0)	0 [X, b, o] (0 - 0)	1 [b] (1 - 1)	4 [a] (4 - 4)
	60 – 130	29.5 [a] (28 - 39)	65.5 [a] (58 - 67)	0 [X, a, o] (0 - 0)	1 [b] (0 - 3)	0 [X, b, o] (0 - 0)	3.5 [a] (1 - 5)

Table 4.8-2: Continued

Time after admission	Age groups (days)	Parameter					
		Lymphocytes	**Neutrophils**	**Bands**	**Eosinophils**	**Basophils**	**Monocytes**
		(%; median, min-max)	(%; median, min-max)	(%; median, min-max)	(%; median, min-max)	(%; median, min-max)	(%; median, min-max)
24 h	**1 – 3**	31 [a] (18 - 72)	56 [a] (8 - 80)	0 [a] (0 - 20)	0 [X, **a, o**] (0 - 0)	0 [X, a, o] (0 - 0)	2 [a] (0 - 13)
	4 – 19	13.5 [b] (9 - 39)	78 [IV, a] (52 - 86)	0.5 [a] (0 - 11)	0 [X, **b, o**] (0 - 0)	0 [a] (0 - 1)	4.78 [II, a] (4, 3 - 8)
	20 – 59	30 [a, b] (11- 49)	63.5 [a] (46 - 81)	0 [X, a] (0 - 0)	2 [a] (0 - 4)	0 [X, a, o] (0 - 0)	4.5 [a] (1 - 8)
	60 – 130	32.5 [a] (19 - 55)	65 [a] (41 - 75)	0 [a] (0 - 1)	0.5 [a] (0 - 1)	0 [X, a, o] (0 - 0)	2.5 [a] (1 - 6)
48 h	**1 – 3**	48 [a] (48 - 48)	32 [a] (32 - 32)	8 [a] (8 - 8)	0 [X, a, b] (0 - 0)	0 [X, **a, o**] (0 - 0)	12 [a] (12 - 12)
	4 – 19	21 [VI, a] (14 - 70)	72 [III, VI, a] (10 - 78)	0 [a] (0 - 20)	0 [a] (0 - 3)	0 [a] (0 - 2)	4.5 [a] (0 - 16)
	20 – 59	36 [a] (12 -46)	61 [a] (48 - 84)	0 [X, a] (0 - 0)	0 [a, b] (0 - 2)	0 [X, **a, o**] (0 - 0)	4 [a] (3 - 4)
	60 – 130	34.5 [a] (22 - 84)	59 [a] (11 - 73)	0 [a] (0 - 3)	1.5 [b] (0 - 4)	0 [X, **a, o**] (0 - 0)	3 [a] (2 - 5)

Table 4.8-2: Continued

I: Values of time point 0 and 12 hours after admission were significantly different ($p<0.05$) within the same age group.

II: Values of time point 0 and 24 hours after admission were significantly different ($p<0.05$) within the same age group.

III: Values of time point 0 and 48 hours after admission were significantly different ($p<0.05$) within the same age group.

IV: Values of time point 12 and 24 hours after admission were significantly different ($p<0.05$) within the same age group.

VI: Values of time point 24 and 48 hours after admission were significantly different within the same age group.

X: The values within the same age group at different time points could not be compared due to too less data.

All other data than listed above showed no differences among the four time points within age groups of ill hospitalized foals.

a, b, c: Age groups with different superscripts differ significantly within time points ($p<0.05$)

o: Age group(s) contained too less or no data, no statistical analyzing could be carried out between groups within the same time point.

4.8.2 Toxic changes score

There were only two healthy foals that had a toxic change score greater than 0 during the whole study period (Fig. 4.8-1). One was 20-59 days old; the other one was between 60-130 days old. Except group A and B that contained too less data, so that no comparison could be carried out, there were no difference found between groups in healthy foals.

Among all four age groups, the ill hospitalized foals had a significantly higher toxic change score than the healthy ones at time point 0. The toxic change score did not vary between the four time points within a same age group among ill hospitalized foals (Fig. 4.8-2).

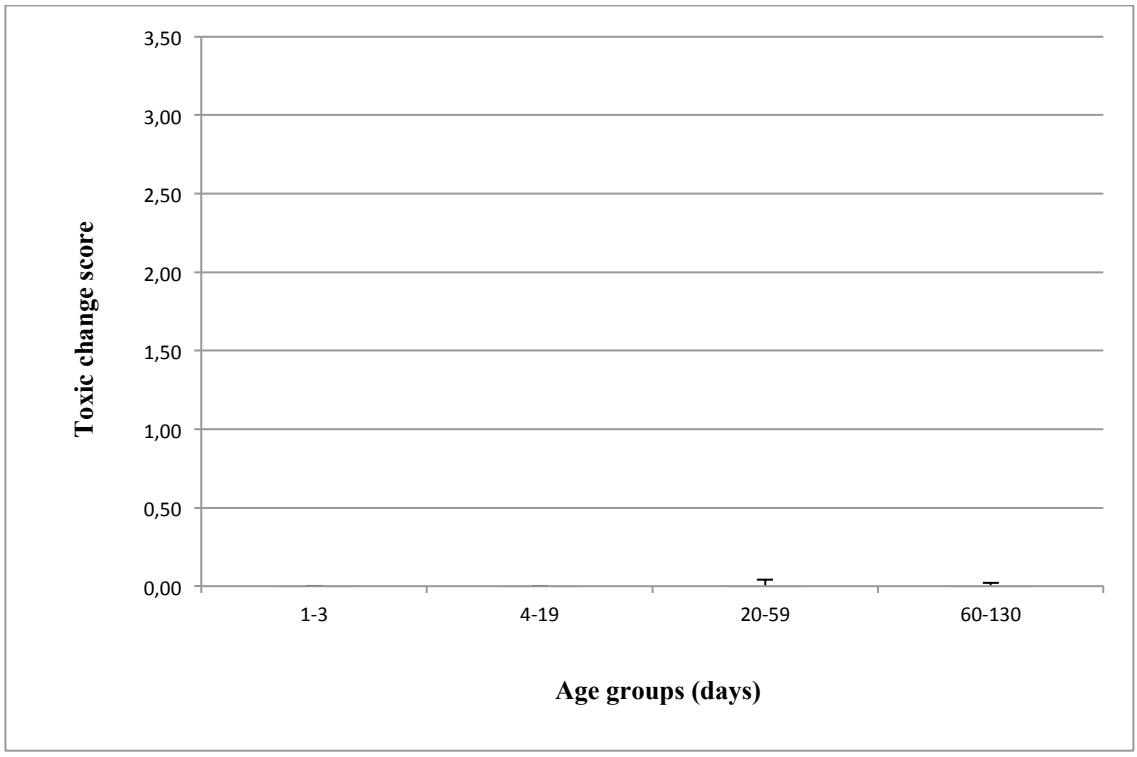
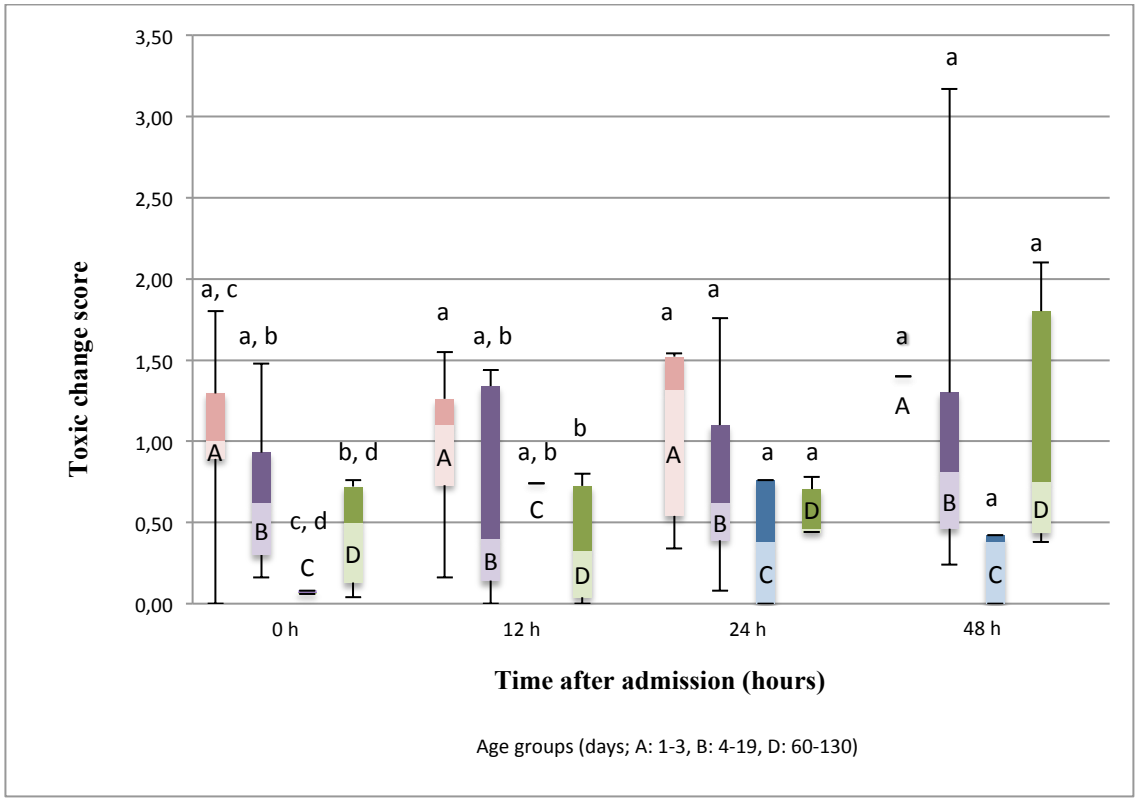


Fig. 4.8-1: Toxic changes score in healthy foals



a, b, c, d: Age groups with different superscripts differed significantly within time points ($p<0.05$)

Fig. 4.8-2: Toxic changes score in ill hospitalized foals

4.9 Blood Immunoglobulin-G values

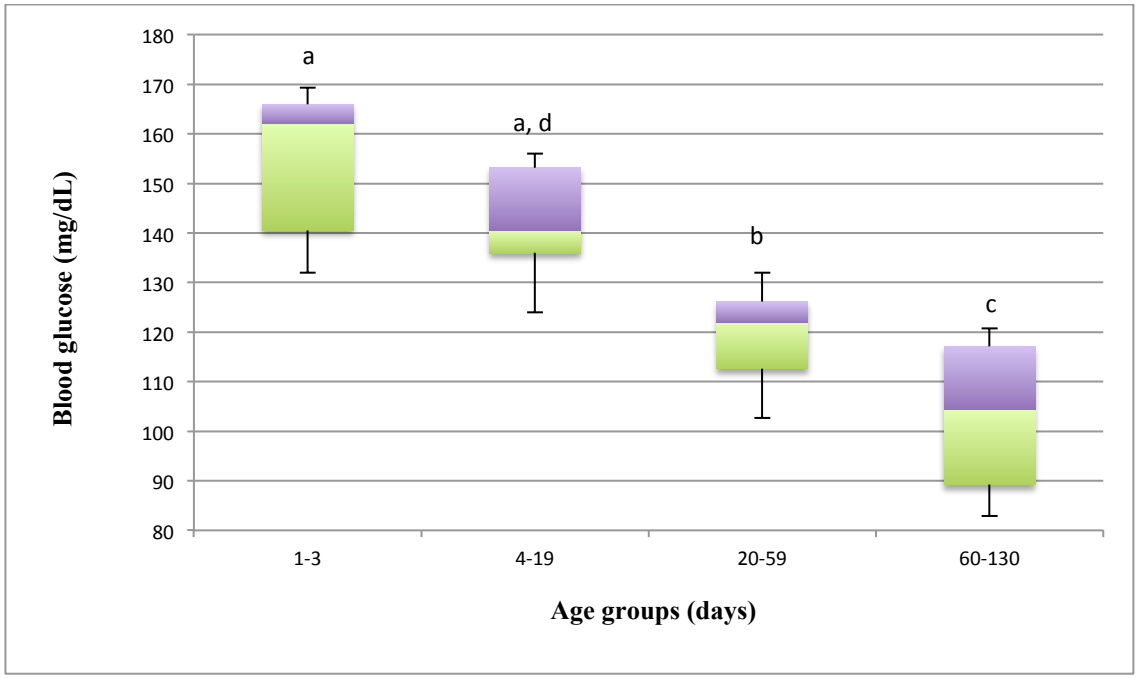
The IgG values from 1-3 days old healthy foals were all above 800 mg/dL. Further IgG value estimations were not carried out. At time of admission, ill hospitalized foals tended to have lower IgG values compared to the healthy ones.

Table 4.9: IgG values of ill hospitalized foals

		Age groups (days)			
		A (1 – 3)	**B (4 – 19)**	**C (20 – 59)**	**D (60 – 130)**
IgG (mg/dL)	> 800	5	4	2	3
	800				
	400 - 800		1		1
	400		1		
	< 400	3	2		
	Total	8	8	2	4

4.10 Blood glucose

Blood glucose concentrations significantly differed among some age groups in healthy foals (Fig. 4.10-1; $p<0.05$). At admission, the 1-3 days old ill hospitalized foals had lower blood glucose values, while the 20-59 and the 60-130 days old ones exhibited higher values (Fig. 4.10-2). The 20 - 59 days old hospitalized foals had similar blood glucose values as the healthy ones. Blood glucose values did not vary among the four time points within the same age group of ill hospitalized foals. The values of the four age groups also did not differ significantly ($p<0.05$) within any of the four time points.



a, b, c, d: Age groups with different superscripts differed significantly ($p<0.05$)

Fig. 4.10-1: Blood glucose of healthy foals

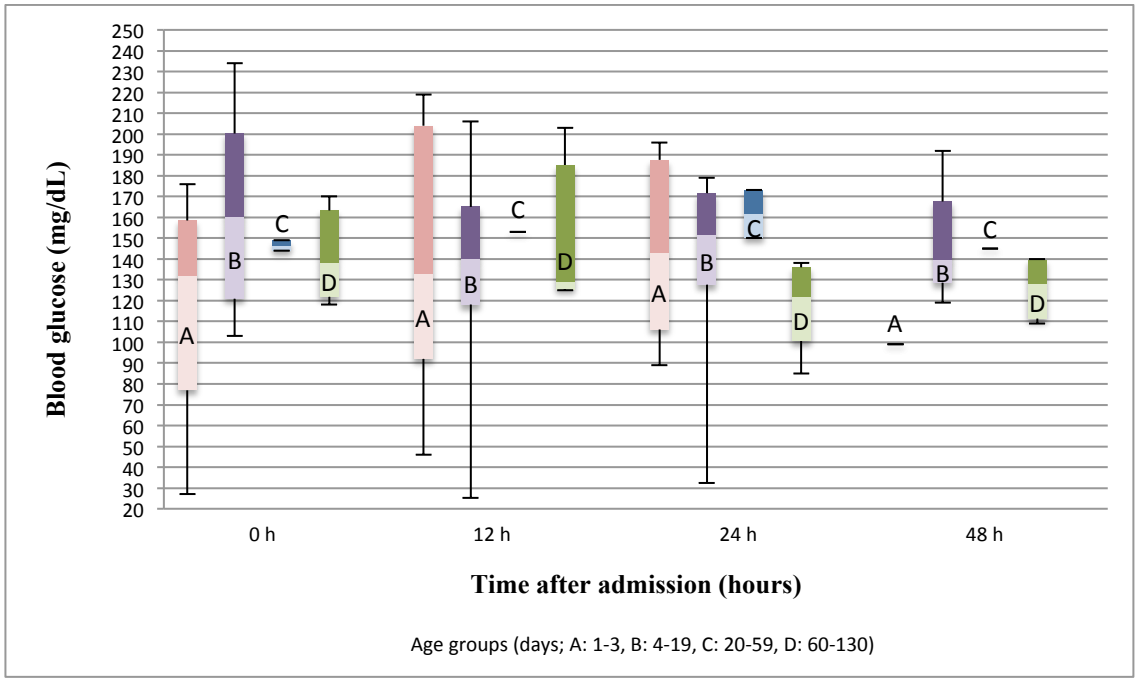


Fig. 4.10-2: Blood glucose of ill hospitalized foals

4.11 Blood lactate

Blood lactate values did not show any differences between age groups in healthy foals (Fig. 4.11-1). Also no differences of blood lactate were seen between healthy and ill hospitalized foals at admission, neither among the four time points within age groups in ill hospitalized foals (Fig 4.11-2).

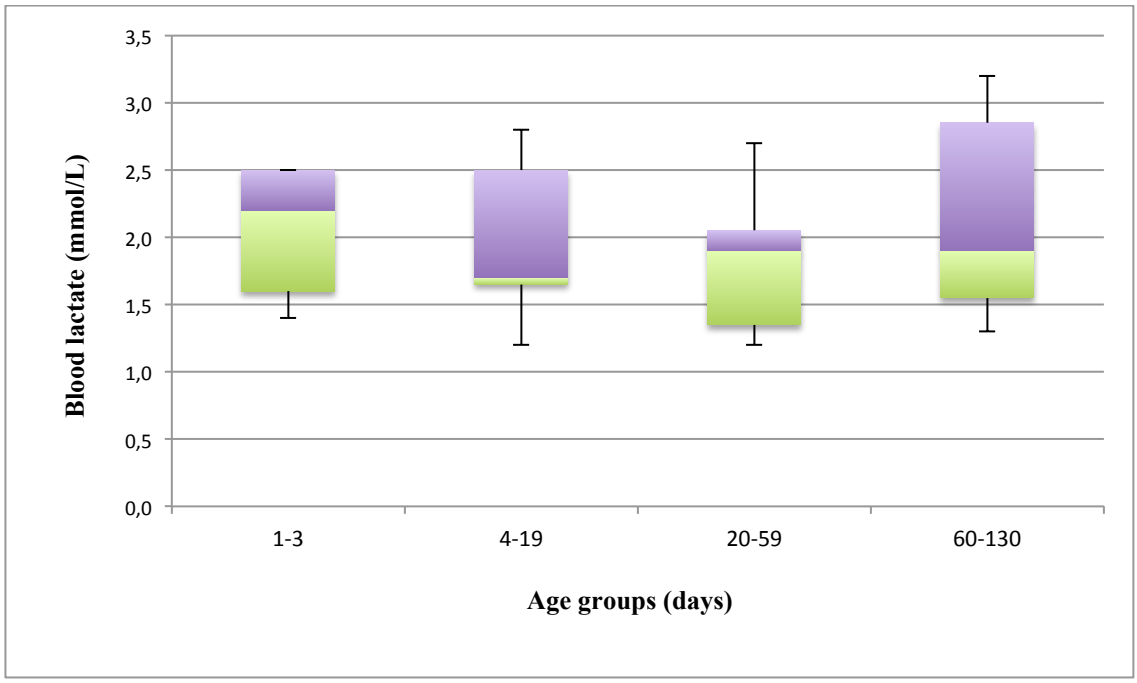
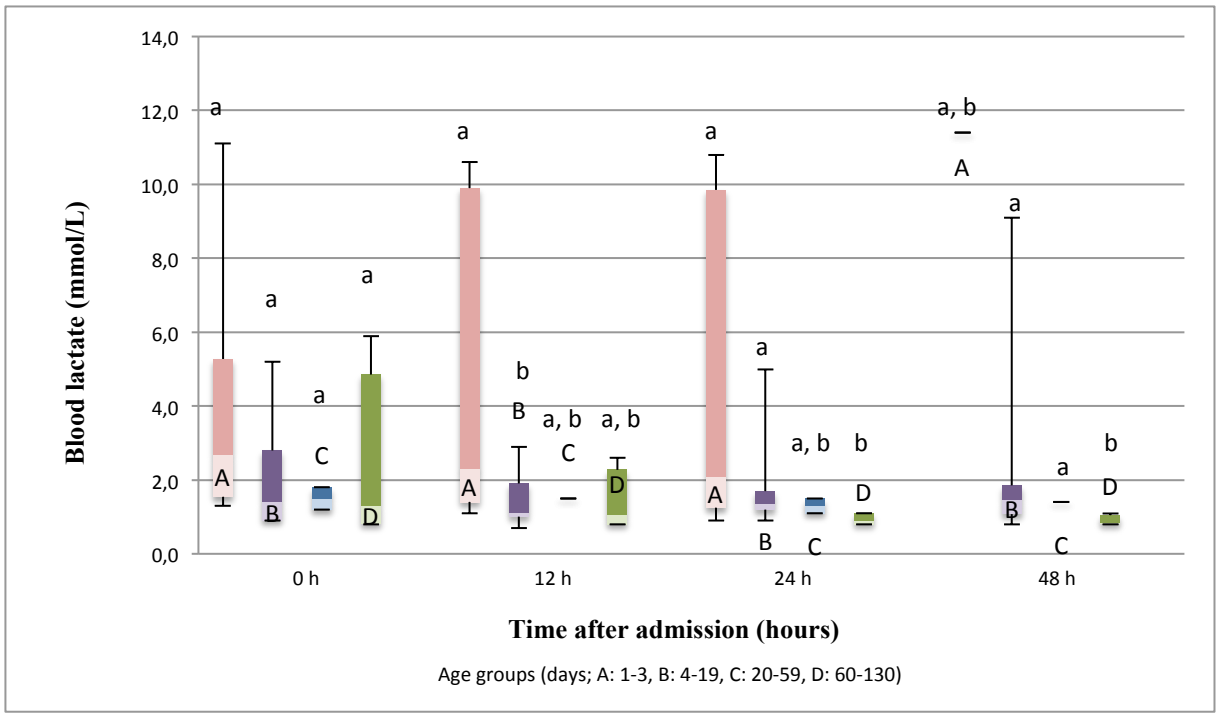


Fig. 4.11-1: Blood lactate concentrations of healthy foals

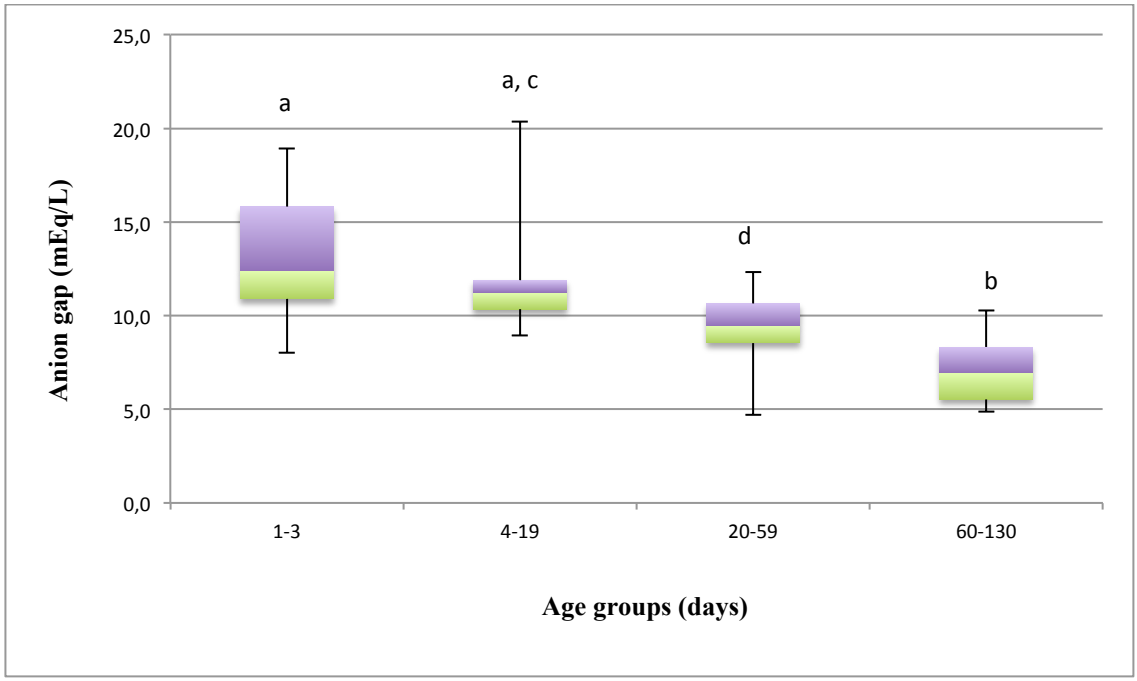


a, b: Age groups with different superscripts differed significantly within time points ($p<0.05$)

Fig. 4.11-2: Blood lactate concentrations of ill hospitalized foals

4.12 Anion gap

The 60-130 days ill hospitalized foals had higher anion gap values at admission than the healthy ones (Fig. 4.12-1, Fig. 4.12-2). The values from the other two groups (4-19 and 60-130 days) were similar. In the three age groups of hospitalized foals, the anion gap values did not show differences among the four time points. The comparisons among age groups within the same time points also showed no significant differences ($p>0.05$).



a, b, c, d: Age groups with different superscripts differed significantly ($p<0.05$)

Figure 4.12-1: Anion gap values of healthy foals

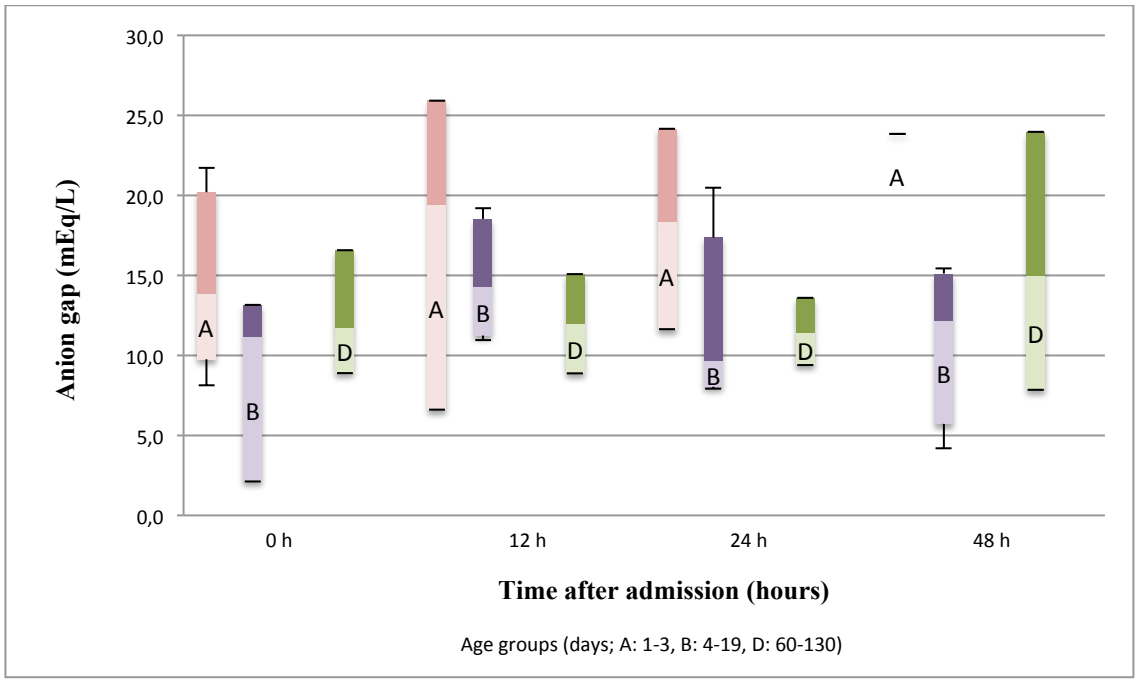


Figure 4.12-2: Anion gap value of ill hospitalized foals

4.13 Plasma haptoglobin

Statistical analysis showed no differences of haptolobin values all age groups in healthy foals (Fig. 4.13-1). At admission, only the 1-3 days old ill hospitalized foals revealed lower haptoglobin values; concentrations of the elder hospitalized foals did not differ from values of the healthy ones. Haptoglobin values also did not vary among the four time points within age groups in ill hospitalized foals (Fig. 4.13-2).

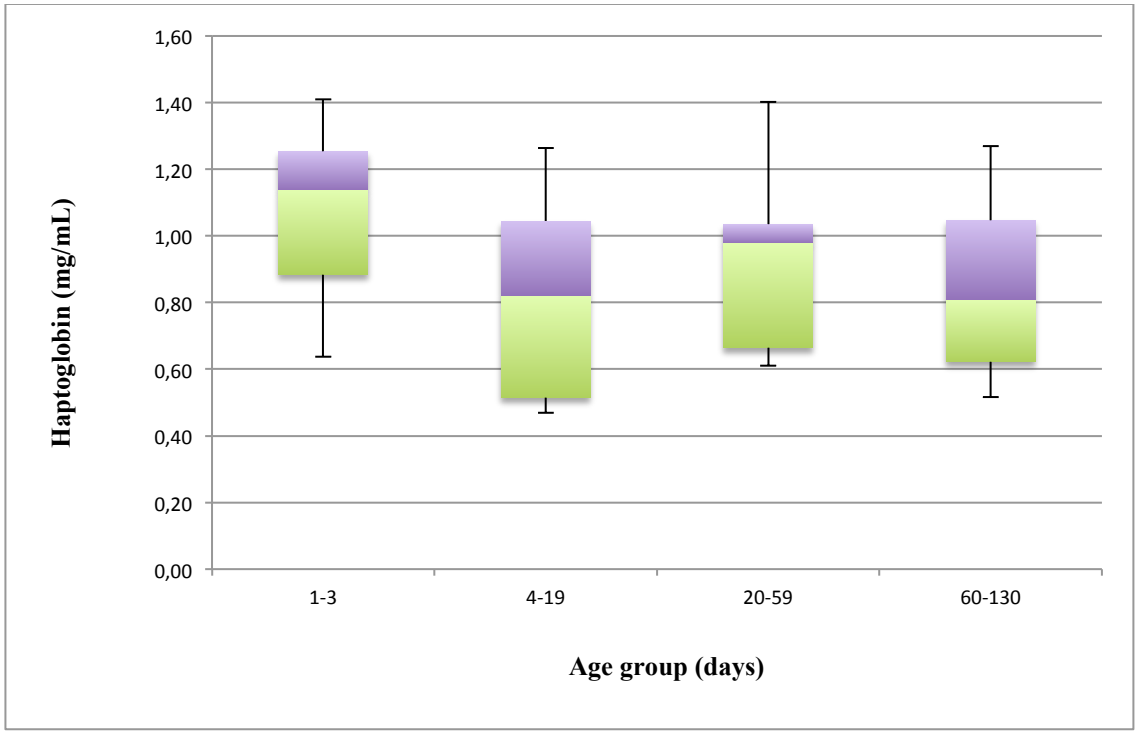
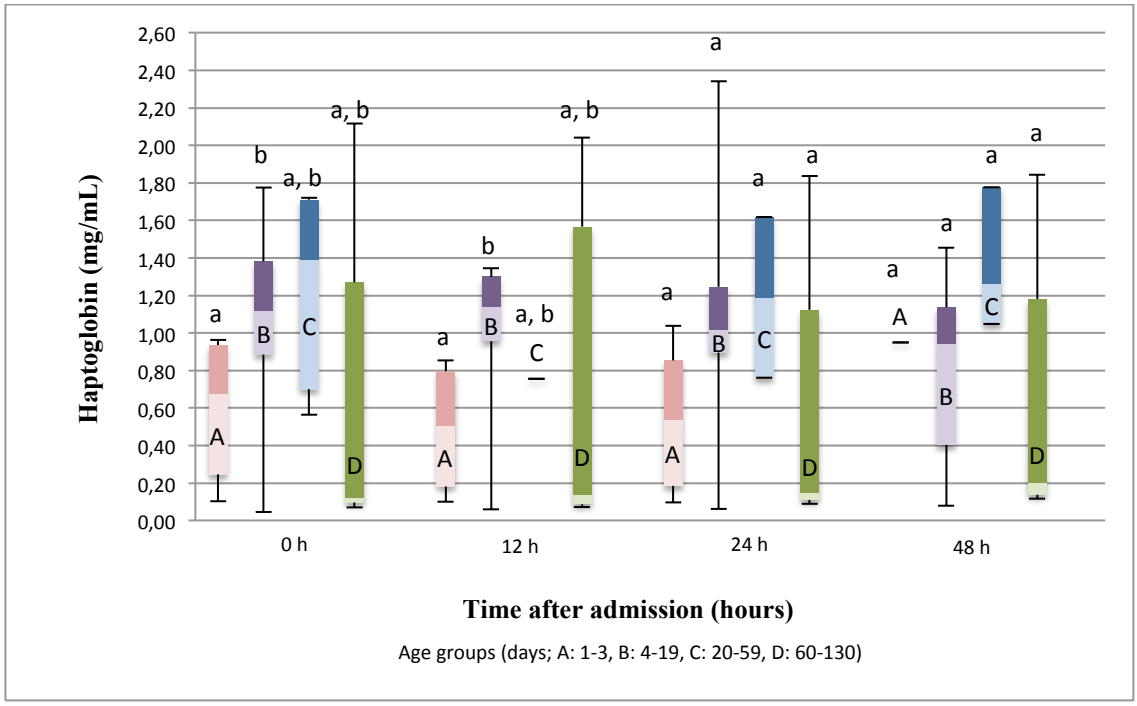


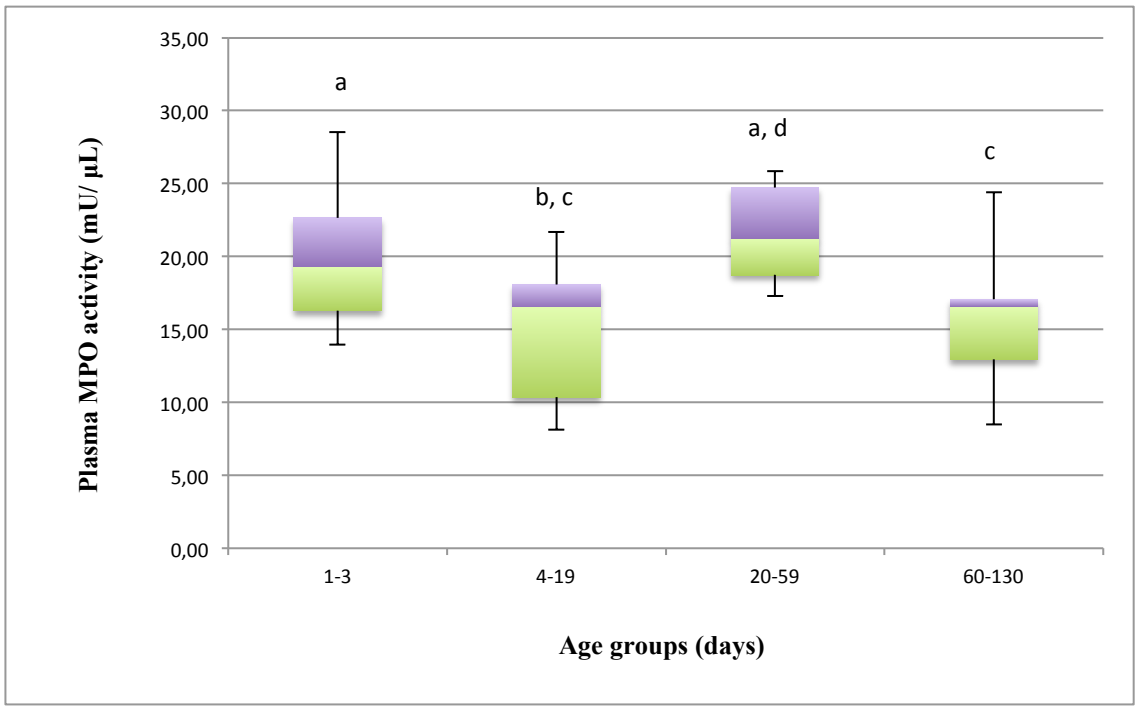
Fig. 4.13-1: Haptoglobin of healthy foals



a, b: Age groups with different superscripts differed significantly within time points ($p<0.05$)

Fig. 4.13-2: Haptoglobin of ill hospitalized foals

4.14 Plasma MPO activity



a, b, c, d: Age groups with different superscripts differed significantly ($p<0.05$)

Fig. 4.14-1: Plasma MPO activity of healthy foals

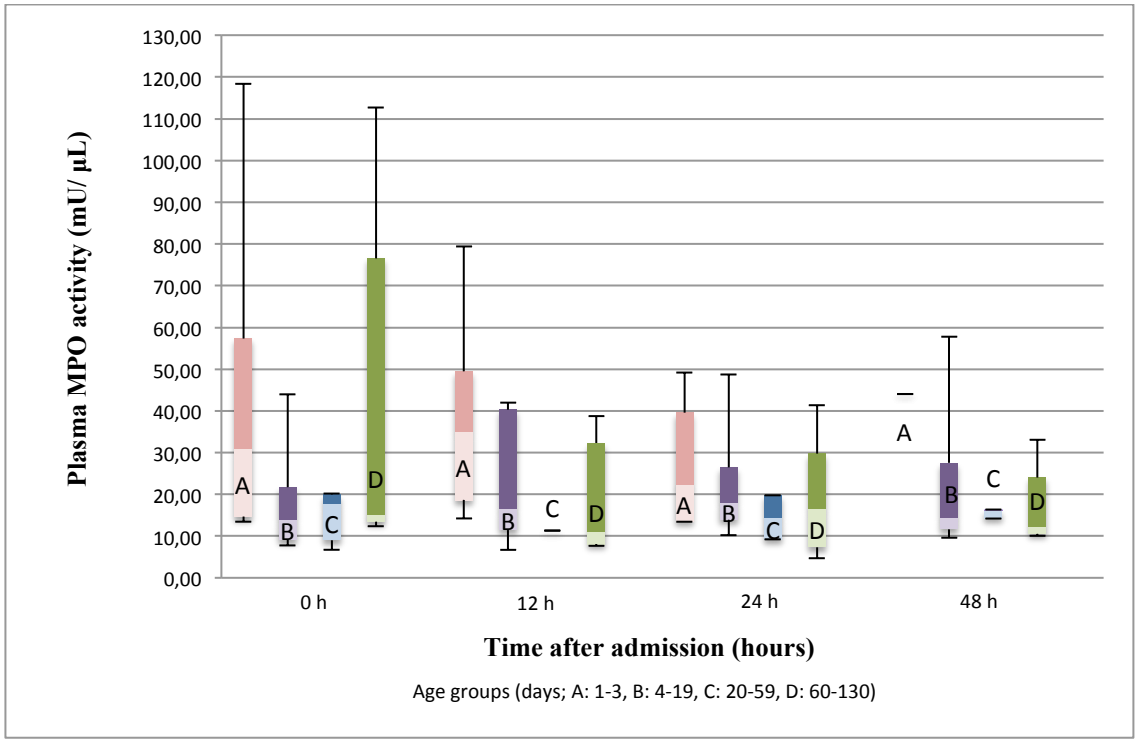


Fig. 4.14-2: MPO activity of ill hospitalized foals

No MPO value differences could be detected between healthy and ill hospitalized foals at time point 0 in all age groups (Fig. 4.14-1, Fig. 4.14-2). The values also did not vary among the four time points within age groups in ill hospitalized foals. Further, MPO values of age groups did not differ significantly ($p>0.05$) within any of the four time points.

5. Discussion

5.1 Clinical examination

All of the healthy foals and the majority of the ill hospitalized foals appeared bright and alert at the time of examination. Other clinical parameters such as rectal temperature, respiratory rate and heart rate did not differ between healthy and ill hospitalized foals. During their stay at the clinic, ill hospitalized foals did not show differences in rectal temperature and respiratory rate, but their heart rates were higher at admission compared to the values at 48 hours after admission. In contrast to the rectal temperature, respiratory and heart rate differed among age groups of the healthy foals. Values of both parameters decreased with increasing age of the foals, which was accompanied by smaller variation of respiratory and heart rates in elder foals.

Published reference values of 1-3 days old foals and foals up to 7 days did not differ much, which is why we pooled them as followed: rectal temperature – 37.0 to 38.9 °C, respiratory rate – 20 to 40 times per min and heart rate – 60 to 100 beats per minute (Baumgartner, 2009; Knottenbelt et al., 2004a; Koterba, 1990; Paradis, 2006). Median values of rectal temperature of our healthy foals remained quite constant among all four age groups, but in foals younger than 60 days the range was narrower (38.1 to 39 °C) than the foals elder than 59 days (37.5 to 38.9 °C). In the 60-130 days old healthy foals, the rectal temperature values were still comparable to that of the literature. However, a non-statistical significant trend of rectal temperature decrease was observed when the foals grew elder.

The respiratory rate of healthy foals up to 19 days was partially consistent to the literature. The majority of foals between 4-19 days had a much higher rate than the younger ones without the values between these two groups being significantly different ($p<0.05$). The possible explanation to this observation is that, most of our healthy foals were from the farm in Frankendorf, the mares and the foals would be restrained in the barn for couple of days after the foal was born. After that, they were put back into the herd, where they could move freely without being disturbed by human beings. For the first sampling, we could approach and restrain the foal relatively easily in the barn; for the second sample we had to sometimes gather the herd together from the yard, in order to be able to approach to our sampling foals. So the foals were on one hand, still excited after a short running and, on the other hand, not used to the handling by humans. Throughout the whole project, we tried our best to get all the

clinical values as objective as possible, but those were the unavoidable factors that we had to take into consideration. The median values of respiratory rate in 20-59 and 60-130 days old foals were significantly ($p< 0.05$) lower than that of the younger foals, which fits well to the findings already published demonstrating the decrease approaching values of adult horses (10-14 times per minute, Baumgartner, 2009).

The heart rate ranges in healthy, 1-59 days old foals were much higher than those published in the literature (Knottenbelt et al., 2004a; Koterba, 1990). This also might be explained by the particular situation in the farm similar to the respiratory rate. Only the range of heart rates of the eldest age group fitted into the reference range for neonatal foals, which was already significantly lower ($p<0.05$) than that of the other three age groups, but yet not close to adults values (28-40 beats per minute, Baumgartner, 2009).

5.2 Haematology

Some haematological parameters such as blood cell count of lymphocytes, eosinophils and basophils as well as N/L-ratio and total protein; the percentages of lymphocytes, neutrophils and band-neutrophil differed significantly between healthy and ill foals. The other parameters (WBC, counts of monocytes, neutrophils, erythrocytes and platelets; percentages of monocytes, eosinophils and basophils; PCV) did not reveal differences between both groups. However, ill foals showed higher degrees of variation concerning these parameters, which might be explained by the wide variety of underlying causes for presentation of the foals to the clinic.

Almost all haematological parameters with the exception of RBC, PCV, platelets and percentages of monocytes and eosinophils differed among age groups in the healthy foals, which is in accordance with the findings of Harvey et al. (1984). While WBC, lymphocytes, increased with age, the N/L-ratio decreased. Interestingly, counts of monocytes yielded highest values in 4 to 19 days old foals in the present study in contrast to the results of Grondin and Dewitt (2010) presenting highest counts in one-day old foals. Similar to the results of Harvey et al. (1984) the foals of the present study presented neutrophil counts in a low-high-low-high pattern. In healthy foals, the lymphocyte percentage rose while the foals grew, similar to lymphocyte counts. Neutrophil percentage, on the other hand, decreased with

the age. Band and juvenile neutrophil could not be observed in healthy foals. Eosinophil and basophil were rarely seen in the blood smears and small amount of monocytes could be almost equally observed in all four age groups (Grondin and Dewitt, 2010).

Eosinophil counts started to rise by twenty days old healthy foals and lead to the highest counts in the foals of the eldest age group, which might be a consequence of the development of the immune system of the foals starting to produce antibodies by themselves (Young and Meadows, 2010). Other researchers also reported increasing eosinophil counts in foals with age (Harvey et al., 1984; Grondin and Dewitt, 2010). The eosinophil counts in the majority of the ill hospitalized foals were also very low, except for one foal with oesophageal obstruction. In a retrospective study on oesophageal biopsy evaluation in children with dysphagia, oesophageal eosinophilia was frequently found in children with food bolus obstruction requiring endoscopic removal (Cheung et al., 2003), which was exactly the referral reason of this foal. Eosinophils play an important role in mucosal immunity of the GI tract and responses to the contact with different kinds of antigens (Young and Meadows, 2010). The bolus that leaded to the obstruction and/or any possible antigens that came along with the bolus could trigger eosinophilic reactions, this could probably explain the extreme high eosinophil number of this foal at admission (3.2 x 10^9/ L). However, since the foal had a significant lower number of eosinophils (0.41 x 10^9/ L) twenty hours after admission, an incorrect measurement of the first sample cannot be totally ruled out.

Young healthy foals (day 1 to day 19) had also almost undetectable basophil counts, while the foals of both elder age groups exhibited higher numbers. However, the median values and measurement ranges of all four age groups were within reference values presented by Harvey et al. in 1984.

The N/L-ratio as a criterion for the maturity of the foals is according to Jeffcott et al. (1982), Rossdale et al. (1982) and Rossdale et al. (1984), who took samples from foals within two to thirty hours after parturition, of clinical importance for the examination of neonatal foals. In the present study, all healthy foals exhibited an N/L-ratio exceeding one with a median value of two, which was in accordance with the results of Jeffcott et al. (1982), Rossdale et al. (1982) and Rossdale et al. (1984).

The RBC median values in all four age groups of healthy foals ranged from 9-10 x 10^{12}/ L and were not significantly different among the four age groups. The 1-3 days old, healthy foals had the highest median PCV value and the widest PCV range, but differences were not significant among the four age groups of healthy foals. Similarly, in our study plasma concentrations of platelets of healthy foals did not differ among age groups and were within the range as reported by Harvey and his co-workers (1984).

In general, most of the ill hospitalized foals had also very low basophil counts, with the both elder age groups presenting higher counts. Two very high values of basophil counts (0.18 x 10^9/ L; 0.07 x 10^9/ L) of the 20 to 59 days old foals at time points 0 and 24 hours were attributed to one foal, which had oesophageal obstruction. Since basophils serve as mediators during allergic responses (Pohlman, 2010) and since this particular foal also had increased eosinophil counts, we supposed that it went through a certain degree of allergic reaction.

In ill foals, only the parameters WBC and lymphocyte counts changed during the duration of hospitalization. All other haematological parameters did not reveal significant differences among time points during hospitalization. The median N/L-ratio values within age groups of ill hospitalized foals at time points zero, twelve and twenty-four hours after admission were above two, however, a much wider range was observed compared with healthy foals. The lowest value at admission was seen in a premature foal, which was born through Caesarean section. The lowest N/L-ratio at twelve hours after admission was attributed to a foal with colon obstipation after surgery, its neutrophil counts dropped from 4.69 to 1.27 x 10^9/L. At time point twenty-four hours, another foal with maladaptation followed by red bag parturition had the lowest level of N/L-ratio, because its neutrophil counts dropped from 2.5 to 0.67 x 10^9/L. Further, N/L-ratios above four were attributed to foals with elevated neutrophil counts. Since neutrocytophilia might also make N/L-ratios look normal at the first sight, this has to be taken into account when using N/L-ratio for the assessment of prematurity.

The lowest RBC value in ill hospitalized foals was seen in one foal with suspected isoerythrolysis. This foals was however, appeared to be healthy through out the hospitalization except for the low RBC value. All other ill foals had a RBC value very close to reference values (Knottenbelt et al., 2004a) and showed no statistical differences between healthy and ill foals within same age groups. Similarly no differences between ill foals at different time points within the same age groups were observed. The highest PCV values in ill

foals were seen mostly in cases of dehydration. After treatments the values normalised. The lowest PCV values were attributed to one foal with suspected isoerythrolysis. The lowest concentration of platelets was noticed in a foal with perforating joint injury, septic joint and multiple skin wounds. Another very low value at 48 hours after admission was attributed to a foal with umbilical fistula. Other than these two foals, the ill hospitalized foals had platelets values above 100, most of them were in accordance with the reference range and the value as stated in the report from Harvey and his co-workers (Harvey et al., 1984).

The lymphocyte and neutrophil percentages in ill hospitalized foals were very variable because of different aetiology of the underlying diseases. Band neutrophils were seen more frequently in ill hospitalized foals, but no juvenile neutrophils were observed. Similar to the healthy foals, eosinophils and basophils were only seldom seen in blood smears of ill foals. Monocytes were more frequently observed in ill hospitalized foals but this wasn't statistically obvious, probably because of the variety of diseases presented.

Basically almost all the healthy foals did not have any neutrophil toxic changes at their four time sampling points with the exception of two foals: one had a shallow skin injury at the age of 28 days and another one exhibiting bilateral conjunctivitis at the age of 88 days. Other clinical and haematological parameters of both foals did not differ from the values of their age groups which. The toxic change they had was classified as mild vacuolization of cytoplasm in 1 of 50 and 2 of 50 neutrophils, respectively. Except from these findings they did not show any abnormalities in all other examinations.

Although not statistically different from the healthy foals, toxic changes were more frequently observed in the majority of the blood smears from ill hospitalized foals in contrast to healthy foals ($p = 0.05$; 83 in 86 and 2 in 38 blood smear samples in ill hospitalized and healthy foals, respectively). We did not specifically evaluate the relationship between neutrophil counts and toxic change scores, but we found that foals with a toxic changes score equal or more than one frequently also had neutrophilia ($>12 \times 10^9$/L) or neutropenia ($<5 \times 10^9$/L). From our data we are able to suggest, that the observation of any abnormalities in neutrophils should be interpreted as an alert for further or more detailed examination of the foal. However, this parameter should only support the CBC evaluation, and one should never use it alone for diagnosis or major treatment decisions.

5.3 Other blood parameters

The total protein values of healthy foals were in a comparable range to the report of Harvey et al. (1984) by using the same method of measurement. Although not correlated, ill hospitalized foals with a total protein value less than 5 g/dL frequently had also reduced IgG values (<800 mg/dL) in our study. Foals with GI-track problems (e.g. meconium obstipation, colon obstipation) had also lower total protein values.

The IgG values in healthy foals were only evaluated at the first sampling at the age of one to three days, because IgG values are known to change with age as foals are going to start their own immune system (Jeffcott, 1974; Knottenbelt et al., 2004a). Since we performed IgG value measurements in hospitalized ill foals just at the time point 0, we compared the results between healthy and ill hospitalized foals in this group only. All the healthy foals had an IgG value equal or above 800 mg/dL, three out of eight ill hospitalized foals exhibited an IgG value less than 400 mg/dL. The latter foals received plasma transfusion, which is why their values were then equal or greater than 800 mg/dL forty-eight hours post transfusion. Two of these three foals were discharged and one of them – the foal with maladaptation followed the red bag parturition – was euthanized based on the poor prognosis.

The blood glucose values in the healthy foals of our study seemed to decrease significantly with age. Bauer (1990) reported glucose values showing a wider reference range in healthy foals from birth to six months age compared to our results. Although the sampling time points in the study of Bauer did not match exactly with those in our study, measures are comparable.

The one to three days old ill hospitalized foals in our study had significant lower blood glucose values at admission compared to their healthy counterparts. According to the reference range (121-223 mg/dL) given by Bauer (1990) for this age group, these foals were hypoglycaemic. In our study ill hospitalized foals exhibiting a glucose value below 121 mg/dL at time point 0 were not nursed properly by different causes. Hypoglycaemia was reported in foals with prematurity, sepsis, insufficient milk intake, reduced gluconeogenesis or other status with increased metabolic demands (Palmer, 2006). Because we did not do bacterial culture or sepsis score in our study group, we could not say for sure if one of those foals was septic. But, according to clinical pathological values, one can suspect that some of them went through a certain level of infective process.

In age groups 4-19 and 60-130 days the blood glucose values of ill hospitalized foals at admission were distinctly higher compared to the values of healthy foals. Foals with sepsis and prematurity may also develop hyperglycaemia (Palmer, 2006). In adult horses, increases of blood glucose values are frequently noticed in cases of acute abdominal diseases (Hollis et al., 2007). Ill hospitalized foals in the age groups of 4-19 and 60-130 days with a glucose value exceeding 156 mg/dL and 121 mg/dL, respectively, were frequently associated with infection.

Hollis et al. (2008a) determined the blood glucose concentrations in 515 critically ill neonatal foals. They concluded from their results, that foals with glucose values <75.6 mg/dL were associated with a worse prognosis for survival to discharge. By application of the same cut off value, we had one premature foal which died, one maladaptation foal which was euthanized based on its poor prognosis and a foal with FPTI but discharged after twenty-four hours based on its owners will, which is why we did not get a follow-up.

In the same report (Hollis et al., 2008a), extreme hyperglycaemia (blood glucose concentration >180 mg/dL) was also associated with a worse prognosis to hospital discharge. This was inconsistent to the observation in our study group. All ill hospitalized foals with glucose values greater that 180 mg/dL survived to discharge. The highest blood glucose value was detected in a Shetland pony with FPTI and bilateral patella luxation. This foal had some laboratory examination abnormalities but appeared clinically sound and vivid during hospitalization and recovered very well after knee surgery.

What we have to point out here is, that four different clinics/institutes were involved in the report of Hollis and co-workers (2008a) and each one seemed to have its own method for the measurement of blood glucose values. Further, only foals under seven days old were included in the study. The reference range (76-131 mg/dL) given was much lower compared to the report of Bauer (1990) and the authors did not specify how the reference range was created. However, younger foals presented with lower blood glucose values most likely are associated with insufficient milk uptake.

Castagnetti et al. (2010) reported a significant higher blood lactate in foals younger than twenty-four hours. The values significantly decreased during the first twenty-four hours and then remained stable. Therefore, they took the cut-off value measured at twenty-four hours *post natum* (2.1 mmol/L) as a reference value. The healthy foals in our study were elder than

24 hours when we took the first sample from them, and our results (median: 2.2 mmol/L) were comparable to the ones Castagnetti et al. (2010) reported. Further, our measurement values were also within the range Castagnetti and co-workers had observed. They collected blood samples from foals from right after birth until the age of three days at twelve hours intervals. After twenty-four hours, the values remained at the same level, which is in accordance with our findings.

Blood lactate values from 0 to 36 hours after admission differed between surviving and non-surviving hospitalized neonatal foals up to seven days age (Castagnetti et al., 2010). High lactate concentrations at admission (>5 mmol/L) were used to predict non-survival. Corley et al. (2005) reported similar observations concerning arterial lactate values in critically ill hospitalized foals younger than seven days. Foals survived to hospital discharge had significantly lower lactate concentrations at admission and at the 18-36 hours period as well. In a report on arterial blood lactate values of adult human patients suffering septic shock (Bakker et al., 1996), not only the initial lactate values were important, but also the decrease of lactate concentrations within the first twenty-four hours of hospitalization and the duration of hyperlactaemia were important. In our study, lactate values of ill hospitalized foals at admission did not differ from healthy foals, which could be due to the diversity of diseases of ill foals. Two of the ill foals of our study had blood lactate concentrations exceeding the cut-off level (5 mmol/L) given by Castagnetti et. al (2010) and Corley et al. (2005) during the first two days of hospitalization. Of these two foals, one died and the other one was euthanized because of the poor prognosis. The agreement of our results with the ones of Castagnetti et. al (2010) and Corley et al. (2005) clearly demonstrates the prognostic importance of the evaluation of blood lactate concentrations repeatedly during hospitalization.

Gossett and French (1983) investigated the relationship between age and anion gap values in ten Quarter horse foals. Younger foals – especially the 2-3 weeks old ones – had higher anion gap values compared with the two-year-old foals. We obtained a similar pattern, although the values of both age groups did not differ significantly, which might be a consequence of the different grouping of the foals in both studies.

The use of anion gap as a single prognostic indicator for prediction of the probability of survival was demonstrated in reports on horses with abdominal pain (Bristol, 1982) and foals of an equine neonatal intensive care unit (Saulez et al., 2007). An elevated anion gap value

was strongly associated with a worse prognosis in both reports. We compared our results with the values from Hoffman et al. (1992), who also evaluated the anion gap from venous samples and used the same formula for calculation. In his report, anion gap <12.7 mmol/L (12.7 mEq/L) at admission was associated with higher survival rates, while anion gap values >24 mmol/L (24 mEq/L) with non-survival. Many of our ill hospitalized foals with anion gap values exceeding 12.7 mmol/L but less than 24 mmol/L at admission were discharged. An anion gap value >24 mmol/L only was seen at time points twelve and twenty-four hours after admission in a premature foal, which died during its hospitalization.

Differences between the findings of our study and the results of Hoffman et al. (1992) might be attributed to differences concerning the selection criteria of the foals. Hoffman and the co-workers (1992) included foals younger than ten days, which required level-two intensive care (e.g. intravenous crystalloid, plasma and antimicrobials, oxygen supplementation, blood gas monitoring, radiography). Since our laboratory purchased a new analyser for blood gas and electrolytes in late 2011, we were able to carry out the anion gap estimation since then, which is why our anion gap data are quite limited. An increased anion gap value is frequently associated with metabolic acidosis (Oh and Carroli, 1977), based on our experience, an elevated anion gap value at any time point of hospitalization should draw the attention on further diagnosis and medical intervention.

5.4 Plasma haptoglobin

So far we are only aware of one report that investigated haptoglobin values very detailed in both, foals and adults horses (Taira et al., 1992). It shows that haptoglobin values remained higher in foals from delivery until the age of twelve months compared with horses elder than eighteen months (7.05 ± 2.07 vs. 5.29 ± 2.34 mg/mL). Whether haptoglobin values differed within each age group was not specified. In the healthy foals of our study, haptoglobin concentrations were not statistically different among the four age groups, ranging from 0.47 to 1.41 mg/ml. In general, haptoglobin values were much lower compared with those of Taira and co-workers study, which might be due to different assays used: a commercial ELISA kit in our study and a single radial immunodiffusion method in the study of Taira and co-workers. The values obtained by Pollock et al. (2005) in thoroughbred horses using a commercial colorimetric method on serum samples showed better comparison to our

measures. Pollock et al. (2005) also observed a decrease of haptoglobin concentrations among foals (< one year old), young adults (one to four years old) and older adults (> four years old). The mean value for foals was 2.2 mg/mL ranging from 1.2 mg/mL - 2.7 mg/mL.

Significantly increased haptoglobin values were seen in foals with acute inflammation (Taira et al., 1992), after surgery (Pollock et al., 2005) and even in calves with chronic respiratory diseases (Tóthová et al., 2010). The highest haptoglobin values were obtained from a neonatal foal twenty-four hours after plasma transfusion and an elder foal with osteomyelitis. Hemolysis proved to be a cause for decreased haptoglobin values (Allen and Archer, 1971); the lowest haptoglobin values were measured in the foal with suspected neonatal isoerythrolysis.

The only haptoglobin value significant difference between the samples of healthy foals and ill hospitalized foal at admission was seen in age group A foals, with the ill ones had a much lower range. Tóthová et al. (2010**)** reported that sick calves with higher haptoglobin values also had significantly higher mean total protein and immunoglobulin concentrations. Not all but many of our neonatal ill hospitalized foals with an initial IgG value less than 800 mg/dL had also lower haptoglobin, which is contrary to the results of Tóthová et al. As much as we know, the relationship between values of IgG and haptoglobin in neonatal foals was not reported elsewhere, there fore, whether decreased IgG values also have effects on haptoglobin should be further investigated.

Allen and Archer (1971) used a haemoglobin binding capacity method for the determination of haptoglobin concentrations in equine serum. They revealed different hemoglobin values between eight crossbred pony foals and six Thoroughbred-foals. Most of our healthy foals were Haflinger or draft horse crossbreds, while the Warmbloods dominated the ill hospitalized foal population. Because of this, we are not able to rule out a predisposed bias of our results due to different haptoglobin concentrations in different horse breeds.

Somehow, we expected higher plasma haptoglobin values in foals with obvious infection or inflammatory processes in our study, but their values were similar to or even lower than those of healthy foals. Controversial values were even seen in one foal, which received plasma transfusion (0.98 mg/mL at admission), which could be due to the limitation of our assay or the long duration of storage of the samples. However, having an in-house reference value of plasma haptoglobin concentration seems to be important for each laboratory.

5.5 Plasma MPO activity

The function of neutrophils in healthy foals was investigated over decades and reported to be lower than in adult individuals and to be changing with age (Bernoco et al., 1987; Wichtel et al., 1991; McTaggart et al., 2001). Christensen and Rothstein (1985) noticed an age related pattern of MPO concentrations in neutrophils in developing rats; MPXI values carried out in infants also revealed significant age variability (Nikulshin et al., 2015). Since MPO is mostly present in neutrophils and released into the phagosome during phagocytosis, which is the primary function of neutrophils (Klebanoff, 1999), it is quite reasonable to suppose that MPO values of foals would differ from adult horses and might change with age. A report on this topic is according to our knowledge, not yet published elsewhere.

Activity of MPO was measured from serum samples in healthy adult horses with the same in-house method (Fietz, 2008a) ranging from slightly above 0 mU/µL to below 25 mU/µL with a median value around 15 mU/µL. In the present study, highest plasma MPO activities were seen in the 1-3 days age group of healthy foals ranging from 13.96 to 28.53 mU/µL with a median value of 19.33 mU/µL. The 20-59 days old foals had similar measurements with a narrower range, while foals of the age groups 4-19 and 60-130 days had significantly lower values (median: 16 mU/µL; range: 8 to 25 mU/µL). These values are within the range of adult horses as reported by Fietz (2008a). An actual study about MPO activity with exactly the same method and same type of samples was not found elsewhere. We compared serum and plasma samples from coinciding time point of a single foal and obtained lower values from serum samples. Since the values derived from plasma samples showed better kinetic curves, we decided to use plasma samples for the evaluation of MPO activity. This could be possibly the cause for the observation that the MPO activity values of the foals in our study were not lower compared with the values in adult horses derived from serum samples.

El-Gammasy et al. (2015) reported a positive correlation between serum MPO activity and WBC count and neutrophil count in human neonates with sepsis. Intracellular neutrophil MPO concentration (10^{-7}/neutrophil) decreased rapidly during the experimental lethal bacterial infection in rats even before neutropenia occurred; during the sublethal experiment, the values however, were double as high as in non-inoculated control animals after forty-eight hours (Christensen and Rothstein, 1985). Intracellular MPO activity was measured with the haematology analyser ADVIA 120 in order to compare the MPXI values among healthy

horses, horses with local inflammation, with sepsis and with SIRS (Schwarz et al., 2011). Horses with SIRS had significantly lowest MPXI values followed by horses with sepsis, while horses with local inflammation had the highest MPXI value and did not differ from that of healthy horses. This study clearly showed that MPXI values are negatively correlated with the severity of disease.

In our study, plasma MPO activity did not differ between healthy and ill, hospitalized foals, among time points and age groups. However, we noticed a much wider range of MPO values in ill individuals. The highest plasma MPO activity was seen in a premature foal, which was born through C-section and died after five days of hospitalization, at time point 0 (118.32 mU/µL). The second highest value (112.77 mU/µL) was obtained from a foal with suspected herbal intoxication at admission, whose MPO values at twenty-four and forty-eight hours were within the range of healthy foals. Another foal with weak ability to suckle and FTPI (initial MPO value: 109.18 mU/µL) was discharged after twelve hours of hospitalization based on the owner's will. According to the owner, this foal survived for at least for one year after being discharged, but it exhibited some developmental restrictions. Three of four foals, which died or were euthanized in our study, had initial plasma MPO values exceeding 40 mU/µL (one missing data was from a foal with colon rupture, whose MPO value was not detectable after at least three attempts). However, whether plasma MPO activity values at admission, nor the values at forty-eight hours seem to be predicative for survival. In our study, sick foals with elevated plasma MPO activities (>40 mU/µL) at admission also had an abnormal neutrophil counts in terms of increased or decreased concentration of neutrophils. Those cases had diagnosis like herbal intoxication, subepiglottic cyst, suckling weakness, osteomyelitis, prematurity and maladaptation.

Interestingly, many of the foals diagnosed with hypogammaglobulinaemia without further systemic complains, obstipation/ meconium obstipation, diarrhoea, omphalitis and umbilical hernia, did not show elevated plasma MPO activities (>40 mU/µL), although the neutrophil concentration was either increased or decreased. The MPO value of the herbal intoxicated foal returned to the range of healthy foals (16,47 mU/µL) after twenty-four hours, although the neutrophil counts was still elevated. Based on those facts that we observed, we could presume that plasma MPO value provides an extra information especially to foals with localised lesion and hypogammaglobulinaemia about whether another potential threat exists or not. And

plasma MPO values might therefore, be different, based on types and the severity of the disease or inflammation.

Because, the number of sick animals in our study was small at the one hand and the causes for presentation at the clinic were manifold on the other hand, statistical analysis on the relationship between plasma MPO activity values and grouping categories was limited. An *in vitro* research revealed that, even different stimuli conditions had effects on the release of MPO by equine neutrophils (Ceusters et al., 2012). In the sublethal animal model study from Christensen and Rothstein (1985), neutrophil MPO concentrations reached the highest value at forty-eight hours after inoculation and decreased gradually from then until ninety-six hours. Concluded all these points together, in order to be able to use plasma MPO activity values as a single prognostic or predictive parameter, we need definitely further studies on foals with more clearly defined inclusion criterions, and, if possible, longer duration of sampling. Nevertheless, our study revealed that the age of the foals has a possible influence on plasma MPO activities; plasma MPO activity values were distinctly increased in some ill foals possibly reflecting the types and the severity of inflammation, which might justify further studies on this topic.

5.6 Conclusion

We found a certain level of consistency to the literature in the majority of our clinical examinations, haematological and other blood parameters in the present study. Ill hospitalized foals had a much wider range of measures compared with their healthy counterparts, which might be the consequence of the variety of diseases included in this study.

The evaluation of plasma haptoglobin indicates the importance of an in-house reference range. Furthermore, the selection criteria for the animals for establishing reference ranges should be properly defined and the population should be bigger. The possible effect of decreased IgG values on haptoglobin concentrations should be further investigated.

In the present study, plasma MPO activity was influenced by the age of the foal. Although we could not use this single parameter as a predictive value in our project, many of the sick foals with elevated plasma MPO activity (>40 mU/μL) also had an abnormal neutrophil count and were suffering from systemic diseases. Sick foals with increased or decreased neutrophil concentrations but without elevated plasma MPO activity had mostly localised lesions or non-complicated complains e.g. hypogammaglobulinaemia. Therefore, we suppose that plasma MPO activity may differ in foals based on the type and severity of the inflammatory process.

6. Summary

Activity of plasma myeloperoxidase (MPO) in healthy and sick foals

Young foals are almost fully developed from the moment of birth, but they also have to go through a lot of transforming periods and their bodies have to adapt to the outer environment constantly. The direct evidences of the adaptation process are the variations of the values of physiological and other laboratory parameters compared to those of the adult individuals.

In equine neonatal medicine, lots of efforts were put into research in order to define the physiological and pathological status, normal and abnormal values of parameters, more accurate and rapid methods as well as diagnostic and prognostic indicators for the outcome of treatments. Myeloperoxidase (MPO) indicating the activity of neutrophils is already routinely used in human medicine and measured by the CBC automatic analyser. This method is also applied in some equine neonatal intensive care units in the world. Other techniques evaluating different sample types and under different physiological or disease status were carried out, mostly in adult horses. Since the majority of the reports showed that MPO is a promising indicator of inflammatory processes, it seemed mandatory to investigate its role in foals. Therefore, we wanted to find answers to the following questions: 1. What is the relationship between MPO activity levels and the age of the foals? 2. Are there any differences of MPO activity patterns between healthy and sick foals within age groups? 3. Do MPO activities change during treatments?

Thirteen healthy foals and thirty ill hospitalized foals were included in this study. They were allocated to four subgroups based on their age at the time of sampling. Samples of healthy foals were taken at the age of 1-3, 4-19, 20-59 and 60-130 days. From ill hospitalized foals samples were collected at 0, 12, 24 and 48 hours after admission if possible. Based on the kinetic test, plasma samples were applied for the MPO photometric activity assay in the present study.

There were statistical differences of plasma MPO activities between different age groups in healthy foals. We therefore inferred that age plays an important role in plasma MPO activity. The comparisons of plasma MPO activity between healthy and sick foals within age groups did not differ in all four groups. However, sick foals had a much wider range of plasma MPO activities; values as much as four times of the highest measurement in healthy foals were seen

in some sick individuals. Unfortunately we could not carry out the statistical comparison between plasma MPO activities and the severity of inflammatory status because of our limited but also multifarious study population. However, foals with elevated plasma MPO activities often also had abnormal WBC and/or neutrophil counts (Leukocytosis/leukopenia; neutrophilia/neutropenia). The majority of the ill hospitalized foals were discharged; yet, each one of them had a different duration of recovery and hospitalization. In the present study we only evaluated the plasma MPO activities until 48 hours after admission, which is why we have no data on complete duration of recovery.

In this study we demonstrated a suitable method of evaluating plasma MPO activities in foals and we were able to demonstrate that plasma MPO activities were affected by age. Sick foals tended to have a wider range of plasma MPO activities; most of the foals with increased plasma MPO activities also had abnormal leukocyte and/or neutrophil counts, which indicated an inflammatory response. Moreover, foals with an increased or decreased neutrophil concentration but normal plasma MPO activity demonstrated a possible correlation between plasma MPO activity depending on the type and/or severity of inflammation. Further investigations with clearly defined inclusion criteria are necessary to answer the questions about the relationships between plasma MPO activity and the severity of the inflammatory status and the response to the treatments.

7. Zusammenfassung

Plasma Myeloperoxidase (MPO)-Aktivität bei gesunden und kranken Fohlen

Fohlen sind vom Moment der Geburt an weit entwickelt und müssen sich an die äußeren Bedingungen anpassen. Dieser Prozess spiegelt sich in der Veränderung der klinischen und Laborparameter wider.

In der Fohlenmedizin wurde viel Mühe investiert, um folgende Punkte besser zu definieren bzw. zu optimieren: Abgrenzung der physiologischen und pathologischen Zustände, normale und abnormale Werte der Parameter, Entwicklung genauerer und schnellerer Messmethoden sowie die Identifikation aussagekräftiger diagnostischer und prognostischer Indikatoren. In diesem Sinn stellt sich die Myeloperoxidase (MPO) als interessanter Indikator der Aktivität der Neutrophilen dar, da sie bereits routinemäßig in der Humanmedizin eingesetzt wird.

Ziel unserer Studie war herauszufinden: 1. Der Zusammenhang zwischen der MPO-Aktivität im Plasma und dem Alter der Fohlen. 2. Der Vergleich der MPO-Aktivität von gesunden und kranken Fohlen gleichen Alters. 3. Der Verlauf der Plasma MPO-Aktivität innerhalb der ersten 48 Stunden nach Einlieferung. Insgesamt wurden dreizehn gesunde Fohlen und dreißig kranke stationierte Fohlen in diese Studie aufgenommen. Von den gesunden Fohlen wurden Proben in allen vier Altersgruppen (1-3; 4-19; 20-59 und 60-130 Tage) entnommen. Die kranken Fohlen wurden einer dieser vier Altersgruppen zugeordnet und Proben wenn möglich jeweils bei der Aufnahme sowie 12, 24 und 48 Stunden danach gewonnen.

Gesunde Fohlen verschiedener Altersgruppen wiesen unterschiedliche MPO-Aktivitäten auf. Im Gegensatz dazu gab es keine Unterschiede zwischen gesunden und kranken Fohlen vergleichbaren Alters. Allerdings zeigten die kranken Fohlen einen viel breiteren Messbereich der Plasma-MPO-Aktivitäten. So waren bei manchen kranken Individuen die Messwerte fast vier Mal so hoch, wie die höchsten Messwerte der gesunden Tiere. Aufgrund der geringen Zahl an Patienten, welche auch sehr heterogene Erkrankungen aufwiesen, war es nicht möglich, einen Zusammenhang zwischen einzelnen Krankheitsbildern und MPO-Aktivitätswerten festzustellen. Allerdings wiesen die Fohlen mit erhöhten Plasma-MPO-Werten häufig auch Veränderungen der Leukozyten- und Neutrophilenzahl auf (Leukozytose/Leukopenie; Neutrophilie/Neutropenie). Der Großteil der kranken Fohlen

wurde nach unterschiedlicher Krankheitsdauer und unterschiedlichen Klinikaufenthalt entlassen. In dieser Studie haben wir die Plasma-MPO-Aktivität nur bis zu 48 Stunden nach der Aufnahme gemessen, weshalb deren Aussagekraft hinsichtlich des Ansprechens der Therapie eingeschränkt ist. Weitere Untersuchungen mit größerer Probandenanzahl und engeren Einschlusskriterien sind nötig, um den Zusammenhang zwischen der Plasma-MPO-Aktivität und Entzündungsprozessen sowie das Ansprechen auf die Therapie zu ermitteln.

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10. Appendixes

10.1 Appendex I: Abbreviations

ACTH	adrenocorticotrophic hormone
AI	artificial insemination
APPs	acute-phase proteins
APR	acute-phase response
BAS	basophil count
BAS%	bsdiphil percentage
°C	degree Celsius
CBC	complete blood count
Cl	chloride
cm	centimetre
CV	coefficient of variation
DIC	disseminated intravascular coagulation
dL	decilitre
DMSO	dimethyl sulfoxide
EDTA	ethylenediaminetetraacetic acid
EHV	*equine* herpesvirus
ELISA	enzyme-linked immunosorbent assay
EOS	eosinophil count
EOS%	eosinophil percentage
FPT	failure of passive transfer
FPTI	failure of passive transfer of immunity
GI tract	gastrointestinal tract
H_2O_2	hydrogen peroxide
HCO_3	bicarbonate
HCT	haematocrit
HGB	hemoglobin
HOCl	hypochlorous acid
HRP	horseradish peroxidase
IgG	immunoglobulin-G
K	potassium
L	litre
LYM	lymphocytes count
LYM%	lymphocates percentage
SAA	serum amyloid A
SIRS	systemic inflammatory response syndrome
max	maximum
µM	micromolar
mM	millimolar
M	molar
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
mg	milligram
min	minimum

µL	microlitre
mL	millilitre
MON	monocytes count
MON%	monocytes percentage
MPO	myeloperoxidase
MPV	meanplatelet volume
MPXI	myeloperoxidase index
Na	sodium
NE	neonatal encephalopathy
NEU	neutrophil count
NEU%	neutrophil percentage
NI	neonatal isoerythrolysis
N:L ratio	neutrophil-to-lymphocyte ratio
nm	nanometre
OD	optical density
PCT	platelet percentage
PCV	packed cell volume
PDWc	platelet distribution width
PLT	platelet cout
RBC	red blood cell
rcf	relative centrifugal force
RDWc	red cell distribution width
RIA	radio-immunoassay
RID	radial immunodiffusion
RNA	ribonucleic acid
rpm	revolutions per minute
SEFED	specific immunological extraction followed by enzymatic detection
SIRD	single radial immunodiffusion
SIRS	systemic inflammatory response syndrome
TCO_2	bicatbonate
TMB	3,3', 5,5'-tetramethylbezidine
WBC	total white blood cell count

10.2 Appendix II: Signalment and clinical history chart

Clinic no.
Date of admission: / / .
Lab no.

A. Signalement and history	
Name of foal filly / colt	Name of dam
Date of birth	Time of birth
Age at time of admission	Time of admission
Breed	Color of fur
Weight at admission	
Prior treatment: ☐ No ☐ Unknown ☐ Yes (If yes state what:)	
Complains/ primary diagnose	
Was the transportation longer than 3 hours? ☐ Yes ☐ No	

- Type of parturition:

B. Birth information

☐ spontanious
☐ assisted
☐ Caesarean section

- Time of sternal recumbency:_____
- Time and method of tearing the umbilical cord:_____
  ☐ by rising of the dam
  ☐ by rising from the foal
  ☐ manual (placenta passed before tearing umbilical cord)
- Time of first standing attempt:_____
- Assistance needed by standing-up: ☐ No ☐ Yes
- Time of first stand-up:_____p. n.
- Time of first searching for udder:_____p. n.
- Time and method of first suckle:_____p. n.
  ☐ by itself
  ☐ with assistance (foal was guided to udder)
  ☐ with feeding bottle:_____ml
  ☐ through nasogastric tube:_____ml
  ☐ colostrum from the dam
  ☐ colostrum bank
- Time of first urination:_____p. n.
- Time when passed first meconium:_____p. n.
  Amount:_____
- Was navel treated? ☐ No ☐ Unknown ☐ Yes _____
- Antibiotics administered ☐ No ☐ Unknown ☐ Yes _____ml/ mg
- When did placenta pass _____p. n. or time:_____.
- Was placenta examined? ☐ No ☐ Unknown ☐ Yes
- Any morphological or pathological alterations of placenta?

☐ No ☐ Unknown ☐ Yes _____

C. Information of the dam

- Date of birth: _____/_____/_____/(date/month/year) or _____ year-old

- Breed: _____
- Latest dates of receiving vaccinations:

EHV:	☐ Yes _____(date)	☐ No	☐ Unknown
Tetanus:	☐ Yes _____(date)	☐ No	☐ Unknown
Influenza:	☐ Yes _____(date)	☐ No	☐ Unknown

■ *Present foal:*

- Date of last service:_____/_____/_____/
  or length of gestation:_____ days
  ☐ Unknown

- Milk leakage during pregnancy: ☐ No ☐ Unknown ☐ Yes _____

- Diseases during pregnancy: ☐ No ☐ Unknown ☐ Yes _____

- Vaginal discharge before parturition: ☐ No ☐ Unknown ☐ Yes _____

10.3 Appendix III: Clinical examination items

Examination item	Method that was performed	Findings
1. General behaviour	Judging by the clinician while the foal presented and before any other examination was performed.	-**bright and alert** -**faint** (apathetic, looks sleepy) -**stupor** (the animal may lei down and be very sleepy, but still react to the pain.) -**comatose** (the animal is difficult to be awaked, reaction to the pain can be delayed or absent.) -**mild/ moderate increased reactive** (the animal show scare-reaction/ tries to kit, bite, lei down, roll or even flight) -**hypotonic** (the animal can not bear its own weight by itself) -**seizures/ convulsion** -**colic** *Status other than listed here was extra noted
2. Suckle reflex	Examined by placing gloved fingers in foals´ mouths, or judging from suckling ability while foal was nursing.	-**very good/ nursing by itself** (continuously and strong suckle) -**good** (strong suckle, but might stop after few suckles) -**weak** -**absent**
3. Heart rate	The clinician held a stethoscope against the left chest of the foal, at the space of ventral part of 3rd to 5th rib behind the elbow joint, and the height between shoulder and elbow joints.	beats per minute
4. Respiratory rate	Calculated by watching the foal breathing from a distance without any fixation, before any other examination was performed.	breaths per minute

10.3 Appendix III: Clinical examination items: Continued

Examination item	Method that was performed	Findings
5. Auscultation of the heart	This was done at the same time that heart rate was calculated.	**-no any abnormal heart sound** **-any abnormalities of heart sound**
6. Auscultation of lungs	The clinician held a stethoscope against both sides of the chest of foals and auscultated different parts of lungs	**-minor/ moderate/ marked increased vesicular lung sounds** **-any abnormalities of lung sounds**
7 Rectal temperature	Measured through anus opening with a digital thermometer.	temperature in °C
8. Eyes	The eyes were examined for he following items with a natural light source: -shape -symmetry -corneal erosions or ulcer/(uveitis) -any congenital defects -presence of any abnormality of the eye or eyelids or any indication of infection	**-normal** **-any abnormalities or congenital defects**
9. Sclera	This was done by placing the forefinger on the upper eyelid and then gently pressed the eyeball; and the thumb pushed the lower eyelid downwards. Thus, the eyeball and the sclera could be well seen	**-not injected** **-mild/ moderate/ marked injected**
10. Membrane colour of eye conjunctiva and muzzle	The upper eyelid was lifted over by placing the forefinger on the upper eyelid and then gently pressed the eyeball, and the thumb pushed the lower eyelid downwards. The membrane colour of eye conjunctiva could be examined like this. Both mouth of the foal were carefully for examining membrane colour of muzzle.	**-pink** **-red** **-jaundiced** **-cyanotic** **-petechiae**

10.3 Appendix III: Clinical examination items: Continued

Examination item	Method that was performed	Findings
11. Colour of tongue	This was performed during examining membrane colour of muzzle.	**-pink** **-red** **-jaundiced** **-cyanotic**
12. Capillary refill time	After examining membrane colour of muzzle, the clinician would press a finger on gingiva for a short second and then remove the finger again. The duration of the site where the finger pressed regained its colour would be counted.	seconds until capillaries were refilled
13. Petechiae	This was performed during examining membrane colour of muzzle.	**-yes** **-no**
14. Mandibular lymph nodes	The following items were examined by carefully touching mandibular lymph nodes: -size -relocatability -pain -swelling -warm	**-normal, no swelling, not warmer the other part of the body, no pain** **-other abnormalities**
15. Auscultation of intestinal peristalsis	Gut sounds were auscultated with a stethoscope at the following four sites: both paralumbar fossae (upper left and right) and both sides of the lower abdomen behind the last rib (lower left and right).	**„-„** for no sound of movement **„+“** for reduced gut sounds **„++“** for normal gut sounds **„+++“** for increased gut sounds
16. Dehydration	Judged from the urine specific gravity by using a refractometer	**-not dehydrated** (urine specific gravity ≤1015) **-dehydrated** (urine specific gravity > 1020)
17. Distensibility of both jugular veins	By compressing a thumb on vena jugularis externa at the base of the neck. Inspect if the vene would distend after a short second.	**-distensible** **-not distensible**

10.3 Appendix III: Clinical examination items: Continued

Examination item	Method that was performed	Findings
18. Cough	Firstly observe if the animal coughs spontaneously. If not, coughing would be induced by placing a hand behind larynx or the first tracheal ring, give a firm pressure through the hand and then release the pressure suddenly.	**-spontaneous** **-inducible** **-non-inducible**
19. Size of internal umbilicus	Examined with palpation by a fresh gloved hand.	**-thumb thick** **-finger think** **-little finger thick** **-pencil thick** **-straw thick** **-string think**
20. Moistness of external umbilicus	Examined with palpation by a fresh gloved hand.	**-dry** **-mild/ moderate/ marked moist**
21. Umbilical management	Information provided from the owner, the stable staff or the clinician.	-type of medical product that was used for umbilical management was noted if it **was performed** **-was not performed** **-unkown** **-other status**
22. Joints and lameness	Palpation of palpable joints was examined for the following items while foals stand: -any deformity -swelling -warm -pain	**-normal** **-any abnormalities**
23. Pattern of faeces	Observe during examine time by the clinician.	**-meconium** **-normal yellow and soft foal faeces** **-type of diarrhea if observed** **-no feces was observed during time of examination**

10.3 Appendix III: Clinical examination items: Continued

Examination item	Method that was performed	Findings
24. Congenital deformities	Observe during examine time by the clinician.	
25. Any wound or trauma	Observe during examine time by the clinician.	
26. Any other abnormality that should be noted	Observe during examine time by the clinician.	

10.4 Appendix IV: Foal clinical examination chart

A. Signalment	
Name of foal	☐ filly ☐ colt
Name of dam	Owner´s name
Date of birth	Time of birth
Age at time of examination	Time of examination
Breed	Color of fur
Weight (Kg)	

B. General examination			
Genernal behaviour (from observation) ☐ bright and alert ☐ faint ☐ stupor ☐ comatose ☐ mild/ moderate increased reactive ☐ hypotonic ☐ seizures/ convulsion ☐ colic ☐ other status:			
Suckle reflex: ☐ very good/ suckles by itself ☐ good ☐ weak ☐ absent			
Heart rate (/min)	Pulse quality	Murmurs audible ☐ Yes ☐ No	
Rectal temperature (°C)	Respiratory rate (/min)	Lung feld *LEFT*	Lung feld *RIGHT*
Eye *LEFT*	Eye *RIGHT*	Membrane color of eye conjunctiva	Sclera injected ☐ Yes ☐ No
Color of muzzle mucus membrane	Cappilary refill time (sec)	Tongue color	
Petechiation ☐ No ☐ Yes: (state location and severtity)		Auscultation of intestinal peristalsis: *Upper left* *Upper right* *Lower left* *Lower right*	
Palpation of lymph node mandibulares		Dehydratoion ☐ No ☐ Yes	
Distensibility of jugular vein *RIGHT* ☐ distensible ☐ not distensible		Distensibility of jugular vein *LEFT* ☐ distensible ☐ not distensible	
Cough ☐ inducible ☐ non-inducible ☐ spontaneous			
Congenital defects ☐ No ☐ Yes (If yes state what):			
Umbilicus ☐ treated with: ☐ not treated ☐ unkown			
Size of internal umbilicus			
Is the external umbilicus moist? ☐ No ☐ Yes: (If yes discribe how humid)			
Palpable joints: (Discribe any abnormalities)			
Pattern of feces: (Consistency/ Colour)			
Any wound or trauma:			
Any other abnormality that should be noted:			

10.5 Appendix V: List of material

A. Hospisept®, Lysoform Dr. Hans Rosemann GmbH, Berlin, Germany.

B. 100 Sterican® (18G x 1 ½", Ø 1.20 x 40 mm), B. Braun, Melsungen, Germany.

C. 100 Sterican® (20G x 1 ½", Ø 0,90 x 40 mm), B. Braun, Melsungen, Germany.

D. Injekt® (20 mL), B. Braun, Melsungen, Germany.

E. Milacath® - Extended Use (14G x 9 cm), Mila Internationl, INC, Erlanger, KY USA

F. High Flow Extension Set (13G x 17 cm), Mila Internationl, INC, Erlanger, KY, USA.

G. Powerflex®, Anover Helthcare, Inc, Salisbury, MA, USA.

H. Optiplaste®-C, BSN medical, Hamburg, Germany.

I. Sodium Chloride 0.9%, B. Braun, Melsungen, Germany.

J. Injekt® (10 mL), B. Braun, Melsungen, Germany.

K. EDTA K (Tube 4 ml), Sarsteadt, Nuembrecht, Germany.

L. Coagulation 9 NC/2 ml (Tube 2 ml), Sarsteadt, Nuembrecht, Germany.

M. Monovette®, Sarsteadt, Nuembrecht, Germany.

N. Serum Z/ 10ml (Tube, 10 ml), Sarsteadt, Nuembrecht, Germany.

O. Floride tube, Sarsteadt, Nuembrecht, Germany.

P. Z 300, HERMLE Labortechnik GmbH, Wehingen, Germany.

Q. ROTOFIX32 A, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany.

R. Tube One® (1,5 ml Natural Flat Cap Microcentrifuge Tube), Starlab, Ahrensburg, Germany.

S. May Gruenwalds eosine-methylene blue solution modified for microscopy, Merk, Darmstadt, Germany.

T. Giemsa azur eosin methylene blue solution for microscopy, Merk, Darmstadt, Germany.

U. OLYMPUS BX 41

V. Immersion oil type-F for (fluorescence/ ordinary) microscopy, Olympus Deutschland GmbH, Hamburg, Germany.

W. Electronic memory counter, Karl Hecht Assistent GmbH, Altnau, Switzerland.

X. Snap® Foal IgG Test Kit, INDEXX GmbH, Ludwigsburg, Germany.

Y. Coagulometer nach Schnitger und Goss, Heinrich Amelung GmbH, Lemgo, Germany.

Z. Tube 3.5 mL, Sarstedt, Nuembrecht, Germany.

a. STA Diluent Buffer, Roche, Mannheim, Germany.

b. Fibrinogen a, Roche, Mannheim, Germany.

c. STA® - Owren-Koller, Stago, Duesseldorf, Germany.

d. FIBRI-PREST® Automate 2, Stago, Duesseldorf, Germany.

e. TECO® Equine Haptoglobin ELISA, TECOmedical GmbH, Bünde, Germany.

f. Bio-Rad Microplate reader, model 550, Bio-Rad laboratories GmbH, Munich, Germany.

g. Microplate Manager 5.2@2002, Bio-Rad laboratories GmbH, Munich, Germany.

h. Citrate (Sodium citrate), Jenapharm-Laborchemie Apolda, Jena, Germany.

i. Hydrogen peroxide solution, 30% (w/w), Sigma-Aldrich Chemie GmbH, Steinheim, Germany.

j. o-Dianisidine, Sigma-Aldrich Chemie GmbH, Steinheim, Germany.

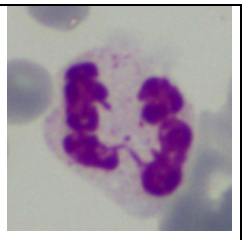
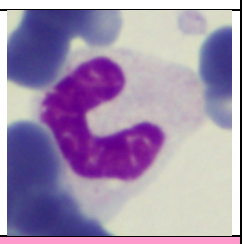
k. DMSO (Dimethyl sulfoxide for molecular biology), Carl Roth GmbH + Co. KG, Kalsruhe, Germany.

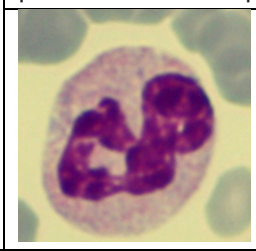
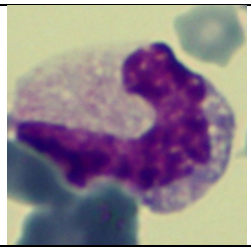
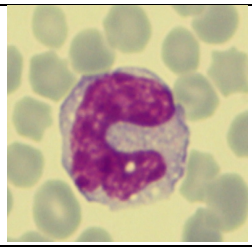
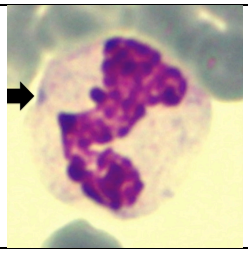
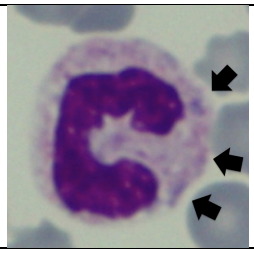
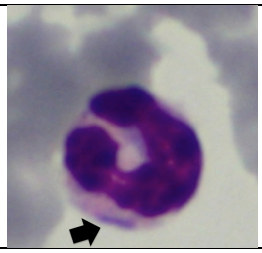
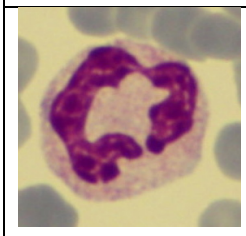
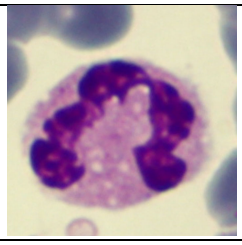
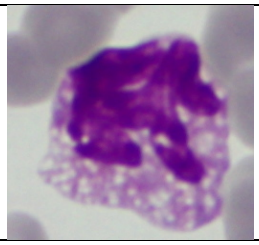
l. Triton® X-100, Ferak Berlin GmbH, Berlin, Germany.

m. Myeloperoxidase Inhibitor-I, Calbiochem, Merck KGaA, Darmstadt, Germany.

n. Mikrotestplatte unsterile, VEB MLW Polyplast Halberstadt, Halberstadt, Germany.

10.6 Appendix VI: Neutrophil toxic change categories and criteria*

Normal neutrophil	The chromatin of the nucleus is condensed (dark-staining clumped areas separated by lighter-staining areas) and segmented (lobulated) and stains blue to purple. Nuclear lobes (usually 2-4) may be joined by fine filaments or simply a narrowing of nucleus between lobes. The nuclear outline is more scalloped in horses. The background cytoplasm generally appears colorless, pale pink or faintly basophilic. Often, there would be some **purple granules** seen in neutrophils from **foals without other evidence of cytoplasmic toxicity**, the clinical significance of which is unclear.	
Band neutrophil	Band neutrophils often have an S- or U-shaped nucleus, with a less condensed chromatin pattern as compared with that of segmented neutrophils. Indentations in the **nucleus do not exceed 50% of the width, suggesting no segmentation**. In horses in which distinct nuclear lobulation is not apparent in health, band neutrophils are identified by a less condensed chromatin pattern and blunting of nuclear membrane projections[b].	

Grad / Typ	**Mild**	**Moderate**	**Marked**
Cytoplasmic basophilia	Presence of a **nonuniform** grayish to light-blue cytoplasm in the neutrophils[c]	Presence of a **uniformly** light-blue cytoplasm[c]	Presence of a **uniformly blue to dark-blue** cytoplasm[c]
			
Döhle bodies	Presence of **1–2** small Döhle bodies per cell[c]	Presence of **3–4** Döhle bodies per cell[c]	A large prominent Döhle body/ **more than 4** Döhle bodies per cell[c]
			
Cytoplasmic Vacuolisation	Loss in cytoplasm clarity and neutral-stained granules[c]	Observation of small cytoplasmic vacuoles[c]	Appearance of intense vacuolisation with grayish reticulation[c]
			

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c. Description from journal publication of Segev, G.; Klement, E.; Aroch, I.: Toxic neutrophils in cats: clinical and clinicopathologic features, and disease prevalence and outcome- a retrospective case control study. J Vet Intern Med 2006;**20**:20-31.
* The categories and criteria here are suitable for evaluating toxic change in neutrophils for sepsis score of foals. They do not include all neutrophil cytoplasmic toxic changes, granules and inclusion in cytoplasm or other morphological abnormalities.

§ All photographs are from foals of our study population in present project.

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12. List of publications

Pan Y-P Y, Neuhauser S, Bondzio A, Einspanier R, Handler J. **Activity of myeloperoxidase (MPO) in healthy and sick foals.** Poster presentation. 13th WEVA Congress 2013: Budapest (Hungary), 3. - 5. October

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14. Selbständigkeitserklärung

Hiermit versichere ich, Yvonne Yu-Ping Pan, die vorliegende Arbeit selbständig und nur auf Grundlage der angegebenen Hilfsmittel und Quellen verfasst zu haben.

Berlin, den 12.10.2016

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