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Occurrence of Equine Parvovirus Hepatitis in Horses in Northern Germany

Diploma Thesis

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Submitted by
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Contents

1 Introduction

Equine serum hepatitis is an acute and often fatal condition in horses, that frequently, but not solely occurs in association with inoculation of blood products. An ultimate causative agent was not determined until in 2018, a new Parvovirus of horses was identified as a possible causative agent (Divers et al. 2018). To date, the virus has been described in horses in the United States of America, Germany, Austria, China and Slovenia (Badenhorst et al. 2019, Divers et al. 2018, Lu et al. 2018, Meister, Tegtmeyer, Brüggemann et al. 2019, Vengust et al. 2020) and in horse sera from New Zealand, Canada, and South America (Meister, Tegtmeyer, Postel et al. 2019). The virus has been described to cause subclinical to clinically apparent hepatitis (Divers et al. 2018, Lu et al. 2018).

The objective of this thesis was to test the hypothesis that equine parvovirus hepatitis (EqPV-H) infection occurs in the horse population presented to the University of Veterinary Medicine of Hannover, Foundation and that evidence of EqPV-H infection can be detected in serum samples of horses housed in Germany. It was further hypothesized that a proportion of horses without clinical signs of hepatic disease are infected with the virus. The study further aimed to identify potential risk factors associated with infection, such as age, breed or sex of the horses and the location where the horses were stabled. The results shall contribute to a better understanding of the frequency of occurrence of EqPV-H in Europe, the geographical distribution in northern Germany, clinical course of infection and potential association with Theiler's disease.

1.1 Parvoviridae

1.1.1 Taxonomy

The family of *Parvoviridae* is categorized into two subfamilies: *Parvovirinae* and *Densovirinae*. *Densovirinae* only infect insects, crustaceans and echinoderms, whereas *Parvovirinae* infect mammals, birds and reptiles. The subfamily of *Parvovirinae* can then be further divided into eight genera: *Amdoparvovirus*, *Aveparvovirus*, *Bocaparvovirus*, *Copiparvovirus*, *Dependoparvovirus*, *Erythroparvovirus*, *Protoparvovirus* and *Tetraparvovirus*. The viruses of the genus *Dependoparvovirus* relay on coinfection with a helper virus such as a herpes- or adenovirus to replicate (Burrell et al. 2017). Other parvoviruses can replicate autonomously and are therefore called autonomous parvoviruses (Cotmore et al. 2019).

1.1.2 Structure of *Parvoviridae*

Parvoviridae range amongst the smallest known viruses with a diameter of only 23-28 nm. The viruses are non-enveloped and the outer structure is formed by an icosahedral capsid, which is made up of 60 copies of structure proteins, named viral protein (VP) 1-4. VP 1 is the largest protein (~80-100kD), VP2-4 are shorter molecules, sharing the same C-terminal sequence, but a different N-terminal sequence and are all encoded from the same reading frame (cap-ORF) (Kailasan et al. 2015). The composition of the variant VPs in the capsid differs by the genus of the virus (Cotmore and Tattersall 2014). The capsid is rough and provides the virus with the endurability to withstand extreme conditions, such as different pH values and great heat (Burrell et al. 2017). The virion can remain infectious for months or even years in the environment (Selbitz et al. 2015). The great durability requires a careful choice of an adequate disinfection agent for contaminated areas (Dubovi and Maclachlan 2016).

1.1.3 The genome and replication

The genome of *Parvoviridae* is rather small with only 4.500 to ~5.500 bases in length and occurs as single stranded linear DNA. Remarkable about the parvovirus' DNA is its unique shape. The middle part contains a long coding region and is framed by so called hairpin termini which can fold and unfold during replication. This dynamic process allows for rolling hairpin replication, a form of DNA-replication that is similar to the rolling circle replication (Cotmore and Tattersall 2014). The genome of *Parvoviridae* usually only encodes for 2 genes (Cotmore and Tattersall 2014). Two major open reading frames (ORF) have been detected: cap -ORF encoding for the viral proteins (VP) 1-4 which are forming the capsid and ns/rep ORF encoding for non-structural proteins (NS) 1 and 2 in autonomous parvoviruses and rep in dependoparvoviruses (Kailasan et al. 2015). Bocaparvoviruses have a third open reading frame for nucleoprotein in between the other two (Selbitz et al. 2015).

NS 1 serves as replication initiator protein and is therefore essential for the replication process. Furthermore, it functions as nickase and helicase in the replication process and also appears to have several cytopathogenic effects such as lysis, apoptosis or necrosis of cells (Kailasan et al. 2015).

Despite this dense packing of necessary proteins for replication, one important step for replication is not obtained within the virus: a mechanism that activates a polymerase (Burrell et al. 2017). The missing ability to drive cells into mitosis leaves the autonomous parvovirus ultimately dependent on the host (Cotmore et al. 2019). However, the host's polymerase will only be active during S phase of the cell cycle. During S phase the cell's DNA is replicated which

results in a doubled genome. The cell can then enter G2 phase and eventually complete mitosis. For replication, *Parvoviridae* are reliant on the host cells eventually entering S phase by themselves or S phase is initiated by a helper virus (Burrell et al. 2017). Then, they can arrest the cell in S phase and make use of the polymerase to transcript their own viral DNA (Cotmore and Tattersall 2014). The dependency on dividing cells explains why most *Parvoviridae* present a high affinity towards rapidly renewing tissues and foetal or neonatal hosts (Cotmore et al. 2019, Kailasan et al. 2015). The lacking ability of actively initiating S phase in infected cells is the likely reason for *Parvoviridae* not being oncogenic (Cotmore and Tattersall 2014).
However, some *Parvoviridae* are capable to replicate in tissue that is not characterized by fast diving cells. The human Bocavirus 1 (HBoV-1) can cause respiratory disease in children (Kesebir et al. 2006). Replication of the virus in fully differentiated, non-dividing cultured epithelial cells was documented (Deng et al. 2016, Dijkman et al. 2009). HBoV-1 has accomplished a different strategy to replicate. The virus replication is dependent on a DNA-damage-response. Instead of a cellular DNA replication polymerase, two polymerases of the Y family are involved (Deng et al. 2016). Y family polymerases are so called translesion polymerases. While other polymerases usually stop synthesizing at damaged DNA sequences, translesion polymerases are capable of synthesizing damaged DNA sequences (Waters et al. 2009). The HBoV-1 is the first known parvovirus that is able to replicate in non-dividing tissue using translesion polymerase of the Y family (Deng et al. 2016).
Parvoviridae also present a high mutation rate, which is probably a result of their unique replication strategy. It allows them to adapt to new hosts or different tissue quite rapidly (Kailasan et al. 2015).

1.1.4 Pathogenicity

Pathogenicity of *Parvoviridae* is very variable. Some *Parvoviridae*, for instance, the feline panleukopenia-virus and the canine parvovirus type 2, are highly pathogenic and can cause fatal diseases in their host species, whereas some *Parvoviridae* only cause mild symptoms or subclinical infection. The NS1 protein seems to contribute significantly to cytopathogenic effects, but many other factors are also involved. Accessory proteins and structural proteins, as well as antibody response, tissue and host tropism contribute to pathogenicity. The exact reasons why some *Parvoviridae* are fatal and others remain subclinical are not fully resolved (Kailasan et al. 2015).

1.1.5 Transmission

In cats, dogs, pigs and mink parvovirus transmission occurs via the oronasal route. Virus shedding occurs mainly via faeces (Miranda and Thompson 2016, Selbitz et al. 2015) but also via other body fluids (Selbitz et al. 2015, Stuetzer and Hartmann 2014). Due to the virus' robustness in the environment, not only direct contact but also living and non-living vectors such as bedding and tools play an important role in virus transmission (Selbitz et al. 2015). Transplacental transmission of parvoviruses is also possible. The human parvovirus B19, the cause for *erythema infectiosum* (Norja et al. 2012), and the porcine parvovirus are examples for diaplacental transmission (Selbitz et al. 2015). Transmission via blood products is reported and can be seen in the human B12 parvovirus (Soucie et al. 2011) and EqPV-H infection in horses (Divers et al. 2018).

1.1.6 Relevant *Parvoviridae* in veterinary medicine

In veterinary medicine, relevant parvoviruses are the canine parvovirus 1 and 2, feline parvovirus (FPV), porcine parvovirus (PPV), mink enteritis virus (MEV), Aleutian mink disease virus (ADV), goose parvovirus (GPV) and Muscovy duck parvovirus (MDPV), chicken and turkey parvovirus (Burrell et al. 2017, Dubovi and Maclachlan 2016).

In horses, at least three parvoviruses are identified so far. In addition to EqPV-H, other equine parvoviruses were found in horses. The equine parvovirus cerebrospinal fluid (EqPV-CSF) was identified in the cerebrospinal fluid of horses with neurological signs in the USA and China (Li et al. 2015, Xie et al. 2019). The eqcopiparvovirus (EqCoPV) was identified in equine plasma, respiratory swabs and cerebrospinal fluid in China (Altan et al. 2019), and the genomes of EqPV-H, EqPV-CSF and EqCoPV have been sequenced (Altan et al. 2019, Divers et al. 2018). Another equine parvovirus named EqPV-Abortion (EqPV-AB), was first isolated in 1982 in Canada in association with multiple abortions (Wong et al. 1985). The genome of this virus has not been sequenced. A different parvovirus associated with synovitis (EqPV-NS) is mentioned to be identified in 2014 in Australia (Ou et al. 2019). However, no further information on these two parvoviruses are currently available.

EqPV-H is associated with equine serum hepatitis (Tomlinson, Tennant et al. 2019). The International Committee of Taxonomy of Viruses (ICTV) lists EqPV-H as a related, unclassified virus within the Genus *Copiparvovirus* (Cotmore et al. 2019). So far, the Genus *Copiparvovirus* includes the bovine parvovirus 2 and the porcine parvovirus 4 (Cotmore et al. 2019). The bovine parvovirus 2 was discovered as contaminant of commercial bovine serum (Cibulski et al. 2016) whilst the porcine parvovirus 4 is currently discussed in context of the multisystemic

wasting complex in pigs (Cibulski et al. 2017). The other mentioned equine parvoviruses are currently not listed by the ICTV.

1.2 Equine Parvovirus Hepatitis

The equine parvovirus-hepatitis is the most recently detected virus in context with equine serum hepatitis. The virus has been first discovered in liver and blood samples of a horse that died of acute liver failure in Nebraska in 2018 (Divers et al. 2018). The virus was also found in the tetanus antitoxin the horse was inoculated with 65 days prior. Experimental infection of two other horses using the contaminated serum was successful. Both horses developed signs of liver disease, proven by elevated liver enzymes and analysis of liver biopsy samples (Divers et al. 2018). In a following case series of 18 horses suffering from Theiler's disease, EqPV-H could be isolated in all affected horses (Tomlinson, Tennant et al. 2019). The virus is thus strongly associated with serum hepatitis (Tomlinson, Kapoor et al. 2019). Until now, the new parvovirus proved impossible to propagate in cell-cultures (Divers et al. 2018). Apart from the USA, the virus has so far been identified in horses in China, Germany, Canada, Austria and Slovenia (Badenhorst et al. 2019, Baird et al. 2020, Lu et al. 2018, Meister, Tegtmeyer, Brüggemann et al. 2019, Vengust et al. 2020). The prevalence of viral DNA in horses varies between 12 % in China, 13 % in the USA and 7 % in Germany (Divers et al. 2018, Lu et al. 2018, Meister, Tegtmeyer, Brüggemann et al. 2019). Depending on presence of diseased horses on farms, prevalence of EqPV-H infection varies. Horses that were in contact with infected horses showed a prevalence as high as 54 % (Tomlinson, Tennant et al. 2019). In Germany, more specific in the federal states Lower Saxony and North Rhine-Westphalia, 35 % of the horses were tested positive for serum antibodies (Meister, Tegtmeyer, Brüggemann et al. 2019). In the USA, 15 % tested seropositive (Divers et al. 2018). The EqPV-H positive horses in China did not show evidence of hepatopathies by presenting liver enzyme levels within the normal range (Lu et al. 2018). That leads to the assumption, that infection with EqPV-H can follow a subclinical course. Potential risk factors are currently under investigation. So far, age and an extended breeding history could be associated with a higher prevalence of infection with EqPV-H. Correlation with travelling history and stock size of the farm could not be found (Meister, Tegtmeyer, Brüggemann et al. 2019).

Another unresolved issue is the route of infection. Iatrogenic infection via contaminated serum products is possible (Divers et al. 2018). Serum products such as tetanus antitoxin (TAT), plasma or stem cells that were administered to horses that fell sick of serum hepatitis were frequently tested positive for EqPV-H as well (Tomlinson, Kapoor et al. 2019). In contrast, none

of the horses that tested positive for EqPV-H in China was treated with any blood products (Lu et al. 2018). Alternative routes of transmission, for instance via nasal or faecal shedding or transmission via vectors are currently discussed (Tomlinson et al. 2020, Tomlinson, Kapoor et al. 2019, Tomlinson, van de Walle, Divers 2019). In her most recent study Tomlinson and coworkers (2020) could show, that infection can not only occur via intravenous but also via intraarticular and intralesional injection. For this purpose, mesenchymal stroma cells and serum were successfully used. Experimental nasal infection failed, while oral infection was successful. Furthermore, it was ascertained that nasal, oral and faecal shedding of EqPV-H is common. Transmission of the virus via horseflies could not be confirmed yet (Tomlinson et al. 2020). A recent study demonstrates, that EqPV-H can commonly be detected in commercial equine serum products (Meister, Tegtmeyer, Postel et al. 2019). Since equine serum products are frequently used in equine medicine, infection of horses with EqPV-H could be a global phenomenon (Meister, Tegtmeyer, Postel et al. 2019). In human medicine, the human parvovirus B19 can also be found in serum products. People exposed to plasma derived products have a higher prevalence of antibodies against parvovirus B19 than people that were not exposed, which leads to the conclusion that transmission of human parvovirus B19 through blood products is possible (Soucie et al. 2011). Therefore, blood products, such as clotting factor concentrates for humans, are scanned for contamination and thresholds have been implied (Soucie et al. 2011). Inactivating these viruses is impossible today, due to their high resistance to heat. It might be necessary to imply similar safety and control measures for equine serum products as well. In the United States of America, the U.S Department of Agriculture is currently working on strategies to eliminate the nonprimate hepacivirus (NPHV) and the EqPV-H from commercial equine blood products (Divers and Tomlinson 2019).

1.3 Diseases of the liver in the horse

The liver's range of function is immensely versatile. To begin with, the liver is essential in the protein metabolism. Up to 90% of all plasma proteins are synthesized in the liver and perform their tasks in blood coagulation, transport, or as acute phase proteins (Divers and Barton 2017). Furthermore, the liver plays a major role in gluconeogenesis, excretion of ammonium, carbohydrate metabolism, fat metabolism, the excretion of bile, detoxification and, with help of the Kupffer cells, also in phagocytosis (Divers and Barton 2017).

The liver can compensate damage very effectively. Not until 60-80% of the liver parenchyma are destroyed, clinical signs may become apparent (Divers and Barton 2017). Distinguishing

between chronic an acute hepatic disease is not always easy, since chronic hepatic pathologies can suddenly lead to acute symptoms, once the compensatory capacity of the liver is exhausted (Divers and Barton 2017).

Clinical signs of liver disease are typically non-specific and include depression, weight loss, anorexia, colic, photosensitization, diarrhoea, icterus, coagulopathy, pruritus, fever, coronitis and hepatic encephalopathy (Divers and Barton 2017, Mair and Divers 2017).

Laboratory findings can be a great support in diagnosing liver disease, as well as in formulating a prognosis. Liver and cholestatic enzymes and markers of functionality of the liver can be analysed. Liver and cholestatic enzymes provide information on structural integrity of the liver and the biliary tract. Commonly analysed enzyme activities are sorbitol dehydrogenase (SDH), gamma-glutamyl transferase (GGT), glutamate dehydrogenase (GLDH), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase. However, only SDH, GGT and GLDH are liver specific enzymes in horses (Mair and Divers 2017). Liver functionality can best be evaluated by testing blood proteins (albumin and globulins), bile acids, triglycerides, urea, glucose, bilirubin and ammonia concentrations (Ambrojo et al. 2013, Mair and Divers 2017).

Ultrasonography and liver biopsy are essential additional diagnostic measures to detect liver pathologies (Divers and Barton 2017).

Hepatic diseases can be caused by multiple mechanisms. They can include infectious agents such as bacteria, viruses and parasites as well as non-infectious agents such as toxins, obstructive, metabolic, or congenital causes (Divers and Barton 2017). Some causes are still unknown today and remain to be investigated further (Divers and Barton 2017, Mair and Divers 2017). In horses, Theiler's disease and pyrrolizidine alkaloid toxicosis are the most common causes for acute liver disease and hepatic failure in the United States (Mair and Divers 2017).

1.4 Theiler's disease

Theiler's disease is named after Sir Arnold Theiler, who first described the disease (Theiler 1918). The condition is also known as serum hepatitis, equine acute hepatic necrosis, idiopathic acute hepatic necrosis, serum sickness or post vaccinal hepatitis. It is regarded as the most common cause of acute hepatitis and liver failure in horses (Tomlinson, van de Walle, Divers 2019). As the various names indicate, Theiler's disease is characterized by a rapid onset of hepatic insufficiency leading to hepatic failure, which is caused by severe necrosis of hepatocytes (Theiler 1918).

When Sir Arnold Theiler first described the disease between1914 and 1918 in South Africa, he eventually recognized, that the disease occurred in association with the vaccination program against African horse sickness that he was running at that time (Theiler 1918).

1.4.1 Aetiology

Serum hepatitis is linked to the administration of equine serum products to horses. Most frequently reported is the prior administration of tetanus antitoxin (Guglick et al. 1995, Messer and Johnson 1994a, 1994b, Robinson et al. 1975, Step et al. 1991). Vaccinations, commercial equine plasma, botulism antitoxin and allogenic stem cells are other causes associated with an outbreak of serum hepatitis (Aleman et al. 2005a, Chandriani et al. 2013, Tomlinson, Kapoor et al. 2019). Serum hepatitis usually develops four to twelve weeks after inoculation with equine serum products (Aleman et al. 2005a, Guglick et al. 1995, Theiler 1918, Thomsett 1971, Tomlinson, Kapoor et al. 2019). Interestingly, in some cases of Theiler's disease, prior inoculation of equine blood products can be precluded (Theiler 1918, Thomsett 1971, Tomlinson, Tennant et al. 2019). However, horses that are in contact with horses that suffered from Theiler's disease can developed hepatitis as well (Tomlinson, Tennant et al. 2019). Furthermore, seasonal increase of cases in late summer and fall could be observed (Thomsett 1971, Tomlinson, Tennant et al. 2019). Therefore, Theiler's Disease appears to be both, infectious and contagious (Divers and Tomlinson 2019). Transmission of disease via nasal or faecal shedding is under investigation (Tomlinson et al. 2020, Tomlinson, van de Walle, Divers 2019). Transmission of EqPV-H was possible in one horse via oral route, but did not trigger the outbreak of Theiler's disease (Tomlinson et al. 2020). The clinical signs can either follow an acute or subclinical course, while some horses are not affected at all (Guglick et al. 1995). The ultimate mechanism of how and why hepatic failure occurs in some horses while other horses are not or only subclinically affected is still to be determined. Prior to the discovery of the EqPV-H, three Flaviviridae were suspected causes for serum hepatitis (Tomlinson, van de Walle, Divers 2019). Since these viruses have been historically linked to Theiler's disease, they will be briefly introduced. To date, however, association with Theiler's Disease seems questionable.

1.4.1.1 Theiler disease-associated virus (TDAV) or Pegivirus D

Theiler disease associated virus, genus *Pegivirus* of the family of the *Flaviviridae*, was initially discovered in context with an outbreak of serum hepatitis after inoculation of antiserum to botulinum toxin (Chandriani et al. 2013). Since then, however, it proved impossible to identify

TDAV in following cases of serum hepatitis (Tomlinson, Kapoor et al. 2019, Tomlinson, Tennant et al. 2019). Prevalence of TDAV in horses appears to be below 1 %, but it can frequently be found in commercial equine sera (Postel et al. 2016). Since the virus could not be isolated in further outbreaks of serum hepatitis, association with Theiler's disease seems rather improbable (Tomlinson, van de Walle, Divers 2019). Interestingly, after discovery of the EqPV-H, the samples of the study of Chandriani, were subsequently tested positive for EqPV-H. Hence, the horses were coinfected with EqPV-H (Divers et al. 2018).

1.4.1.2 **Equine pegivirus or Pegivirus E**

Pegivirus E (EPgV), genus *Pegivirus* belongs to the family of *Flaviviridae* as well and was isolated from horses with clinical signs of hepatitis (Kapoor et al. 2013). The prevalence of pegivirus E is higher than of pegivirus D. Pegivirus E RNA is found in 1 %-32 % of horses and up to 66 % of horses were seropositive (Kapoor et al. 2013, Lu et al. 2016, Lyons et al. 2014). To date, no statistical association between infection with pegivirus E and hepatitis could be found (Divers et al. 2018). It seems unlikely, that the equine pegivirus is a cause of clinical liver disease (Kapoor et al. 2013). EPgV can also frequently be found in commercial horse serum (Postel et al. 2016).

1.4.1.3 **Nonprimate hepacivirus**

Non primate hepacivirus (NPHV) or equine hepacivirus is also a member of the family of the Flaviviridae. It belongs to the genus hepacivirus and is categorized as hepacivirus A (Simmonds et al. 2017). It was first discovered in dog's airways, but horses seem to be the virus's primary host. NPHV has caused great interest in research, as it is the closest genetically related virus to the human pathogenic Hepatitis C Virus. Hepatotropism and pathogenicity could be demonstrated (Pfaender et al. 2015). However, in most cases disease seems to develop subclinically, with only mild elevation of liver enzymes (Pfaender et al. 2015, Ramsay et al. 2015, Ramsay et al. 2019, Scheel et al. 2015). In Germany, the prevalence of viraemic horses is 2,5 % and approximately 31 % of horses are seropositive (Pfaender et al. 2015). Seroprevalence in other countries varies and can be as high as 84 % in young South African Thoroughbreds (Badenhorst et al. 2018). Similar to EqPV-H, TDAV and EPgV, NPHV can be found in commercial horse sera (Postel et al. 2016).

1.4.2 Distribution/Occurrence

Theiler's disease occurs worldwide. Sir Arnold Theiler first discovered the disease in South Africa (Theiler 1918). Since then, the majority of cases are reported from the United States of America (Aleman et al. 2005b, Guglick et al. 1995, Kopper et al. 2018, Tomlinson, Kapoor et al. 2019, Tomlinson, Tennant et al. 2019). In Europe, most reports are from Great Britain (Robinson et al. 1975, Thomsett 1971). In Hungary, one case of suspected serum hepatitis was investigated in association with NPHV (Reuter et al. 2014). Most recently, 12 cases of Theiler's disease in Slovenia could successfully be linked to EqPV-V infection (Vengust et al. 2020).
Serum hepatitis is a disease of the adult horse. Foals are usually not affected. Scarce reports of affected yearlings are available (Smith et al. 1991). Lactating mares seem to be overrepresented in some studies (Messer and Johnson 1994a).
Serum hepatitis occasionally also occurs without a history of prior administration of blood products (Theiler 1918, Tomlinson, Tennant et al. 2019). This form is suspected to appear seasonally. Most cases of this form are reported during late summer and autumn (Thomsett 1971, Tomlinson, Tennant et al. 2019). A mechanical transmission via vectors is currently under discussion (Mair and Divers 2017, Thomsett 1971, Tomlinson, Tennant et al. 2019). Horseflies were not able to contract EqPV-H infection so far (Tomlinson et al. 2020).

1.4.3 Clinical signs

Theiler's disease usually follows an acute and severe clinical course (Theiler 1918). Per-acute deaths can occur (Guglick et al. 1995, Robinson et al. 1975). A chronic course of disease is also possible (Guglick et al. 1995, Messer and Johnson 1994b). Signs of Theiler's disease are consistent with those of acute hepatic disease and hepatic insufficiency. Early reported signs are dullness, anorexia, jaundice, hyperhidrosis (Hjerpe 1964, Theiler 1918). The clinical course can quickly worsen into hepatic encephalopathy with signs including blindness, aimless walking, head pressing, recumbency, coma, ataxia and photosensitivity (Hjerpe 1964, Theiler 1918, Thomsett 1971). Theiler's disease often ends fatally (Aleman et al. 2005a, Guglick et al. 1995, Robinson et al. 1975, Theiler 1918, Thomsett 1971, Tomlinson, Tennant et al. 2019). Some horses develop a fever (Messer and Johnson 1994a) while others remain afebrile (Tomlinson, Kapoor et al. 2019). Signs of colic are reported in some cases (Aleman et al. 2005a, Robinson et al. 1975, Tomlinson, Kapoor et al. 2019). Sporadically, subcutaneous oedema of the distal extremities (Messer and Johnson 1994a, 1994b), respiratory symptoms (Messer and Johnson 1994b) and abortion are reported (Guglick et al. 1995).

1.4.4 Laboratory findings in Theiler's disease

In Theiler's disease GGT, SDH, AST, arginase and ammonium are consistently elevated above reference ranges (Divers and Barton 2017, Guglick et al. 1995, Messer and Johnson 1994b, Tomlinson, Kapoor et al. 2019). Bilirubinaemia and bilirubinuria are usually present (Divers et al. 2018, Divers and Barton 2017, Guglick et al. 1995, Tomlinson, Kapoor et al. 2019). Glucose is normal to low (Messer and Johnson 1994b, Tomlinson, Kapoor et al. 2019). Metabolic acidosis can occur (Mair and Divers 2017, Messer and Johnson 1994b). Blood urea nitrogen can be low, while creatine kinase was elevated in some reports (Guglick et al. 1995, Messer and Johnson 1994b). The haemogram can show leucocytosis, neutrophilia and haemoconcentration (Messer and Johnson 1994b, Tomlinson, Kapoor et al. 2019). In addition, prothrombin time and partial thromboplastin time can either be prolonged (Divers and Barton 2017) or stay within normal range (Messer and Johnson 1994b). Laboratory test results can also help formulating a prognosis for the patient. Mair and Divers state that AST values of above 4000 IU/L in horses appear to have a poor prognosis of survival (Mair and Divers 2017). In horses with hepatic disease, serum globulin above 60-70 g/l might indicate a poor prognosis as well (Ambrojo et al. 2013, Durham et al. 2003). Also, great increase of GGT above 399 IU/l is linked to an increased risk of death (Durham et al. 2003, McGorum et al. 1999). SDH and AST can be regularly checked to assist evaluation of the recovery progress (Mair and Divers 2017). In epizootics, laboratory screening can be useful to identify horses with subclinical serum hepatitis. Since there is continuously increasing evidence, that EqPV-H is the causative agent for serum hepatitis, testing for EqPV-H via PCR might be useful. Subclinically affected horses will show elevated GGT and AST enzyme activity (Guglick et al. 1995).

1.4.5 Necropsy

Characteristic for serum hepatitis is a liver reduced in size and of firm or friable consistency (Theiler 1918). The lobular architecture of the liver is replaced with cellular debris. Haemorrhage is possible (Tomlinson, Kapoor et al. 2019). Centrilobular necrosis is another characteristic (Robinson et al. 1975, Theiler 1918). Centrilobular hepatocytes degenerate and show vacuolar changes (Guglick et al. 1995, Tomlinson, Kapoor et al. 2019). Biliary hyperplasia is reported (Aleman et al. 2005b, Guglick et al. 1995, Theiler 1918, Tomlinson, Tennant et al. 2019) and mild to moderate inflammatory cell infiltration is common (Robinson et al. 1975, Tomlinson, Kapoor et al. 2019, Tomlinson, Tennant et al. 2019). Furthermore, Robinson and colleagues (1975) noted changes in the kidney in two horses, presumably a consequence of

pigmenturia. In the brain, Alzheimer Type II astrocytes were reported several times in horses presenting hepatic encephalopathy (HE) (Aleman et al. 2005b, Tomlinson, Kapoor et al. 2019, Tomlinson, Tennant et al. 2019). Alzheimer Type II astrocytes are typical for hepatic encephalopathy and develop as a result of hyperammonaemia (Butterworth 2010).

1.4.1 Morbidity and Lethality

Morbidity is rather low and ranges between 1 % and 18 % (Sturgeon 2017). Aleman and coworkers estimate the prevalence of serum hepatitis following plasma transfusion with 0,4 % (Aleman et al. 2005b). Lethality of clinical cases ranges between 30 % and 88 % (Messer and Johnson 1994a, Thomsett 1971, Tomlinson, Kapoor et al. 2019).

1.4.1 Diagnosis

The definitive cause of Theiler's disease is yet to be proven and the diagnosis is based on history, clinical signs, interpretation of serum levels of liver enzyme activities, liver biopsy results and post mortem necropsy (Mair and Divers 2017). In historical data, prior inoculation with equine serum products should be enquired. Ultrasonographic imaging may be of limited value and shows a homogeneous liver reduced in size (Divers and Barton 2017). Often, other causes for secondary acute hepatitis in horses such as intoxication, metabolic or neoplastic causes need to be ruled out before a diagnosis of Theiler's disease can be made (Tomlinson, van de Walle, Divers 2019).

1.4.1 Treatment

Treatment of serum hepatitis is limited to supportive care (Mair and Divers 2017). Intravenous fluids, correction of electrolyte imbalances and avoiding hypoglycaemia are most important (Divers and Barton 2017). If hepatic encephalopathy is present, adequate treatment should be provided. Additional to the measures already mentioned, mechanical protection and chemical sedation of the horse to reduce self-inflicted injuries can be necessary (Divers 2015). Stress should be avoided, and tranquilizers be used if necessary. To reduce any further increase of blood ammonium concentrations, production of ammonia in the bowel can be reduced using lactulose or neomycin (Mair and Divers 2017). Antioxidant and anti-inflammatory therapy should be administered. Reducing cerebral oedema is another therapeutic goal (Divers and Barton 2017, Mair and Divers 2017).

This study aims to acquire further knowledge of EqPV-H, the potential causative agent of Theiler's disease. The research will focus on occurrence of EqPV-H in horse serum collected in northern Germany and on determination of potential risk factors for infection with EqPV-H. To contribute to a better insight of the clinical course of infection of EqPV-H, serum samples of infected horses were screened for manifestations of hepatitis. With increasing knowledge on cause, contributing factors and clinical course of the disease, potentially, therapy can be improved, and prophylactic managements can be established.

2 Material and Methods

2.1 PCR

For the study, a total of 2471 frozen serum samples were available for testing for EqPV-H. The samples were sourced from diagnostic samples of 2273 horses admitted to the Clinic for Horses of the University of Veterinary Medicine Hannover, Foundation, between 2014 and 2017. All samples were collected as part of the diagnostic work-up of the patients in the clinic. After centrifugation and separation of serum, the samples were stored immediately at -80 °C until further processing. Frozen samples were shipped on dry ice to the Department for Molecular and Medical Virology of the Ruhr University Bochum, Germany, where PCR analyses were performed. Pools of ten serum samples subsequently were created.

To test whether viral DNA were contained within the serum samples, a quantitative real time polymerase chain reaction was done (RT-qPCR). Firstly, viral DNA was extracted with the viral DNA Kit Qiagen (Cat. No. 1048147, Hilden, Germany). The extraction process was performed according to the manufacturer's directions. The PCR protocol was provided by Dr. Amit Kapoor (Divers et al. 2018). Two genes, the NP gene and the VP gene, are detected in the PCR process. Abiding to the protocol of Dr. Kapoor, four different primer pairs were used to detect the NP and the VP gene. For the non-structural protein, EqPV ak1 (5′-GGAGAA-GAGCGCAACAAATGCA-3′) and EqPV ak2 (5′-AAGACATTTCCGGCCGTGAC-3′) were used in the first round and EqPV ak3 (5′-GCGCAACAAATGCAGCGGTTCGA-3′) and EqPV ak 4 (5′-GGCCGTGACGACGGTGATATC-3′) were used in the second round. For the detection of the VP gene, in the first round primer pairs EqPV ak5 (5′-GTCGCTGCATTCTGAG-TCC-3′ and EqPV ak6 (5′-TGGGATTATACTGTCTACGGGT-3′) and EqPV ak 7 (5′-CTG-CATTCTGAGTCCGTGGCC-3′) and EqPV ak 8 (5′-CTGTCTACGGGTATCCCATACGTA-3′) in the second round were used (Divers et al. 2018).

To obtain standards for quantification, a dilution row was generated using a plasmid that contained genetic information of the VP1 of EqPV-H. For Quantification of the amount of viral DNA, a LightCycler (480 LightCycler 480 Roche, Germany) together with a fluorescent (BRYT Green® Dye, Promega Corporation, United States) was used. Five different steps were run in the light cycler. At first, one circle with 37 °C for 15 seconds for reverse transcription was run. Then, 50 circles of each of the following steps were carried out. First, denaturation (95 °C, 10 sec) were used to split the double cDNA strand. Annealing of the primer to the DNA fragment (54 °C, 30 sec) and elongation of the DNA can take place (72 °C, 10 sec). Then, one circle of a melting curve follows (95 °, 1 sec, 65 °,15 sec, 95 °C). Cool down program is carried out for 30 seconds by 40 °C. The originate number of templets could then be

calculated. If serum pools contained more than 875 copies per millilitre, the individual sampled were analysed again to identify the infected horses. Single samples containing more than 1000 copies per millilitre were considered positive.

2.2 Data collection and analysis

All horses admitted to the hospital were registered in the patient management system (EasyVet®,Veterinärmedizinisches Dienstleistungszentrum (VetZ) GmbH, Hannover, Germany). Each horse was assigned an individual number by the software. All relevant information about the patient will be saved in the electronic index card. The serum samples were labelled with the horses' number and date of collection. A list with the numbers of all sampled horses and sample date was transferred into Microsoft Excel365 (Microsoft Office Corporation, Redmond, WA, USA). To obtain further information on these horses' data, lists containing information about breed, date of birth, sex, reason for admittance to the clinic, postal code of the owner and laboratory results of all patients that were treated between 2014 and 2017 were extracted from the EasyVet software. These lists were converted into Excel files. Those lists could then be compared with the list of sampled horses.

2.2.1 Clinical course of infection

Medical records of positive tested horses were extracted from the electronic patient index. Horses were anonymised and numbered from one to twelve. Medical history was reviewed individually and summarized in tables (Table 4).

2.2.2 Age

Year of birth was subtracted from the year of presentation to calculate the age of the horse at the time of sampling. If horses were sampled multiple times, same procedure was rerun. Five age groups were created: Horses sampled under the age of 5 (n= 590), sampled between age 5 and 10 (n=696), between 11 and 15 (n=516), between 16 and 20 (n=362) and age older than 20 (n=297).

2.2.3 Breed

Eight breeds were analysed: Arabic horses (n=74), Draft horses (n=34), Islandic horses (n=167), Ponies (n=215), Standardbreds (n=26), Thoroughbreds (n=24) and Warmblood

horses (n=1275). Horses with another breed than those were combined in the group “other” (n=394).

2.2.4 Sex

Horses were assigned to four groups depending on their sex; mares (n=1053), stallions (n=196), geldings (n=988). For 36 horses, no record on sex was found in the record system. They were grouped as “unknown”.

2.2.5 Location

To obtain information on location of housing of the horses, postal codes of horse owners were integrated into a Map using Microsoft Excel365 (Microsoft corporation, Redmond, WA, USA supported by Bing GeoNames, HERE, MSFT, Wikipedia). To improve visibility, areas were highlighted using Microsoft Paint3D (Microsoft corporation, Redmond, WA, USA).

2.2.6 Diagnosis

To evaluate if diagnosis of the horse and infection with EqPV-H correlate, the horses were assigned to groups depending on their diagnosis listed in the electronic patient data. Ten Groups were created to combine different diseases (Table 1) and analysed.

Table 1 *Diagnosis of sampled horses*

Diagnosis	Number of sampled horses
Diseases of the digestive tract	842
Others	731
Diseases of the cardiovascular and respiratory tract	344
Diseases of the tooth, head, sinus systems and the mouth	319
Diseases of the locomotor system	310
Diseases of the eye	199
Diseases of the urogenital tract and neonatology	177
Diseases of the skin	89
Neurological diseases	53
Metabolic and endocrinologic diseases	18

2.3 Laboratory Test

All positive sera (n=12) were tested for liver enzyme activities at the central diagnostic unit of Vetmed Uni Vienna. Blood concentration of urea, albumin, bilirubin, bile acid, cholesterol and iron as well as enzyme activity of ALP, AST, ALP, GLDH and GGT were analysed.

3 Results

3.1 Occurrence of EqPV-H in horses in northern Germany

A total of 2471 blood samples were taken in the period from 2014 to 2017. The samples originate from 2273 different horses admitted to the equine hospital of the University of Veterinary Medicine Hannover Foundation. Serum was tested for EqPV-H DNA via PCR as previously described. Out of the 2273 horses, serum samples of twelve horses were tested positive for EqPV-H DNA. This equates to an occurrence of 0,5 % in all tested horses.

Most of the sampled horses were stabled in the German federal state of Lower Saxony, where the University of Veterinary Medicine Hannover Foundation is located (Fig.1). Details of the individual horses can be found in Table 4. Most positive horses were localized in Lower Saxony (Fig.1).

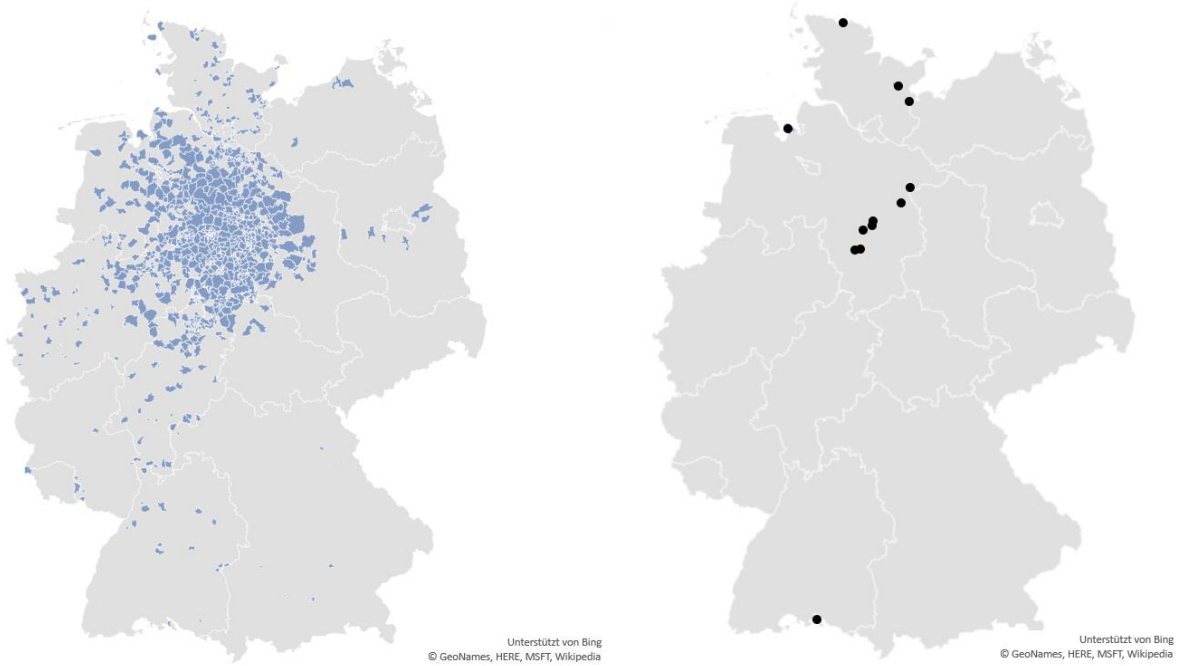

Figure 1
Left: Blue areas mark residential area of all sampled horses
Right: Black dots mark residential areas of EqPV-H positive horses

3.2 Age as a potential risk factor for EqPV-H

Out of all sampled horses 24 % (n=590) were under the age of five. 28 % (n=696) were between five and ten years old, 21 % (n=522) between eleven and fifteen. 15 % (n=364) were between 16 and 20 years old. 12 % (n=285) were older than 20 years (Figure 2).

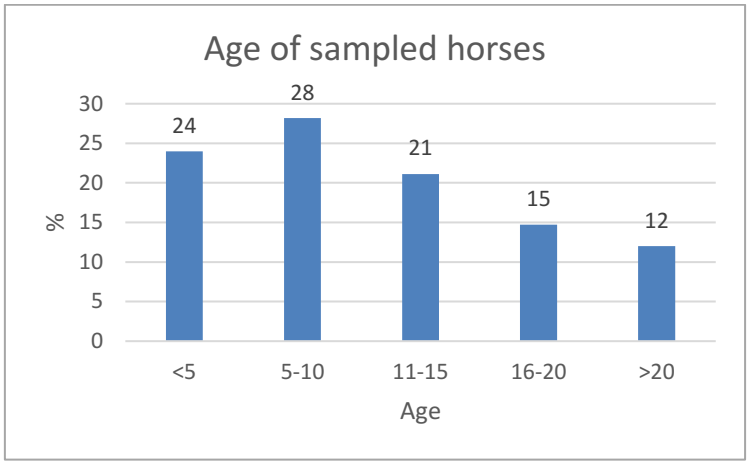

Figure 2 *Distribution of age of all sampled horses in percent*

Among the twelve horses that were tested positive for EqPV, two horses were under the age of five years (0,34 % of the group). In fact, one was a foal of just one month of age. Six horses were between eleven and 15 years old (1,15 %), two between 16 to 20 (0,55 %) and two between 21 to 25 (0,87 %) were tested positive (Figure 3).

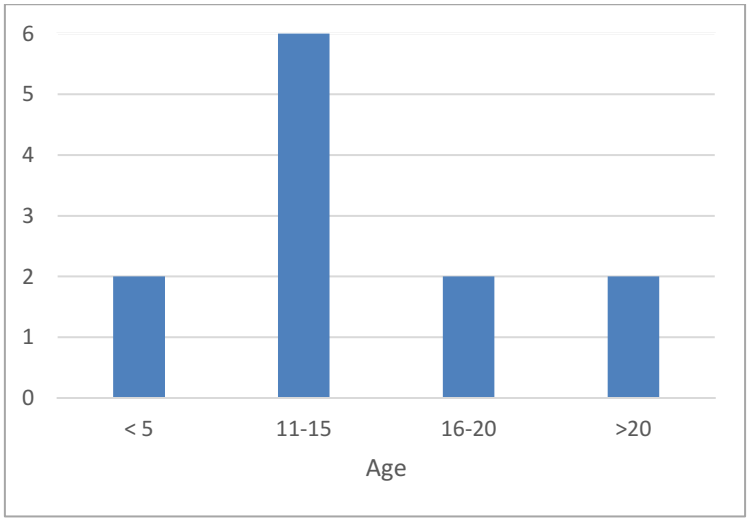

Figure 3 *Quantity of EqPV-H positive horses regarding age*

3.3 Sex as a potential risk factor for EqPV-H

The cohort consisted of 46 % mares, 44 % geldings and 10 % stallions. Eight of the 12 EqPV-H DNA positive horses were mares and four were geldings (Fig.4). None of the stallions tested positive.

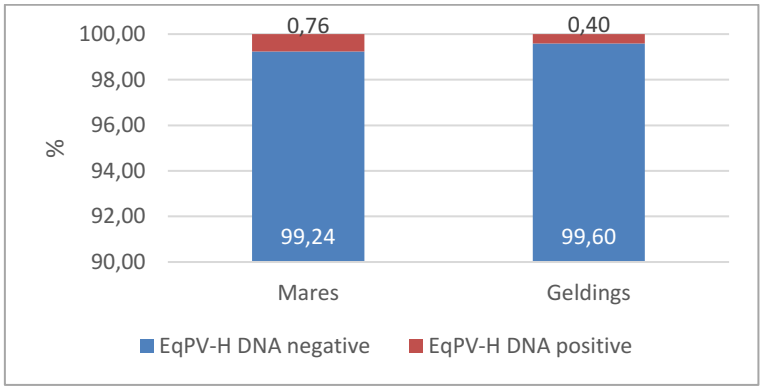

Figure 4 *Ratio of EqPV-H positive and negative tested mares and geldings*

3.4 Breed as a potential risk factor for EqPV-H

Horses were categorized according to their breed. Among the 12 positive horses, nine were Warmbloods. However, 56 % of total samples were collected from Warmblood horses. In relation, 0,71 % of all Warmblood horses were tested positive for EqPV-H. That ranges above the overall occurrence of 0,48 % in all tested horses. In addition to the positive Warmbloods, one Icelandic horse, one pony, and one Thoroughbred tested positive. Leading to occurrences between 0,47 % in ponies and 0,6 % in Icelandic horses.

Table 1 *Breed of sampled horses and their test results*

Breed	Number of Samples (%)	EqPV-H positive samples (%)
Warmblood Horse	1275 (56 %)	9 (0,7 %)
Other Breeds	394 (17 %)	0
Pony	215 (9 %)	1 (0,5 %)
Islandic Horse	167 (7 %)	1 (0,6 %)
Arabian Horse	74 (3 %)	0
Quarter horse	64 (3 %)	0
Draft horse	34 (1 %)	0
Standardbred	26 (1 %)	0
Thoroughbred	24 (1%)	1 (4,2 %)
Total	**2773**	**12**

3.5 Infection with EqPV-H often follows a subclinical course

3.5.1 Reason for admittance to the hospital

None of the EqPV-H-positive horses were admitted to the clinic in context with hepatic disease. Four of the horses were admitted for dental pathologies, two because of intestinal problems, two horses due to ophthalmologic disease, one for removal of a sarcoid, one because of guttural pouch tympany and two were admitted for non-clinical reasons (Table 4). Horse Number 1 (N1) was readmitted in 2019, three years after the first admission, for the treatment of a large colon displacement, due to reported fever and colic signs. Fever was not present in clinic. Again, the horse was diagnosed with left dorsal displacement of the left colon and treated conservatively just like the first time. No blood sample was drawn at the second time of admission.

Six out of the 12 horses were admitted more than once during the study period. However, serum samples for repeated EqPV-H testing were only available in two cases. Eleven horses were positive at the time of first admission. One horse (N2) was tested negative at the first admission in 2016 and was tested positive at the second time of admission one year later. One horse (N8) was positive at the first admission in 2016 and was tested negative at the second admission to the clinic in 2017. All horses returning to the clinic, did so for reasons not associated with hepatic disease.

3.5.2 Hospital stay and clinical course of EqPV-H positive horses

All horses stayed at the clinic between four and 22 days. Ten horses could be discharged to their owners, while two horses had to be euthanized. One because of bad condition of the teeth and one was enrolled in a scientific study, was hospitalized for 22 days at the clinic and was euthanized in context of that study. This horse never had signs of liver disease or clinical pathological abnormalities that could be associated with hepatic infection.

Regarding clinical signs of chronic liver disease, horse Number 8 was slightly underweight. Most common clinical sign in EqPV-H DNA positive horses was nasal discharge. Five horses were presented with nasal discharge at admittance. In three horses, nasal discharge was linked to dental diseases (Table 2).

Table 2 *Overview of EqPV-H positive horses. Signs indicated in bold are potential clinical signs for hepatitis HR: heart rate, RR: respiratory rate.*

	Reason for Admittance to clinic	**Diagnosis**	**Clinical signs at admittance**
Horse 1	Colic signs	Nephrosplenic entrapment of colon ascendens	HR 44, RR 16/min, decreased bowel sounds, nasal discharge
Horse 2	Nasal discharge, cough, equine odontoclastic tooth resorption and hypercementosis	Equine odontoclastic tooth resorption and hypercementosis, Equine Asthma, Sialolithiasis	Nasal discharge, slightly stressed abdominal respiration
Horse 3	Expert report		Slightly harshened respiratory sounds
Horse 4	Colic signs	Obstipation of the large colon	Absent bowel sounds
Horse 5	Guttural pouch tympany	Guttural pouch tympany	Enlarged guttural pouches, mild stridor
Horse 6	Lump in neck area	Sarcoid	Total protein 76g/L
Horse 7	Enrolled in scientific study	Strongyloidosis	Lnn. mandibulares slightly enlarged, slightly harshened respiratory sounds, right eye presented mild exophthalmos, **Leucocytosis (12G/L)**
Horse 8	Enucleation of the right eye	Equine recurrent Uveitis	**Leucocytosis (10,4 G/L),** total protein 54g/L, erythrocytopenia (14,72 G/l), haemoglobin: 234 l/l, haematocrit: 72% **thrombocytopenia (88G/l)** mild nasal discharge
Horse 9	Nasal discharge, dental fracture	Multiple dental pathologies	Nasal discharge, **slightly underweight**
Horse 10	Referral for surgery of paranasal cyst	Paranasal cyst, equine odontoclastic tooth resorption and hypercementosis	
Horse 11	Referred because of corneal ulcer	Corneal ulcer, corneal oedema	**Leucocytosis (10,4 G/l)**
Horse 12	Nasal discharge	Odontogenic sinusitis, Pulpitis 209	Nasal discharge, slightly enlarged and painful Lnn. mandibulares

3.5.3 Laboratory test results

Clinical reports of the infected horses were reviewed. Haematology was unremarkable in five horses. Four horses had hemograms with abnormal results. Three horses did not receive a hemogram at admittance. Leucocytosis was found in two horses. Haematocrit and erythrocytes were elevated in horse N8, haemoglobin and thrombocytes were low (Table 2, Table 4). Except for haemoglobin (below reference range at the time of discharge from the hospital) all blood parameters for this horse returned to normal at the end of its hospital stay. No clinical or laboratory signs indicated hepatic disease in any of the twelve horses in their clinical reports. Infection with EqPV-H went utterly subclinical. The serum samples of the infected horses were now screened for hepatitis. Serum levels of GGT were elevated in only one horse. Serum levels of GLDH were elevated in two horses and one horse showed elevated levels of GLDH, GGT, AST and ALP. Nine horses did not show elevation of liver specific enzymes, despite infection with EqPV-H. Patient records from horses that were tested negative were reviewed for laboratory findings of biochemistry (Table 3).

Table 3 *Liver enzymes of EqPV-H positive horses, values outside the reference value are printed in bold*

Horse	Reference	1	2	3	4	5	6	7	8 a	8 b	9	10	11	12
Urea	20-40 mg/dl	36,9	26,2	21,7	28,8	**8,9**	25,9	31,1	**19,1**	32,6	28,7	25	**18,3**	23,9
Total protein	5,5-7,5 g/dl	5,56	6,82	6,45	5,83	**4,19**	7,44	6,97	6,57	**4,78**	5,98	6,15	6,72	5,77
Albumin	2,4-4,5 g/dl	3,31	3,48	3,31	3,44	2,56	3,5	3,09	3,13	2,16	2,61	3,26	3,67	3,2
ALP	<250 U/L	69	153	178	98	**622**	162	**538**	169	146	142	83	143	123
AST	<550 U/L	399	328	438	359	225	303	**1193**	442	385	484	283	353	243
ALT	<30 UL	13	8	7	12	14	7	14	10	10	30	8	15	9
GLDH	<13 U/L	1,97	2,36	3,27	1,33	2,74	4,66	**269,56**	5,24	**13,13**	**23,84**	1,04	3,61	4,37
GGT	<30 U/L	12	15	**190**	18	24	15	**402**	16	13	30	15	20	15
Bilirubin	0,7-3,1 mg/dl	1,36	1,23	1,72	2,76	1,11	0,92	**0,55**	0,96	0,79	0,88	1,13	1,12	1,21
Bile acid	<20 µg/dl	2	5	12	2	2	5	5	13	13	9	5	3	6
Cholesterol	90-170 mg/dl	92	**73**	**86**	97	136	**74**	**81**	**76**	**65**	**178**	**72**	**85**	**79**
Iron	80-240 µg/dl	184	119	194	124	82	100	131	237	189	108	149	143	158

4 Discussion

In this investigation, it was shown that equine parvovirus-hepatitis was found in samples of horses presented to a university teaching hospital in Germany. Most viraemic horses were found in lower Saxony, where most of the sampled horses were stabled. Interestingly, the overall occurrence in the cohort was rather low with only 0,5 % positive horses versus 7-13 % prevalence in the United States of America (Divers et al. 2018), China (Lu et al. 2016), northern Germany (Meister, Tegtmeyer, Brüggemann et al. 2019) and Austria (Badenhorst et al. 2018). A similar occurrence rate as in the study of Meister et al. (2019) was expected in the north west of Germany. However, occurrence was considerably lower with only 0,5 % compared to a prevalence of 7 % (Meister, Tegtmeyer, Brüggemann et al. 2019).

One possible reason could be a difference in the detection method. In this study, given to the large number of samples, serum samples were pooled for PCR. In this process, sera containing low numbers of viral DNA might become diluted below the threshold through negative samples and the pool was than considered negative. It might be necessary to re-evaluate the cut-off line for serum pools. Furthermore, the principle of sampling is different to previous studies. Lu et al. (2018) and Meister et al. (2019) sampled from farms, rather than single horses admitted to a hospital. It may be possible, that prevalence of EqPV-H is higher on stud farms than in the average horse population. Divers and colleagues (2018) tested serum samples of convenience submitted for nonclinical reasons and Badenhorst et al. (2018) included patients of an equine hospital as well as privately owned healthy horses. Their sampling methods resemble the sampling method used in this study. They still documented a considerably higher prevalence of 8-14 % compared to the occurrence of EqPV-H in this study. Another factor could be that in Lu et al. (2018) and Meister et al. (2019) studies many samples originate from the same farm. Lu et al. (2018) tested horses from five different farms, with an average of 28 sampled horses per farm. Another difference between this study and previous studies of Lu et al. (2018) and Meister et al. (2019) is, that the horses' breed differs. In their studies, mostly Thoroughbreds were tested. Since Thoroughbreds show a significantly higher prevalence of NPHV viraemia (Pfaender et al. 2015), it could be suspected that the difference in overall occurrence of EqPV-H could also be connected to the breed. Meister et al. (2019) included only Thoroughbreds in her study and Lu et al. (2018) tested mostly Thoroughbreds but also Warmblood horses. In contrast, a study including 802 actively racing Thoroughbreds could demonstrate a prevalence of only 2,9 % (Ramsay et al. 2019). In this study, only 24 Thoroughbreds were included. One was tested positively for EqPV-H.

No potential risk factors for certain breeds could be determined in this study. Due to the low number of EqPV-H positive horses (n=12), only descriptive statistical analysis was performed in this study. Calculation of correlation using statistical test was not carried out, since the cohort of positive horses was considered too small for reliable results.

Rather than breed, the use or career of the horses could be a factor of interest. In this study, no information on the use of the horses was obtained. But one could speculate that a higher prevalence could be found on breeding farms than in the average horse population, since studies that sampled on stud farms present higher prevalences compared to this study. It remains unclear, if the career of horses plays a role in risk of infection or if breeding horses are at higher risk of infection than eventing horses or leisure horses. The reason why overall occurrence remains low in this cohort cannot be solved. Considering that EqPV-H can frequently be found in commercial equine serum pools (Meister, Tegtmeyer, Postel et al. 2019), the prevalence in our study appears to be rather low. However, sera are often pooled or sourced from one donor herd (Meister, Tegtmeyer, Postel et al. 2019). If sera are pooled, infected horses could contaminate the final serum product, depending on the detection limit. If infected horses live in donor herds, horizontal transmission is conceivable.

Considering age as a risk factor, we were able to show that 50 % of the seropositive horses were within the age of eleven and 15 years. This is consistent with a recent study by Meister et al. (2019) that showed most viraemic horses were between eleven and 15 years old. Additionally, we were able to find EqPV-H genome sequence in a foal at the age of 4 weeks. To date, no vertical transmission of EqPV-H was detected (Meister, Tegtmeyer, Brüggemann et al. 2019, Tomlinson et al. 2020). Routes of transmission for foals may be the same as for adult horses but are to investigate further. In our study, the foal did not show any clinical signs of liver disease. The serum levels of ALP, AST and GGT were considered normal in foals during the first months. These parameters should not be used in diagnostics for hepatopathies in foals during that time (Christian 2017). No indication for hepatic disease could be found. For this foal, infection with EqPV-H was completely asymptomatic.

When taking a look at reports of Theiler's disease, we learned that foals of mares that were suffering from Theiler's disease remained asymptomatically (Guglick et al. 1995, Hjerpe 1964, Messer and Johnson 1994a). Even when foals received tetanus antitoxin of the same batch as the mares, foals remained healthy (Guglick et al. 1995, Hjerpe 1964). In one case, a foal of a mare that suffered from serum hepatitis, showed high activities of AST and GGT, however foals of one clinical and two subclinical affected mares did not show elevated enzymes (Guglick et al. 1995).

Since, despite the large cohort, only twelve horses tested positive for EqPV-H, results must be interpreted with caution. Analysis of risk factors was limited due to the low number of cases, so that no definite assertion regarding risk factors for EqPV-H infection can be made.

Horses in this study did not present signs of acute hepatic disease at time of admission and during the stay at the clinic. Since clinical signs of mild or chronic hepatitis are often nonspecific, some clinical signs of infected horses could either be linked to hepatitis or to the condition the horses were admitted to the hospital for. One horse was readmitted three years after sampling to the hospital for fever. Fever can occur in context with hepatic disease. However, no serum sample was drawn at time of readmission and no liver values were determined. Therefore, it remains unclear, if fever was linked to an underlying hepatitis. The definition of hepatitis is not universally set. If hepatitis is defined as two liver enzymes elevated above the reference, like Tomlinson et al. (2020) propose in their most recent paper, we can identify only one of the EqPV-H infected horses with hepatitis. Clinical and biochemical findings suggest, however, only subclinical hepatitis. None of the infected horses showed signs of Theiler disease. This is consistent with other studies that showed that infection can occur entirely subclinical (Baird et al. 2020, Lu et al. 2018).

Incubation time of EqPV-H is rather long with up to ten weeks or more until hepatitis develops (Divers et al. 2018, Tomlinson et al. 2020). Viremia can also persist at lower levels after hepatitis resolved (Divers et al. 2018, Tomlinson et al. 2020). Since longitudinal sampling was not part of this study, it remains difficult to determine if those horses never developed hepatitis or if, at time of sampling, hepatitis had not yet occurred or is already resolved.

Of the EqPV-H positive horses, the liver enzyme activity of GGT was elevated in one horse, GLDH was elevated in two horses and both enzymes were elevated in one horse. The results of this study correspond with prior results of EqPV-H positive horses. Tomlinson et al. (2019) reported that 48 % of infected horses did not present with hepatitis. In another study, the liver enzymes of all the EqPV-H positive equines were within the refence range (Lu et al. 2018). In experimental infection of horses, subclinical to clinical hepatitis could be documented (Divers et al. 2018). It is further suggested that infection with the virus can be persistent (Divers et al. 2018). In this study, one horse tested negative one year after a positive PCR result. The horse either cleared the infection completely or viral load decreased below the limit of detection. In this cross-sectional study the other horses were only tested at one time point.

The retrospective nature of this study results in several limitations that should be critically addressed. Analysing the postal code of owner to assume where the horse is stabled, is not fully

accurate. Usually, one would assume that the owner and the horse live relatively close in most cases. However, this has not been proven in the present study. Clinical signs of the horses were not completely unequivocal since patients were admitted for different underlying illnesses. So, distinguishing between possible effects from EqPV-H and overlying disease is difficult. Nevertheless, signs of severe hepatic insults would have been noticed by clinicians. In addition, analysis of liver enzymes was done to detect underlying hepatitis. We could not obtain information about the route of infection. No blood product administration was recorded in the patient files. However, inoculation with blood products outside of the University of Veterinary Medicine Hannover, Foundation cannot be ruled out. Horses might be treated from different veterinarians as well, since the equine clinic of the University of Veterinary Medicine Hannover acts as referral clinic. We mostly did not obtain a complete clinical record of the horses, but only a record about the time the horse was treated at the hospital. Therefore, we cannot provide information on transmission of EqPV-H, which however, was not the main goal of this study. Conclusion: Nevertheless, it was that occurrence of EqPV-H in horses admitted to the clinic was very low compared to other studies. We could not detect risk factors in association with breed and sex. In this study the age group between 11 and 15 was affected the most. Mild hepatitis was only found in one horse. Overall occurrence of EqPV-H appears to be very variable depending on tested horse population.

5 Summary

Almost 100 years after the detection of Theiler's disease, the likely cause for the condition may finally be discovered. The equine parvovirus hepatitis was recently found highly plausible for causing this fulminant hepatopathy. Apart from acute hepatitis, subclinical or asymptomatic courses of EqPV-H infection are also reported. This work's objectives were to determine the occurrence of EqPV-H infection in horses in northern Germany, as well as identifying risk factors for infection, such as age, sex, breed and location of where the horse is stabled. Furthermore, it was determined if clinical or biochemical indications for hepatic disease in infected horses were present.

The serum samples were sourced from horses admitted to the University of Veterinary Medicine Hannover, Foundation. They were screened for EqPV-H via rt-PCR and corresponding patient records were reviewed. In addition, liver enzymes of EqPV-H positive horses were analysed to detect hepatitis. Occurrence of EqPV-H in the tested horses was 0,5 %. Risk factors regarding sex and breed could not be determined. However, horses with an age of eleven to 15 years were overrepresented in this study. None of the infected horses showed clear clinical signs of hepatic disease. One infected horse was identified with mild hepatitis through laboratory testing of blood samples.

Further research is necessary to improve insight in the clinical course of infection and to determine why the clinical course of EqPV-H varies widely from asymptomatic to fulminant.

6 Zusammenfassung

Theiler's-Disease wurde im Jahre 1918 in Südafrika entdeckt. Nach mehr als 100 Jahren wurde nun mit dem equinen Parvovirus-Hepatitis die wahrscheinliche Ursache dieser schweren und akut verlaufenden Hepatopathie entdeckt. Das EqPV-H scheint neben der Theiler's-Disease jedoch auch subklinische Hepatitiden auszulösen oder verläuft gänzlich asymptomatisch. In dieser Arbeit wurde die Häufigkeit einer EqPV-H-Infektion in einer norddeutschen Pferdepopulation ermittelt sowie der Frage nachgegangen, ob bestimmte Faktoren, wie Alter, Pferderasse, Geschlecht oder die Region in der das Pferd lebt, begünstigende Faktoren für eine Infektion darstellen können. Außerdem wurde eruiert, ob es bei infizierten Pferden klinische oder blutchemische Hinweise auf Lebererkrankungen gab. Dazu wurden Serumproben von Pferden, welche der Pferdeklinik der Stiftung Tierärztliche Hochschule Hannover vorgestellt wurden, mittels PCR auf Virus-DNA untersucht und die zugehörigen Patientendaten ausgewertet. Bei positiv getesteten Pferden wurden aus den Serumproben zusätzlich die Leberwerte bestimmt. Bei 0,5 % der untersuchten Pferde konnte eine Infektion mit dem EqPV-H festgestellt werden. Bestimmte Risikofaktoren bezüglich Pferderasse und Geschlecht konnten nicht identifiziert werden. Jedoch war die Gruppe der Pferde im Alter von elf bis 15 Jahren überrepräsentiert. Von den infizierten Pferden zeigte keines eindeutige klinische Anzeichen einer akuten Lebererkrankung. Da Symptome für Lebererkrankungen selten eindeutig sind, ließen sich einige Symptome sowohl einer Lebererkrankung als auch der zum Zeitpunkt der Vorstellung vorherrschenden Erkrankung zuordnen. Bei einem Pferd zeigte die Untersuchung der Leberenzyme im Serum das Vorliegen einer milden Hepatitis.

Weitere Forschungen zum equinen Parvovirus-Hepatitis sind nötig, um den Infektionsverlauf besser zu verstehen, sowie Erklärungen für den unterschiedlichen Verlauf der Infektion, welcher von asymptomatisch bis fulminant reicht, erklären zu können.

7 Appendix

Table 4 *Summery of clinical history of infected horses*

Horse 1	
Postal code	30171
Breed	Warmblood
Sex	mare
Age	12 years
Year of blood sample drawing	2016
Vaccination Status	Influenza, EHV1,4, Tetanus, Rabies
Length of stay	4 days
Diagnosis	Nephrosplenic entrapment of the ascending colon
History	colic symptoms, received Metamizole, nasogastric intubation, deworming 1 week prior, was treated at clinic the year before due to large colon impaction
Abnormal clinical findings on arrival	heart rate: 44/min, respiratory rate: 16/min, decreased gut sounds on the right ventral side, dorsal left without gut sounds. moderate seromucous nasal discharge, spleen lifted from abdominal wall, intestine palpable in between and in the nephrosplenic space
subsequent clinical finding	sonography: slightly increased amount of abdominal free fluid, stomach expanded to ICR 13
Abnormal laboratory findings on arrival	pH 7,43 blood Lactate 1,4 mmol/l leucocytes: 13.1 G/l
subsequent laboratory findings	pH returned to normal (7,38) blood lactate 1 mmol/l
Clinical course	all blood parameters return to normal at time of discharge, colic resolved conservatively, horse is discharged with incomplete relocation of the colon since horse appears comfortable and no further improvement is noticed with continued therapy
Therapy	starving, adrenalin IV, IV-fluids, NSS and fluids neostigmine
Follow up	returns to clinic 3 years later due to colic and fever, diagnosed with left dorsal displacement of colon, no cause for fever identified. No fever in clinic, colic conservatively resolved. leucocytes: 13.1 G/l, pH 7.47 blood lactate: 1,8 mmol/l, thrombocytes: 43 G/l all parameters return to normal at time of discharge besides the thrombocytopenia (56 G/l)

Horse 2	
Postal code	**29221**
Breed	Warmblood
Sex	gelding
Age	19 years
Year of blood sample drawing	2016, 2017
Vaccination Status	influenza, EHV1,4, tetanus, rabies
Length of stay	4d
Diagnosis	equine odontoclastic tooth resorption and hypercementosis, chronic obstructive bronchitis, sialolithiasis
History	nasal discharge, coughing, equine odontoclastic tooth resorption and hypercementosis
Abnormal clinical findings on arrival	heart rate: 44/min, respiratory rate: 20/min, slightly stressed abdominal respiration, bilateral serous nasal discharge
subsequent clinical finding	endoscopy: moderate amount of mucous found in trachea radiographs: slightly increased density of lung tissue, slightly thickened bronchial walls
Abnormal laboratory findings on arrival	hemogram normal
subsequent laboratory findings	
Clinical course	
Therapy	extraction of 103, 203 Flunixin-Meglumine Clenbuterol, Dembrexine information on optimizing housing
Follow up	2017: dental appointment, horse appears underweight, mild nasal discharge on the left side, blood without abnormalities. 2018: horse appears underweight, bilateral seromucous nasal discharge, leucocytes 10.3 G/L, extraction of 101, 201, 202

Horse 3	
Postal code	23568
Breed	Warmblood
Sex	mare
Age	12 years
Year of blood sample drawing	2016
Vaccination Status	influenza, tetanus
Length of stay	7 days
Diagnosis	came for a report, orthopaedic patient. no further information available
History	
Abnormal clinical findings on arrival	slightly harshened respiratory sounds
subsequent clinical finding	
Abnormal laboratory findings on arrival	hemogram normal
subsequent laboratory findings	
Clinical course	
Therapy	
Follow up	

Horse 4	
Postal code	31171
Breed	Thoroughbred
Sex	gelding
Age	21 years
Year of blood sample drawing	2016
Vaccination Status	unknown
Length of stay	2 days
Diagnosis	impaction of the large colon
History	colic symptoms, no improvement after spasmo-analgetics, sent to clinic
Abnormal clinical findings on arrival	respiratory rate: 20/min, absent gut sounds
subsequent clinical finding	sonography: Stomach until 13th intercostal space
Abnormal laboratory findings on arrival	blood calcium ++ 1,16 mmol/l blood lactate 1 mmol/l base excess 3,8 mmol/l
subsequent laboratory findings	
Clinical course	impaction resolved, returned to owner.
Therapy	placement of nasogastric tube, fluids, paraffin oil, walking, Xylazine, Butorphanol
Follow up	

Horse 5	
Postal code	**26969**
Breed	Warmblood
Sex	mare
Age	4 weeks
Year of blood sample drawing	2016
Vaccination Status	unknown
Length of stay	9 days
Diagnosis	guttural pouch tympany
History	
Abnormal clinical findings on arrival	heart rate: 100/min, respiratory rate: 44/min, Internal body temperature: 38,3 °C bilateral enlarged guttural pouches, occasional mild stridor
subsequent clinical finding	
Abnormal laboratory findings on arrival	hemogram normal
subsequent laboratory findings	
Clinical course	
Therapy	guttural pouch surgery, Flunixin meglumine, trimethoprim-sulphonamide
Follow up	

Horse 6	
Postal code	24955
Breed	Warmblood
Sex	mare
Age	11 years
Year of blood sample drawing	2016
Vaccination Status	influenza, EHV-1.4, tetanus
Length of stay	12 days
Diagnosis	sarcoid
History	lump in neck area
Abnormal clinical findings on arrival	all normal
subsequent clinical finding	
Abnormal laboratory findings on arrival	total protein 76g/L
subsequent laboratory findings	total protein returned to normal
Clinical course	incomplete wound dehiscence occurred d 6 after surgery
Therapy	excision of sarcoid Flunixin-Meglumine, TMS, 5-Flourouracil
Follow up	

Horse 7	
Postal code	30559
Breed	Warmblood
Sex	mare
Age	2 years
Year of blood sample drawing	2016
Vaccination Status	unknown
Length of stay	22 days
Diagnosis	Strongyloidiasis
History	enrolled in study unrelated to clinical and laboratory findings
Abnormal clinical findings on arrival	heart rate: 48/min, Lnn. mandibulares slightly enlarged
subsequent clinical finding	respiratory sounds bilateral slightly harshened, right eye showed mild exophthalmos
Abnormal laboratory findings on arrival	
subsequent laboratory findings	stool sample presented infection with Strongyloides, moderate amount of sand, leucocytosis (12 G/L)
Clinical course	
Therapy	Ivermectin, Praziquantel, Prednisolone
Follow up	euthanasia in context of a study on day 22

Horse 8	
Postal code	31303
Breed	Islandic Horse
Sex	gelding
Age	14 years
Year of blood sample drawing	2016, 2017
Vaccination Status	influenza, tetanus, rabies
Length of stay	14 days
Diagnosis	
History	bilateral equine recurrent uveitis
Abnormal clinical findings on arrival	mild serous nasal discharge
subsequent clinical finding	
Abnormal laboratory findings on arrival	erythrocytes :14,72 G/l haemoglobin: 234 l/l haematocrit: 72% thrombocytes 88G/l
subsequent laboratory findings	Urea level normal blood parameters return to normal range; however, haemoglobin is low (102-96 l/l) Leptospira PCR neg in corpus vitreous
Clinical course	
Therapy	bilateral Vitrectomy, Trimethoprim sulphonamide, Flunixin-Meglumine, eye ointment (Dexamethasone, Neomycin sulphate, Polymyxin-B-sulphate), Atropine, vaccination against tetanus and influenza
Follow up	returns 2017 for standing trans palpebral enucleation of right eye, leukocytes 10,4 G/l, total protein 54 g/l

Horse 9	
Postal code	29594
Breed	Warmblood
Sex	Mare
Age	22 years
Year of blood sample drawing	2016
Vaccination Status	unknown
Length of stay	3 days
Diagnosis	multiple dental pathologies
History	nasal discharge, dental fracture
Abnormal clinical findings on arrival	mild bilateral sero-mucous nasal discharge slightly underweight
subsequent clinical finding	inspection of oral cavity: multiple diastases, infundibular caries, wave mouth, ramps
Abnormal laboratory findings on arrival	
subsequent laboratory findings	
Clinical course	
Therapy	euthanasia
Follow up	

Horse 10	
Postal code	23715
Breed	Warmblood
Sex	mare
Age	17 years
Year of blood sample drawing	2017
Vaccination Status	tetanus, influenza, EHV-1,4
Length of stay	16 days
Diagnosis	paranasal cyst, equine odontoclastic tooth resorption and hypercementosis
History	referral for surgery of paranasal cyst
Abnormal clinical findings on arrival	heart rate:44/min, respiratory rate:16/min swelling in area of crista facialis and dorsum of the nose
subsequent clinical finding	cardiac murmur (1.-2/5 systolic, mitral valve) slightly bilateral enlarged Lnn. mandibulares, various dental pathologies
Abnormal laboratory findings on arrival	hemogram normal blood albumin normal
subsequent laboratory findings	albumin 26 g/l haematocrit: 51% thrombocytes 100 G/l total protein: 48 g/l thrombocytes 89 G/l leucocytes 12,7 G/l total protein 52 g/l
Clinical course	
Therapy	computed tomography in general anaesthesia bone flap surgery, removal of cyst Flunixin-meglumine, Trimethoprim sulphonamide

Horse 11	
Postal code	29386
Breed	Pony
Sex	mare
Age	15 years
Year of blood sample drawing	2017
Vaccination Status	influenza, herpes, tetanus, dermatophyton
Length of stay	3 days
Diagnosis	right eye: corneal ulcer, corneal oedema
History	corneal ulcer, corneal oedema
Abnormal clinical findings on arrival	heart rate:44/min right eye with severe conjunctivitis, severe cornea defect, epiphora
subsequent clinical finding	mild bilateral serous nasal discharge
Abnormal laboratory findings on arrival	leucocytes: 10,4 G/l
subsequent laboratory findings	
Clinical course	horse discharged from hospital at owner request. No improvement yet.
Therapy	eye ointment: Atropine, Regoluctan, Cepemycin, serum, n-acetylcysteine eye drops Flunixin-meglumine
Follow up	return to clinic in 2017 with suspected twin pregnancy, no clinical abnormalities despite mild nasal discharge

Horse 12	
Postal code	78476
Breed	Warmblood
Sex	gelding
Age	12 years
Year of blood sample drawing	2017
Vaccination Status	influenza, tetanus, EHV1, 4
Length of stay	9 days
Diagnosis	left sided odontogenic sinusitis
History	nasal discharge
Abnormal clinical findings on arrival	moderate sero-mucous nasal discharge, Lnn. mandibulares slightly enlarged and painful
subsequent clinical finding	pulpitis 209
Abnormal laboratory findings on arrival	hemogram normal
subsequent laboratory findings	
Clinical course	
Therapy	extraction of 209, trepanation of sinus and flushing, Flunixin-Meglumine, Acetylcysteine, Trimethoprim sulphonamide, Metronidazole
Follow up	one month later: satisfying healing process

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