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Pre- or Perioperative Antibiotic Prophylaxis in Horses Undergoing Aseptic, Elective Orthopaedic Surgery

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Statement of authorship

I hereby declare that I have submitted this dissertation independently and without any unauthorized help and only with the help specified in this dissertation.
All passages, literal or analogous from published or unpublished writings, all information based on oral information are indicated as such. In my studies, I obeyed the principles of good scientific practice as established in the *Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis*.

Berlin, 13th of November 2018
Sabita Diana Stöckle

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Abbreviations

CBC	Complete blood count
dl	Deciliter
EDTA	Ethylendiaminetetraacetic acid
G	Giga
g	Gram
h	Hour
i.v.	Intravenously
kDa	Kilo Dalton
kg	Kilogram
l	Liter
µg	Microgram
µl	Microliter
mg	Milligram
min	Minute
ml	Milliliter
MRSA	Methicillin-resistant *Staphylococcus aureus*
PAP	Perioperative antimicrobial prophylaxis+
Pen-b	Penicillin-based antibiotics
SAA	Serum amyloid A
sec	Second
SSI	Surgical site infection
TMS	Trimethoprim-sulfadiazine
WBC	White blood cells

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1 Introduction

Due to increasing worldwide problems with bacterial resistance, the use of antibiotics in veterinary medicine has entered the public eye (Johns et al. 2012). Despite fierce discussions, there has not been a lot of research on the necessity of the perioperative application of antibiotics in equine surgery. In addition to bacterial resistance developing against antimicrobial agents, antibiotic prophylaxis can also cause adverse effects in horses such as diarrhoea and/or colitis (Weese and Cruz 2009). Information on an ideal perioperative antimicrobial agent for equines is not available (Weese and Cruz 2009, Southwood 2014) although penicillin and gentamicin are widely used in combination (Southwood 2014).

German guidelines on antibiotic use in animals specify, that complicated and long surgical procedures should be the sole indication for antibiotic prophylaxis in horses (Bundestierärztekammer 2015). In clean orthopaedic surgeries of equines, in the 1980s, an infection rate of 8.1% was shown (MacDonald et al. 1994). However, rates close to 1% for the development of – potentially life-threatening – septic arthritis after arthroscopy was reported in newer studies (Olds et al. 2006, Borg and Carmalt 2013). Despite overall low infection rates in clean arthroscopic procedures, many equine surgeons still prefer to administer pre-/perioperative antimicrobials due to the severe consequences of a joint infection (Borg and Carmalt 2013). However, each use of antimicrobials will feed the development of resistant bacterial strains, development of superinfections, and nosocomial infections (Olds et al. 2006). Especially for clean procedures in veterinary medicine, further research is needed on the necessity of antimicrobial prophylaxis because its benefit still remains unclear (Weese 2008).

The aim of the two studies presented here was to elucidate possible differences in the complications developing in horses undergoing clean, elective, orthopaedic surgical procedures, whether receiving pre- or perioperative antimicrobial prophylaxis or not.

2 Literature survey

2.1 Perioperative antimicrobial prophylaxis

Nosocomial infections are a widespread problem: According to a study on antibiotic prophylaxis in humans at 72 German hospitals, 3.5% of all patients develop a hospital-acquired infection, of which approximately 15% occur at surgical sites (Ruden et al. 1997). The proliferation of bacteria will be suppressed when antibiotics are administered before the tissue is contaminated (Burke 1961). According to Cruse and Foord (1980), wounds are classified as clean, clean-contaminated, contaminated, or dirty. In a ten-year prospective clinical study of a total of 62,939 wounds, an infection rate of 1.5% was determined for 47,054 wounds classified as “clean”. In dirty wounds (2,093), defined by the presence of pus, a perforated viscus, or a traumatic wound older than four hours, the infection rate reached 40% (Cruse and Foord 1980). Nowadays, the administration of prophylactic antibiotics for arthroscopy in humans is no longer recommended. However, an antibiotic prophylaxis in human orthopaedic surgery is still indicated when implants remain in the body (Széll et al. 2006). On the other hand, a recent prospective randomised controlled study of antimicrobial prophylaxis in 100 patients undergoing lesser toe fusion surgery with K-wire implants did not find any significant difference between a wound infection developing in antibiotic-receiving (6.2 % infection rate) and in control (1.9% infection rate) patients (Mangwani et al. 2017).

The timing of an antibiotic application is critical because it is mandatory to have sufficient drug levels in the tissue where the antibiotic concentration must exceed 90% of the minimum inhibitory concentration (Kujath et al. 2006). Using β-lactam antibiotics for prophylaxis, the administration has to be timed between one hour prior to surgery and making the initial incision (Burke 1961, Classen et al. 1992). Already in 1992, the incidence of surgical site infections in patients receiving antibiotics two to three hours prior to surgery or 24 hours after surgery, was more than twice as high than in patients being treated during two hours before and three hours after the initial incision (Classen et al. 1992). A retrospective analysis of 5,023 case files of paediatric surgery, including general, cardiac, or spinal surgery, found a 1.7-fold higher risk of developing a surgical site infection (SSI) when administering perioperative antimicrobial prophylaxis incorrectly (Shah et al. 2014).

As the tissue drug levels need to remain effective in longer surgeries, a repetition dose might be necessary. Administering a repetition dose after two half-lives of the pharmacon seems reasonable (Kujath et al. 2006). A repetition dose is not necessary in orthopaedic surgeries that do not exceed three hours (Széll et al. 2006). A benefit of continuing an antimicrobial prophylaxis after wound closure was not brought to proof (Dellinger et al. 1994).

Although a lot of information has been provided on a correct perioperative antimicrobial prophylaxis in human medicine (Hohmann et al. 2012), individual differences in the timing and duration of antibiotic prophylaxis cannot be denied (Martin and Pourriat 1998). A study of 5,064 surgeries conducted at seven German clinics indicates, that the guidelines for perioperative antimicrobial prophylaxis were followed in 70.7% of all the cases (Hohmann et al. 2012).

During a prospective clinical study which monitored the perioperative antimicrobial prophylaxis conforming to the guidelines, there was a significant increase in the correct timing of the first antibiotic dose, and the excessive duration of perioperative antimicrobial prophylaxis (in 1,187 procedures) was significantly reduced compared to four months prior to the study (1,211 procedures). Full compliance with the guidelines was achieved in 40.2% of the cases. This was significantly higher (15.5%) than before the study. Also, the incidence of surgical site infections dropped from 18.5% to 12.0% (Bozkurt et al. 2013).

Boaz et al. (2014) conducted a single-centre, cross-sectional study to compare the incidence of complications prior to and following implementation of the Guidelines for Safe Surgery checklist. After introducing this checklist, postoperative fever was reduced significantly from 10.6 to 5.3%. Furthermore, the incidence of surgical site infections decreased by 34%, though this was not significant (Boaz et al. 2014).

Several guidelines define the risk factors for developing a surgical site infection in humans. For example, advanced age is mentioned as well as extremes in nutritional status, diabetes, colonization of the nasal vestibule with *Staphylococcus aureus*, coexisting infection at a remote body site, altered immune response, and preoperative hospitalization (World Health Organization 2009, Wacha et al. 2010, Bratzler et al. 2013). The surgery-associated risk factors listed are inadequate skin preparation, inappropriate preoperative shaving, inadequate surgical team hand and forearm antisepsis, contaminated operating room environment, inappropriate surgical attire and drapes as well as inadequate sterilization of instruments, excessive duration of the surgery, poor surgical technique, and inappropriate or untimely administered antimicrobial prophylaxis (Wacha et al. 2010, Bratzler et al. 2013). In creating wounds in equines, the surgeons themselves are the proximate cause of bacterial contamination by the surgical incision (Santschi 2006). Usually, the nasal vestibule is the reservoir for methicillin-resistant *Staphylococcus aureus* (MRSA), but the hands of medical personnel may also transfer bacteria, for example after contact to contaminated patients and surfaces. In German human hospitals, MRSA-positive personnel are asked to refrain from treating or taking care of the patients (Robert Koch Institut 2016). Also, surgical theatre clothes should be changed regularly. On their surface was higher contamination after being worn for two hours than initially. This suggests that the skin flora is carried over from the body to theatre clothing. A definite limit of time for wearing the clothes cannot yet be given because this requires further research (Sivanandan et al. 2011) but a regular change of theatre clothes apparently is indicated.

In equine surgery, there is no information on the ideal antibiotic agent although a combination of penicillin and gentamicin is used regularly (Southwood 2014). For colic surgery, in 23 German equine hospitals, a combination of penicillin and gentamicin (34.8%) was most commonly used, followed by penicillin alone (21.7%), cefquinome (8.7%), marbofloxacin (4.3%), sulfonamide/trimethoprim (4.3%), amoxicillin and gentamicin (4.3%), and amoxicillin alone (4.3%) (Teschner et al. 2015).

In equine castrations, the contamination of the wounds cannot be avoided as covering these wounds to avoid direct contact with bacteria, e.g. during lying down, is practically not possible. The increase of serum amyloid A (SAA) in castrates that received penicillin additionally to an NSAID (n=24) was not as great as in horses without perioperative antimicrobial prophylaxis (n=26, Busk et al. 2010). Another study concerning equine castrations (n=47) showed that a three day course of intramuscular procaine penicillin (n=23) was more effective than a single shot of intravenous penicillin G sodium (n=24) in reducing post-surgical scrotal swelling, wound drainage and body temperature on day 8 after surgery. Also, β-haemolytic *Streptococcus* spp. was found more frequently in the single shot group (Haucke et al. 2017).

2.2 Antibiotic resistance – a worldwide emerging health concern

2.2.1 Antibiotic resistance

Antibiotics and chemotherapeutics are substances that inhibit bacterial growth. Typically, antibiotics are produced by fungi or bacteria for protection against other microorganisms. Chemotherapeutics are produced synthetically (Bundesministerium für Gesundheit et al. 2015). In the following, the term “antibiotic” refers to both, antibiotics and chemotherapeutics.

Antibiotic resistance means that bacteria are not affected by antibiotics. This is subclassified in natural and acquired resistance (Kayser and Böttger 2010, Valentin-Weigand 2010). If strains of bacteria are not affected by an antibiotic agent because of a characteristic quality of their species/family, it is called natural resistance (Kayser and Böttger 2010). For example, the outer cell membrane of gram-negative bacteria is responsible for their resistance to β-lactam antibiotics (Kroker et al. 2010). In the case of a genome change in primarily susceptible bacteria (Kayser and Böttger 2010) through mutations, transformation, transduction, and conjugation (Kroker et al. 2010, Valentin-Weigand 2010), the antibiotic resistance is called acquired resistance. Through the so-called horizontal gene transfer, resistance can even be exchanged between different species of bacteria (Ventola 2015).

Mechanisms of resistance include modification of target structures such as the penicillin-binding Protein 2a (PBP2a), which is the principle of resistance to β-lactam antibiotics in MRSA and other coagulase-negative *Staphylococcus* species (Mallardo et al. 2013). Important as well is the enzymatic inactivation of the antibiotic and the minimisation of the intracellular concentration of the antibiotic by decreased influx and/or increased efflux (Kayser and Böttger 2010, Valentin-Weigand 2010).

The clinical resistance is of major importance for the veterinarian. Bacteria are clinically resistant when they are not susceptible to the concentration of the antimicrobial that can be reached in the tissue (Kayser and Böttger 2010).

Antibiotic resistance emerged even before the antibiotic era (D'Costa et al. 2011). Scientists confirmed a connection between the 30,000-year-old VanA, which is the enzyme responsible for resistance to vancomycin and the structures found in present-day bacteria (Courvalin 2006, D'Costa et al. 2011). Whenever antimicrobials are used, resistant bacteria obtain a selective advantage (Kayser and Böttger 2010, Kroker et al. 2010, Valentin-Weigand 2010, Mather et al. 2013). The spread of resistance, despite the development of new antimicrobials, is not only a question of mutation and horizontal gene transfer but also a question of the frequency and prudence of the new drug substance’s use (D'Costa et al. 2011). Therefore, a reasonable administration is important for maintaining the effect of antibiotic agents (Hughes et al. 2013).

2.2.2 Antibiotic resistance in humans

In the United States of America, more people die each year from infections caused by MRSA than from HIV/AIDS, Parkinson’s disease, emphysema, and homicide combined. The first MRSA was isolated in the United Kingdom (Ventola 2015), 34 years after the discovery of Penicillin by Sir Alexander Fleming in 1928. Another 53 years later, infections with antimicrobial resistant bacteria have become a global problem (Ventola 2015).

The administration of antibiotic agents is an important component of modern therapies, for example, in neonatal care or in organ transplantations (Perez and Villegas 2015). As patients are weakened through disease or surgical procedures, infections in hospitals are typically caused by resistant bacteria (Ventola 2015). The on-going distribution of bacterial strains resistant to most or even all available agents underlines the need for progress in the development of new antimicrobial agents and, most of all, the prudent use of available antibiotics (Perez and Villegas 2015).

A high risk of getting infected with multi-drug resistant bacteria, other than the consumption of antibiotics or hospitalization, has been attributed to travel to Africa and Greece as well as to having had contact with pets (Schmiedel et al. 2014). MRSA has been isolated from both companion animals and livestock (Loncaric et al. 2014). Coagulase-negative *staphylococci* that were isolated from animal infection sites turned out to be the same species as those causing nosocomial infections in humans (Kern and Perreten 2013). Interestingly, isolates from cats, dogs, and horses typically belong to human *Staphylococcus aureus* lineages, whereas farm animals are infected with non-human MRSA strains (Loeffler and Lloyd 2010, McCarthy et al. 2012). Resistance to ertapenem, an antimicrobial that is not approved for use in livestock and only restrictedly allowed in companion animals, was found in several isolates of 19.7% of the animals (35/178) and 24.5% of the humans (45/183). Of these isolates, 95.6% were segregated from cats and dogs, which suggests that these bacteria have been transferred from owner to pet (Schmiedel et al. 2014). For animals, repeated admission to a veterinary practice/hospital and usage of antimicrobials were noted as a risk factor to obtain MRSA. For humans and animals living in the same household with an MRSA carrier, hospitalization and surgery increase the risk of MRSA acquisition (Weese et al. 2006).

Furthermore, antibiotic resistance is also of great immediate political interest. During the G7-summit in 2015, the G7-states agreed on supporting the global action plan of the World Health Organization concerning antibiotic resistance as well as on developing national action plans. Furthermore, they will promote research in the area of epidemiology, infection prevention and control, as well as the development of new antibiotics, alternative therapies, vaccines, and rapid test (G7-Gipfel 2015).

2.2.3 Antibiotic resistance in horses

With antibiotic resistance considered to be one of the most severe developing worldwide health concerns, the antibiotic use and resistance in veterinary medicine are subject to public (Johns et al. 2012) and political attention (G7-Gipfel 2015). However, the focus of antibiotic resistance in veterinary medicine is rather on production animals than on horses (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit und Paul-Ehrlich-Gesellschaft für Chemotherapie e.V. 2016, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit 2016, Bundesministerium für Gesundheit et al. 2015), but antibiotic use in horses was restricted as well with the latest novella of the "Verordnung über tierärztliche Hausapotheken (TÄHAV)". For any off-label use of an antibiotic in horses, or the administration of third and fourth generation cephalosporins and fluorquinolones, a bacteriological culture including an antibiogram is mandatory (TÄHAV § 12 c).

Development of resistant bacteria during a course of antibiotic treatment was shown previously. A study performed at a veterinary teaching hospital indicated that 21.9% of the participating horses (n=105) had developed nosocomial gram-negative aerobic infections with high rates of resistance against three of the most frequently prescribed antibiotics gentamicin, kanamycin, and trimethoprim/ sulfadiazine. *Escherichia coli* and *Klebsiella* sp. isolated on day 7 were resistant to a significantly higher number of antibiotics than day 1 isolates (Koterba et al. 1986). A newer study also examined the faecal samples of 32 horses admitted to hospital: 13/32 horses were treated with antimicrobial agents prior to admission, and 21/32 horses were treated with antimicrobial agents during their hospitalization. In these horses, significant alterations in specific in-vitro equine faecal *E. coli* antimicrobial resistance profiles were found after hospitalisation for 7 days. Also, multi-drug resistant *E. coli* were isolated significantly more often after 7 days of hospitalisation (Williams et al. 2013).

Not only in a hospital but also in the field, an elevated risk of developing commensal *E. coli* resistant to antimicrobials was found in horses treated with antibiotics. Enrolled in this study were 80 horses: 56 were hospitalised and treated with antibiotics, 14 were not hospitalised and treated with antibiotics, and 10 were not treated with antibiotics and hospitalised (control). Samples were collected within 24 hours of admission/first treatment, five days after admission/initiation of treatment, two weeks after discontinuation of the antibiotic, and six weeks thereafter. There was no significant difference between hospitalised and non-hospitalised horses. The odds of a sample to contain a resistant isolate increased significantly on day 5 in the treated horses, but not in the controls. Two weeks after discontinuation, the odds to identify a resistant isolate returned to baseline in the non-hospitalised horses, but not in the hospitalised horses in which resistant bacteria were significantly more often isolated than before treatment. Pre-treatment levels were reached two months after the treatment in the antibiotic receiving, hospitalized horses (Johns et al. 2012).

A survey in the United Kingdom showed that over 60% of the antibiotics prescribed were not dosed correctly. 56.9% of the antibiotics were dosed too high, whereas 5.8% were given under the recommended dose (Hughes et al. 2013). In Germany, Austria, and Switzerland the selected dosage for antibiotics is insufficient in 12 % (manufacturer data) – 72 % (BEVA recommendations, https://www.beva.org.uk/protectme). These data suggest, that in these countries veterinarians rather follow manufacturer's data than scientific information (Schwechler et al. 2016). It must be considered problematic, that label dose instructions are not always consistent with current principles of prudent antimicrobial use and may promote antimicrobial resistance (Weese et al. 2015).

Antimicrobial resistance is a critical issue in equine medicine. Strategies for the adequate use of antibiotics need to be developed (Bowen 2013). As a pioneer, the British Equine Veterinary Association (BEVA), developed the "Protect ME"-strategy to improve antibiotic use in equine medicine and to encourage the introduction of "Responsible antibiotic use policies" in every practice (British Equine Veterinary Association 2018).

Therefore, the aim of this research project was to confirm the hypothesis that perioperative antimicrobial prophylaxis does not lead to a reduction in post-operative infection in equine clean elective uncomplicated orthopaedic surgery. This should contribute to the targeted use of antibiotics and at least help to decrease the progression of antibiotic resistance.

2.3 Equine acute phase parameters

As a protective mechanism, the inflammatory reaction dilutes, isolates, and at best eliminates the cause of tissue damage and repairs the defect through healing. This is the body's own capacity to react to endogenous and exogenous noxae. The five cardinal signs (tumour, rubor, dolour, calor, functio laesa) of an inflammatory reaction are commonly known (Ackermann 2009) but not always clinically visible (Jacobsen and Andersen 2007).

Proteins whose plasma level increase/decrease during an inflammation by ≥ 25%, are called acute-phase proteins. These proteins are mainly synthesized in the liver and mediate or inhibit the inflammatory reaction (Jain, Gautam, and Naseem 2011; Baumgärtner and Schmitt 2010; Ackermann 2009). Production of Serum Amyloid A (SAA), C reactive protein (CRP), and haptoglobin can also be found in the lungs (Yang et al. 1995, Ramage et al. 2004, Upragarin et al. 2005). During inflammation, in many species the plasma concentrations of CRP, α1-acid glycoprotein, haptoglobin, mannose-binding protein, fibrinogen, and α1-antitrypsin, as well as the concentration of the complement factors C3 and C4 augments, whereas the concentration of albumin and prealbumin decreases (Ackermann 2009). Ferritin, transferrin, and ceruloplasmin are also mentioned as acute phase proteins (Baumgärtner and Schmitt 2010).

Besides SAA and fibrinogen, haptoglobin has been reported to be an important acute phase parameter in horses (Taira et al. 1992).

2.3.1 Serum amyloid A

As the major acute phase protein in horses, the heterogeneous apolipoprotein (Uhlar et al. 1994) serum amyloid A is a reliable parameter of inflammation or infection in the horse (Baumgärtner and Schmitt 2010, Belgrave et al. 2013). Beside the synthesis in liver and lungs, the mammary gland (colostrum) and joints were found to produce SAA (McDonald et al. 2001, Jacobsen et al. 2006). SAA concentrations in the serum of foals diagnosed with bacterial infections (n=8) were reported to be higher than in foals with non-bacterial or uncertain diagnosis (n=17) (Hulten and Demmers 2002). The degradation of SAA takes place in the liver (Bausserman et al. 1987). Its plasma half-life is very short; in laboratory rodents, it reaches from 30 to 120 min (Hoffman and Benditt 1983, Uhlar and Whitehead 1999). Approximately six hours after the onset of inflammation, the plasma level of SAA starts increasing by more than a hundred-fold (Jacobsen and Andersen 2007). The maximum plasma level is reached 48 hours after the triggering stimulus (Jacobsen and Andersen 2007). The kinetic profile and diagnostic sensitivity make it an ideal marker for inflammation and tissue damage in the horse (Crisman et al. 2008). It is possible that the pattern of expression is regulated by different inflammatory stimuli (Raynes and McAdam 1991, Rygg et al. 1993). Reactions to SAA include enhancement or inhibition of leukocytes, T cell chemotaxis (Xu et al. 1995), inhibition of proliferation of endothelial cells and lymphocytes, inhibition of platelet aggregation, and phagocytosis (Crisman et al. 2008). During inflammation, the observed intra-articular synthesis of serum amyloid A could lead to the induction of metalloproteinase activity and cyclooxygenase metabolite formation as well as to a chemical attraction of leukocytes (Badolato et al. 1994, Malle et al. 1997, Vallon et al. 2001, O'Hara et al. 2004).

Non-infectious arthritis was induced by aseptic injection of amphotericin B (20 mg) and sterile water (4 ml) into the right mid-carpal joint of 24 horses. 16 hours after injection, all horses had elevated SAA concentrations which returned to baseline within 15 days. In this study, the relative maximum increase of SAA was significantly higher than that of the other markers (haptoglobin, fibrinogen, α_2-globulin (Hulten et al. 2002). After an intrasynovial injection of 1 µg or 3 µg LPS diluted in 2 ml saline, respectively, the serum and synovial fluid SAA levels peaked 48 hours after injection and then declined rapidly. The SAA response depended on the LPS dose injected into the joint: 1 µg LPS was found to cause lower SAA levels than 3 µg LPS (Jacobsen et al. 2006). Comparing the white blood cells (WBC) and SAA concentrations after initiating the non-infectious arthritis, the WBC showed an earlier augmentation (4-8 hours after induction) and peak (12 hours after induction). The course of WBC reflected the clinical signs with a return to the base-level 48-72 hours after initiating the inflammation. The SAA peaked 48 hours after arthritis induction when the clinical signs started to disappear. Despite its quick decline, the augmented levels of SAA lasted distinctly longer than the leucocytosis and levels were back to baseline level after 144 hours (Jacobsen et al. 2006).

In 24 horses undergoing arthroscopies, the SAA peaked twelve hours after the procedures. Seven days after surgery, the SAA concentration returned to its reference interval (Miller 2006). Another study measuring the inflammatory response after arthroscopy (n=11) found significantly higher levels than the preoperative baseline levels of SAA on the first day after surgery. Baseline levels were again reached five days after surgery (Jacobsen et al. 2009).

2.3.2 Fibrinogen

Beside its role in the coagulation process, several functions have been attributed to the plasma glycoprotein fibrinogen (Crisman et al. 2008). Due to its relatively high concentrations in healthy individuals (200-400 mg/dl) and the lengthy response period, fibrinogen is regarded as a fairly insensitive acute-phase protein. 24-72 hours after being exposed to an inflammatory stimulus, the plasma fibrinogen concentrations show a 1-10-fold increase (Crisman et al. 2008). The peak concentration is reached after 72-144 hours (Jacobsen and Andersen 2007). The augmentation remains elevated for up to 7 days after the end of the inflammation (Crisman et al. 2008) and is, therefore, not as sensitive as SAA as an inflammatory marker. Degranulation, phagocytosis, and antibody-dependent cytotoxicity as a result of an intracellular signal cascade after binding to CD11 and CD18 (cell surface integrins) are attributed to this β-globulin (Crisman et al. 2008).

Thirty-six hours after the induction of arthritis by injection of 20 mg Amphotericin B into the right mid-carpal joint of 24 horses, all probands had increased serum fibrinogen levels. These concentrations returned to normal levels after 15 days (Hulten et al. 2002).

After arthroscopy in eleven horses, fibrinogen concentrations were significantly higher on day one than preoperatively. The fibrinogen concentration remained elevated throughout the eleven day observation period of the study (Jacobsen et al. 2009). Another study examining the response of 24 horses undergoing an arthroscopy found a significant increase of fibrinogen concentration 24 hours after surgery, which was even more distinct 48 hours after surgery (Miller 2006). Like SAA, it declined to normal levels after 7 days (Miller 2006).

2.3.3 Iron

The plasma iron concentration decreases in horses during systemic inflammation (Feldman et al. 1981, Smith and Cipriano 1987, Borges et al. 2007). After lipopolysaccharide infusion, less iron enters the circulation due to hepcidin, which prevents the export from the mononuclear phagocytic system and from enterocytes (Oliveira-Filho et al. 2012). In mice, the iron resorption from the gut is also affected by hepcidin as a negative regulator (Nicolas et al. 2001, Pigeon et al. 2001). In mammals, iron is important for oxygen transport, phagocytosis, and the mitochondrial electron transport chain (Marx 2002). Iron is essential for bacterial proliferation (Marx 2002) and viral replication as part of the ribonucleotide reductase (Drakesmith and Prentice 2008). During infection, host and microbe require iron, so they compete for the host's iron stores. As a response to inflammation and chronic infection, iron sequestration is a well-recognized defence mechanism which leads to hypoferraemia and anaemia (Kent et al. 1994, Weinberg 1996). Hypoferraemia and anaemia are common symptoms of this iron sequestration, which is a well-recognized defence mechanism of the host (Brosnahan et al. 2012). On the day after arthroscopy, the iron concentration decreased significantly in eleven horses but returned to baseline level the next day. Post-surgical iron concentrations were also affected by the patient's age with older horses having higher iron levels (Jacobsen et al. 2009).

2.3.4 Haptoglobin

The α_2-globulin haptoglobin can be consistently detected in equine blood. Its concentrations are increased during inflammation (Jacobsen and Andersen 2007, Cray and Belgrave 2014). The function of the α_2 globulin is to prevent iron loss. It forms stable complexes with the free haemoglobin in the blood. Free haemoglobin stimulates the synthesis of haptoglobin so that it can be collected efficiently, which prevents the loss of iron and limits the oxidative damage to tissue caused by free haemoglobin. The complexes formed by haptoglobin and haemoglobin reduce the renal filtration of both haemoglobin and iron. These complexes are removed by hepatocytes allowing the reutilization of iron and amino acids so that the components can be reused. It is also believed that haptoglobin has a bacteriostatic effect since it limits the availability of iron which is essential for bacterial growth (Crisman et al. 2008). During the acute phase reaction, it shows a 1-10-fold augmentation and is therefore considered a moderate acute-phase protein (Crisman et al. 2008). The concentration of free haptoglobin can be affected by events other than inflammation such as acute haemolysis, during which its plasma concentration decreases (Crisman et al. 2008).

After inducing non-septic arthritis by injecting 20 mg Amphotericin B into the right mid-carpal joint of 24 horses, the relative haptoglobin concentration increased 16 hours after injection. An absolute increase was observed 24 hours post-injection. The peak in haptoglobin concentration was observed three days after induction of the non-infectious arthritis. On day five, the mean haptoglobin concentration returned to the reference interval (Hulten et al. 2002). A recent study examined haptoglobin as an inflammatory parameter in serum and synovial fluid. Non-septic arthritis was induced in 12 Shetland-pony geldings with 25 mg amphotericin B which was diluted in 3 ml of sterile water and injected aseptically into one radio-carpal joint. Prior to arthritis induction, the haptoglobin in the serum was significantly higher than in the synovial fluid ($p=0.0021$). Both in the serum and synovial fluid, the haptoglobin concentration was significantly higher 15 days after inducing non-septic arthritis than before ($p<0.0001$). Fifteen days after injecting the amphotericin B, the concentration of haptoglobin in the serum was not significantly higher than in the synovial fluid (Barrachina et al. 2016).

After arthroscopy in 24 horses, the haptoglobin concentration showed a slight increase after 48h as well as a decisive augmentation after 72 h (Miller 2006). Whereas these values decreased seven days after arthroscopy, they continued to increase in horses that were castrated and suffered from wound healing disturbance (n=12) as well as in horses that underwent laparoscopy (n=27) (Miller 2006).

2.3.5 Albumin

Albumin is a 66 kDa protein that is invariably found in plasma (Gassmann and Lutz 2005). 60% of the plasma protein fraction is albumin (Horn et al. 2005). The protein without a prosthetic group is mainly responsible for maintaining the oncotic pressure and also for unspecific transport (e.g., bilirubin, fatty acids, bivalent cations) (Gassmann and Lutz 2005). The concentration of albumin decreases during the acute phase of an inflammation (Ackermann 2009, Baumgärtner and Schmitt 2010); therefore, it is a so-called negative acute phase protein. However, in 24 horses undergoing arthroscopy, the albumin concentration increased by 1% over 7 days (Miller 2006).

3 Material and methods

3.1 Retrospective data analysis

The aim of the retrospective part of this study was to evaluate whether or not an antibiotic prophylaxis was able to decrease surgery-associated complications in elective, clean equine orthopaedic surgery.

3.1.1 Data collection

Electronic patient charts (easyVET, IFS Informationssysteme GmbH/Hannover, Germany) were reviewed of horses that underwent an elective, clean orthopaedic surgery at the equine clinic in Lüsche (Tierklinik Lüsche GmbH, Bakum, Germany) between June 8th, 2011, and January 9th, 2015 (3 years and 7 months). June 8th, 2011 was chosen as a starting point because from that time on, a special record of horses that did not receive antimicrobial prophylaxis before the elective surgery was started at Tierklinik Lüsche GmbH. The start of the prospective study (see section 3.2) determined the end of the retrospective study.

3.1.2 Study population

All data of horses that underwent elective, clean orthopaedic surgery were considered for inclusion in the retrospective analysis. Arthroscopy, fasciotomy, neurectomy, tendovaginoscopy, tenotomy, extraction of fractured splint bones (Mc II, Mc IV, Mt II, or Mt IV), bursoscopy, and combinations of these were defined as elective, clean orthopaedic surgeries. Documentation was considered complete if information was available about the pre-anaesthetic examination on the day prior to the operation, as well as the type of surgery, the number of operated joints/legs, the antimicrobial usage, and the antibiotic agent applied. Case files with incomplete documentation were not considered for statistical evaluation. Horses that had contracted a disease not associated with the surgery or which had already left the hospital on the day of surgery were not included. Patients that were euthanized during surgery due to irreparable findings (e.g. extreme cartilage destructions) were also not incorporated in the retrospective study.

3.1.3 Data extraction

The case details extracted comprised signalment, type, year, and season of the surgery, as well as the number of legs/joints operated on. The horses included in the statistical evaluation stayed for at least one night after surgery. At the discharge of each patient, the clinic asked the owners to contact them when complications occurred. The data were noted on antibiotic usage and any substances used as well as on the time and type of complications. The veterinary surgeon in charge decided on the antibiotic treatment based on his personal experience and preferences.

Complications included joint/wound infections, neuroma and seroma, dehiscence, any kind of wound discharge and its character, moderate and severe swelling as well as fever and inflammation of the subcutaneous tissue. Therefore, details were necessary of the regular surgical wound evaluation during bandage changes and the daily measurement of the rectal temperature. Fever was defined as a rectal temperature of at least 38.6°C – at this temperature, the horses were not only monitored more closely, but also received additional medications. Fever was either listed as being the sole postoperative complication or occurring associated with a complication at the surgical site or associated with alterations at the incision site plus suspected respiratory/gastroenteric disease.

The responsible orthopaedic surgeon and assistants were noted for all surgeries. Veterinarians that had participated in less than 30 surgeries were merged into one group for statistical analysis. The duration of postoperative hospitalisation and outcome was also excerpted. Possible outcomes were discharge from the clinic, second orthopaedic surgery, or euthanasia due to surgery-related complications. If available, the white blood cell count of the day prior to surgery was extracted from the patient file as well.

The information was manually transferred from the patient chart into an excel file. After checking it twice, the data was encoded for the statistical analysis. For this purpose, antibiotic agents belonging to the same class, as well as agents and combinations of those with a similar spectrum of efficacy, were listed together. Procaine-benzylpenicillin, procaine-benzylpenicillin plus dihydrostreptomycin, and amoxicillin were grouped together as “penicillin-based antibiotics”. If these agents were combined with gentamicin, they were grouped as “gentamicin combined with penicillin-based antibiotics”. Also, the cephalosporins (ceftiofur, cefquinome, and cefalexin) were incorporated into the group “gentamicin combined with penicillin-based antibiotics” due to their expected broad spectrum efficiacy. Trimethoprim/sulfadiazines formed the third group. A fourth group consisted of other combinations such as gentamicin with trimethoprim/sulfadiazine or gentamicin with cefquinome. Antimicrobial treatment usually started on the evening prior to surgery. The second dose was maximally given six hours before induction of anaesthesia. Antibiotics were applied for 3-5 days.

3.1.4 Statistical analysis of the retrospective study

The statistical assessment of the data was supported by Dr. Klaus Failing and Marion Sparenberg from the Unit for Biomathematics and Data Processing, Justus Liebig University, Gießen/Germany.

During the retrospective part of the study, the data were statistically compared of the two independent groups, (a) horses that underwent clean orthopaedic surgery and received antibiotics (the group with perioperative antibiotic prophylaxis: PAP) versus (b) horses without any application of antimicrobial drugs (control). The main target parameter was the rate of postsurgical complications.

Most of the statistical tests were conducted using BMDP/Dynamic, Release 8.1 (Dixon 1993) (Statistical Solutions Ltd., Cork/ Ireland). For Fisher’s exact test and the exact Wilcoxon-Mann-Whitney test, StatXact-9 (9.0.0, Cytel, Cambridge, Massachusetts/USA) (Cytel 2010) was additionally used. The Fisher’s exact test was conducted when there were any differences in qualitative parameters between the horses receiving antibiotics and the controls, such as the occurrence of complications and the occurrence of exudation and its character. Furthermore, the Fisher’s exact test was applied to determine possible differences between the groups and the types of surgery, surgeon and assistant, and the year and season of surgery. This test was also used to detect differences in types of complications, development of fever, the outcome as well as gender and breed between the treated animals and the controls. The Wilcoxon-Mann-Whitney test was used to determine the differences between the groups in quantitative parameters like hospitalisation time, the day after surgery on which complications arose as well as the age of the horses. The exact Wilcoxon-Mann-Whitney test was applied to determine differences between the PAP and control patients in the number of joints/legs operated on and the severity of the complication.

Additionally, a multifactorial ANOVA was performed which considered the overall effects of possibly influencing variables like the kinds of surgical procedures, the number of surgical lesions, the surgeon, and the season.

Microsoft® Excel® for Mac 2011 (Version 14.5.2) was used for graphically presenting the data. The mean ± standard deviation is given for describing the data when not mentioned otherwise. The significance level was set at $p < 0.05$ as usual.

3.2 Prospective clinical study

The prospective part of the study was performed to compare the development of surgical site infections in equine clean orthopaedic surgery with and without antimicrobial prophylaxis. Except for the preoperative antimicrobial prophylaxis, the study participants followed the same medication and evaluation protocols. Another aim of the study was to evaluate the course of inflammatory parameters of the horses with and without preoperative antimicrobial prophylaxis and to evaluate their possible use as predictive and/or diagnostic parameters for surgical site infection.

3.2.1 Study plan

For this prospective randomized clinical trial, horses that underwent elective orthopaedic surgery were repeatedly examined over a period of seven days (Figure 1). To determine the number of animals needed per group, the Sample Size Calculator (http://clincalc.com/Stats/SampleSize.aspx) was used as a study power calculator. An expected complication rate of 8% was set for the antimicrobial-treated horses (MacDonald et al. 1994) and of 20% for the control group. The alpha value was set at 0.3, and the statistical power as 60%, with the result of a minimal sample size of 28 horses per group. The horses were randomised into an antibiotic-receiving and a non-antibiotic-receiving (control) group. The probands were assigned to the groups with the help of a randomization list that was created beforehand in Microsoft® Excel® 2011 (Version 14.5.2, Microsoft Corporation, Redmond, USA). Data were collected from mid January 2015 to October 2015.

In comparison to the usual procedures in the clinic, no additional examinations or additional blood samples were taken, just the blood volume was increased to a total volume of 25-30 ml per sample. According to the relevant authority (Regierungspräsidium Gießen), at that time, the animal welfare laws in Germany therefore did not require an approval procedure. Owners were informed about the additional blood examinations, but only charged for the usual creatinine analysis.

The horses included were randomised on the evening prior to their surgeries. If there was more than one surgery per day, the patients were listed in the same succession as on the surgery plan. On July 6th, 2015, a second randomization list was created in order to reach the required number of horses in each group since 15 horses had to be excluded from the study due to reasons mentioned in chapter “4.2.1.1 Drop-outs". The author has personally examined 60 of the horses. However, due to the end of the author’s internship at Tierklinik Lüsche GmbH in mid July 2015, two colleagues clinically examined the remaining 15 horses, and the laboratory team (2 persons) of the clinic was responsible for the laboratory evaluation (Table 5). All persons involved became acquainted with the procedures in detail while the author was working at the clinic.

The wounds were evaluated three times postoperatively on days 1, 3, and 5 (Figure 1) according to the specific criteria of a scoring system prepared beforehand (see chapter 3.2.4.4).

Also, the inflammatory parameters SAA, haptoglobin, iron, albumin, as well as total serum protein, were determined on the same days the wounds were evaluated. Due to the regular postoperative monitoring of creatinine at Tierklinik Lüsche GmbH (Figure 1), there were no additional venipunctures. A maximum of 24 ml of blood was withdrawn per horse. The inflammatory parameters were determined at the central laboratory of the department of veterinary medicine of the Justus Liebig University, Gießen.

Day -1 Day prior to surgery	Pre-anaesthetic examination Haematology, Creatinine & IP **Randomization**
Day 0 Day of surgery	Pre-surgical check – up Surgical intervention Post-surgical check – up
Day 1 after surgery	Postural control **New bandage/wound evaluation** 2x rectal temperature Creatinine & IP
Day 2 after surgery	Postural control 2x rectal temperature
Day 3 after surgery	Postural control **New bandage/wound evaluation** 2x rectal temperature Creatinine & IP
Day 4 after surgery	Postural control 1x rectal temperature
Day 5 after surgery	Postural control **New bandage/wound evaluation** 1x rectal temperature Haematology, Creatinine & IP

Figure 1: Timing of clinical and laboratory examinations in the prospective study

IP: inflammatory parameters (SAA, haptoglobin, iron, albumin, total protein)

3.2.2 Study population

The study population consisted of horses that were presented to the Tierklinik Lüsche for an elective orthopaedic intervention. The study probands had to be clinically healthy except for the orthopaedic reason for surgery. Horses with chronic disease such as RAO or PPID were considered clinically healthy when only mild abnormalities were detected in the clinical examination and/or haematology. The participating horses had to be at least three months old. Surgeries took place at the equine clinic in Lüsche (Tierklinik Lüsche GmbH) except for five surgical interventions that were performed at Sanakena (part of Tierklinik Lüsche GmbH), an orthopaedic clinic for horses with a rehabilitation centre attached in Pinneberg. At Tierklinik Lüsche GmbH, the interns had to rotate to Sanakena where the surgery protocols are the same as at Tierklinik Lüsche GmbH. These five surgeries were conducted during the author's rotation to Sanakena.

The same examination pattern was applied to all horses. This included the clinical examinations and the laboratory tests (Figure 1).

Drop-out criteria were a contraction of a disease not associated with the surgical procedure, such as infection of the respiratory tract or a surgical colic. Horses that followed a different examination schedule or had their perioperative antibiotic protocol changed by the responsible surgeon during or directly after the procedure, were also dropped out of the study. If the horses required antibiotic treatment within the study period but not due to the above mentioned reasons,, they stayed in the study. Patients leaving the hospital less than five days after surgery were also dropped out.

3.2.3 Medication protocol

3.2.3.1 Antibiotics

Patients in the antibiotic group received 10 mg/kg amoxicillin (Belamox®, bela-pharm, Vechta, Germany) and 6.6 mg/kg gentamicin (Genta 100mg/ml, CP-Pharma, Burgdorf, Germany). The drugs were administered once via an I.V. catheter, which was placed by the anaesthetist directly (< 1 min), prior to using it to induce the general anaesthesia. Between the administration and the initial incision, 30 to 45 minutes passed. The horses of the control group were neither administered antibiotics nor a placebo.

3.2.3.2 Analgesia

Either phenylbutazone (Phenylbutariem® 100 mg/ml Suspension zum Eingeben für Pferde und Ponys, Ecuphar NV/SA, Oostkamp/Belgium) or meloxicam (Metacam® 15 mg/ml, Suspension zum Eingeben für Pferde, Boehringer Ingelheim, Ingelheim, Germany) were used. Study participants which were not considered for food production according to their equine passport received 2.5 mg/kg phenylbutazone orally twice daily, which is the recommended maintenance dose of the manufacturer. Horses that were intended to possibly serve for food production received 0.6 mg/kg meloxicam orally once per day as recommended by the manufacturer. The medication started on the evening prior to surgery and ended on the morning of the fourth day after surgery.

3.2.3.3 Anaesthesia

At Tierklinik Lüsche GmbH and in Sanakena, a standardized anaesthesia protocol existed for orthopaedic surgeries, which was applied to all participants of the study. Before being taken to the induction room, a catheter (Braunüle® MT, B. Braun Vet Care GmbH, Tuttlingen/Germany) was placed in the jugular vein. One patient had both jugular veins thrombosed, and therefore, the catheter was placed in the right cephalic vein. Through the catheter, the horses received 0.4 mg/kg xylazine (Xylazin 2%, CP Pharma, Burgdorf/Germany) while they were still in the stable. After being led to the induction box, the horses received 0.8 mg/kg xylazine (Xylazin 2%, CP Pharma, Burgdorf/Germany) and L-Polamivet® (MSD Tiergesundheit, Unterschleißheim/Germany). L-Polamivet® contains levomethadone (2.5 mg/ml) in combination with fenpipramide (0.125 mg/ml). The levomethadone was dosed at 0.05 mg/kg, which meant 2 ml of L-Polamivet® per 100 kg. To induce a general anaesthesia, 2.2 mg/kg ketamine (Ketamin 10%, CP Pharma, Burgdorf/ Germany) and 0.08 mg/kg diazepam (Diazepam Ratiopharm®, Ratiopharm GmbH, Ulm/Germany) were given intravenously. The anaesthesia was maintained by inhalation of isoflurane (Isofluran CP®, CP Pharma, Burgdorf/Germany) in oxygen (Westfalen AG, Münster/Germany) through an orotracheal tube. While the surgery field was being prepared antiseptically and during the orthopaedic procedure, patients received an infusion of isotonic sodium chloride solution (Isotonische Natriumchlorid-Lösung ad us. vet. B. Braun, B. Braun Vet Care GmbH, Tuttlingen/Germany). Dobutamine (Dobutamin-hameln 5 mg/ml Injektionslösung, Hameln Pharma Plus GmbH, Hameln/Germany) was added to the solution when indicated. As a postoperative sedation, xylazine was used (0.2 mg/kg). After recovering from surgery, each patient received 200 ml of plasma prepared from horses owned by the clinic. The catheter was removed after the plasma transfusion.

3.2.4 Clinical examinations

3.2.4.1 Pre-anaesthetic health check

The horses were stabled at the clinic for at least one day before the elective surgical procedure. Gender, weight and age of every patient was recorded. In the evening before the surgical intervention, the study participants were checked for posture, behaviour, and body condition. The oral mucous membranes were checked for colour, moisture, and capillary refill time. The conjunctival membranes and sclerae were examined for their colour and status of their vessels. Nostrils were inspected for abnormal discharge. The discharge was considered abnormal when it appeared other than mild and serous. The pulse was palpated at the facial artery and evaluated for frequency and quality. The mandibular lymph nodes were palpated for size, consistency, and possible sensitivity to pain. By slightly compressing the first tracheal ring manually, it was tested whether a cough was induceable. Both jugular veins were investigated for integrity and venous pulse. The trachea and lungs were auscultated for abnormal respiratory noises. The respiratory rate was counted by closely watching the thorax and the abdomen for motions associated with breathing (8-16 breaths per minute were considered normal). The heart was carefully examined by auscultation, and the heart frequency was noted (28-40 bpm was considered normal). The intensity of the heart sound was evaluated as well as the regularity of the heartbeat, the distinction between the first and second heart tones, and any heart murmurs. Peristalsis was evaluated by listening to gut movement in the quadrants. The posture and weight bearing of all four limbs were checked. The pulses were also palpated digitally. The body temperature was measured rectally using a digital thermometer (Thermometer-Microlife VT 1831, Lehnecke, Schortens/Germany). A temperature between 37.0 °C and 38.0 °C was considered normal. Temperatures above 38.5 °C were defined as fever. In foals up to an age of three months, temperatures up to 38.5 °C were considered normal and temperatures above 39.0 °C were defined as fever. In mares, the vulva was checked for abnormal swellings and/or discharge; in stallions, the testicles were examined for complete descent, signs of inflammation, and hernia. In both stallions and geldings, the prepuce was investigated for signs of inflammation such as swelling.

3.2.4.2 Pre- and post-operative check-ups

The horses were evaluated immediately before and again on the evening after surgery. Behaviour and posture were examined, and the heart rate, respiratory rate, and rectal temperature were determined. The oral mucous membranes and capillary refill time were also checked. On the evening after surgery, the vein into which the catheter had been placed was checked for integrity and signs of inflammation.

During the next five days, the horses were examined daily, but the results of the general examinations were not included in the statistical analysis. The patients were also checked daily for posture, behaviour, and weight bearing. For the first three days after surgery, the rectal temperature was measured mornings and evenings, and on days four and five, only in the morning.

3.2.4.3 Bandage change

Gloves (Vasco® Basic, Braun Vet Care, Tuttlingen/Germany) were worn while changing the bandages. The distal limb bandages consisted of a layer of mull (Vetrol Mullwatterolle, Farm and Stable KG, Wiehl/Germany) fixed with an elastic bandage (Raucolast®, Lohmann-Rauscher, Neuwied/Germany) and then covered with a self-adhesive bandage (Prowrap, Farm and Stable KG, Wiehl/Germany). If the operated joint was the coffin joint, the bandage was fixed distally by adhesive tape to the hoof (Gewebe Reparaturband silber, engelbert strauss GmbH, Biebergemünd/Germany). After tarsus arthroscopies, the bandages consisted of a layer of mull and an adhesive bandage (Optiplaste®-C, BSN medical GmbH, Hamburg/Germany). A bandage was not applied on patients recovering from knee surgery. Instead, the incision sites were covered with a Zinc Cover (ESS GmbH, Bakum/Germany). These surgical lesions at the knee were looked after daily, but the evaluation sheet was filled in only on the first, third, and fifth days after surgery as well as after all other procedures.

3.2.4.4 Wound evaluation

For evaluating the wounds, a semi-quantitative scoring system was developed by the author, which was applied during the bandage changes on the first, third, and fifth day after surgery. Before starting this clinical part of the study, the scoring system was tested on patients that had undergone orthopaedic surgery. If a patient had more than one surgical lesion, the highest score determined was entered into the evaluation sheet. The appearance of the wound was judged for wound exudation (Table 1), swelling and pain (Table 2), skin temperature (Table 3), and suture dehiscence (Table 4).

Exudation (Table 1) was scored according to its character. The amount of exudate was not considered because it was only present in the amount absorbed by the bandage material and according to experience, only in minimal to slight amounts. Non-purulent exudate was scored with 1 point. Purulent exudate was considered severe and was therefore scored with 3 points. For this parameter, a score of 2 points did not exist.

A swelling (Table 2) was scored with 1 point if the area operated on was only slightly thicker than it had been before surgery and was not painful by touch to the horse. The swelling was scored with 3 points if the distension of the tissue was drastic, the sutures were notching the swollen tissue, and touching the area was painful. The back of one’s gloved (Vasco® Basic, Braun Vet Care, Tuttlingen/Germany) hand was used to evaluate the temperature of the skin (Table 3). One score point was considered when the skin felt slightly warmer than the surrounding area. The increase in skin temperature was considered severe (3 points) when the skin felt hot.

The surgical wounds were closed in a simple interrupted pattern. Minimally invasive procedures required 1-2 sutures per incision. For other interventions, the number of sutures depended on the length of the surgical incision. Usually, 5-7 sutures were placed. Dehiscence was scored with 3 points when more than half of the sutures were out of place (Table 4). For non-minimally invasive interventions where less than 50% of the sutures were dehiscent, a score of 1 point was given when there were only 1-2 sutures dehiscent, and a score of 2 points if more than 2 sutures were involved.

For calculating the total score, the score points for exudation, swelling and skin temperature were added up. Dehiscence was not included in the total score, as clinical improvement was not possible in this parameter. A statistical analysis used the score points of each parameter as well as the sum of the score values.

Table 1: Evaluation of exudation

Character of the exudate	Clinical score
Dry and clean	0
Serous	1
Serosanguinous	1
Purulent	3
Haemopurulent	3

Table 2: Evaluation of swelling and pain

Extent of swelling and pain	Clinical score
No swelling	0
Low-grade swelling, not painful	1
Middle-grade swelling, mildly painful	2
High-grade swelling, severely painful	3

Table 3: Evaluation of skin temperature

Extent of calor	Clinical score
Normal skin temperature	0
Mild increase	1
Moderate increase	2
Severe increase	3

Table 4: Evaluation of dehiscence

Severity of dehiscence	Clinical score
No sutures dehiscent	0
1-2 sutures dehiscent	1
≤ 50% of sutures dehiscent when more than 2 sutures were applied	2
> 50% of sutures dehiscent	3

An evaluation sheet which was placed in front of the patient's stable had to be filled in by the author after every bandage change and wound evaluation. After the author's internship at Tierklinik Lüsche GmbH, the wounds of 15 horses were examined by two colleagues who were very well acquainted with the scoring system. Together with the author, they evaluated the surgical wounds of at least five of the horses before evaluating the wounds on their own.

3.2.5 Laboratory studies

3.2.5.1 Blood collection and preanalytic procedures

Blood for the laboratory tests was obtained by puncturing the jugular vein on the day prior to surgery as well as on day one, three, and five after surgery. A gauze swab (ES Kompressen unsteril 5x5 cm 8fach 100 ST PZN 01447157, Paul Hartmann AG, Heidenheim/Germany) was moistened with alcohol (kodan® Tinktur forte farblos, Schülke & Mayr GmbH, Norderstedt/Germany), and the puncture site of the jugular vein was disinfected. A 20G needle (Terumo® Agani Needle 20G x 1½" Yellow, Terumo Deutschland GmbH, Eschborn, Germany) was placed into the vein at a 45° angle. A syringe (BD DISCARDIT™ II, BD, Heidelberg/Germany) was then attached and blood was aspirated. If the patient had thrombosed jugular veins, the blood was taken from the sinus venae transversae faciei. After disinfecting it, a 20G needle was inserted perpendicular to the head directly under the facial crest, and a vacuum was created.

Directly after recovering from the surgery (before plasma transfusion), blood was obtained through the intravenous catheter. Of the blood obtained, the first 20 ml were discarded. The blood thus collected was directly transferred into tubes (VACUETTE® Röhrchen, Greiner Bio-One GmbH, Frickenhausen/Germany). After collecting each blood sample, the blood was filled into a Lithium-Heparin tube and a serum tube (Z Serum Sep Gerinnungsaktivator, VACUETTE® Röhrchen, Greiner Bio-One GmbH, Frickenhausen/Germany). An EDTA tube (K3E K3EDTA, VACUETTE® Röhrchen, Greiner Bio-One GmbH, Frickenhausen/Germany) was collected on the day prior to surgery as well as on day 5.

The blood was processed within two hours. EDTA tubes were inverted several times in order to achieve an equal cell distribution throughout the sample before blood was transferred into the LaserCyte® tubes or the VACUETTE® tubes were inserted into the ProCyte®. To separate the blood cells from the plasma, the Lithium-Heparin tubes were centrifuged for 5 minutes at 865 g (EBA 20, Andreas Hettich GmbH & Co. KG, Tuttlingen/Germany). The heparin plasma was used for measuring the creatinine. The serum containers were centrifuged for 10 minutes at 865 g. The serum was decanted into multi-purpose tubes (Röhre 13ml, 95x16.8mm, PS, Sarstedt, Nümbrecht/Germany) and stored at -18°C. The blood samples of horses operated in Sanakena were similarly centrifuged. The heparin plasma and serum were decanted into multi-purpose tubes (Röhre 13ml, 95x16.8mm, PS, Sarstedt, Nümbrecht, Germany), frozen at -18°C and transported frozen to Lüsche on the next day. The EDTA samples were stored at 4°C and transported cooled to Lüsche on the day after collection, too. The laboratory investigations (CBC, creatinine) were performed immediately after delivery. In order to prove that there were no valid differences between haematology values of the same sample directly after having been obtained and after having been refrigerated for 24 hours, a storage experiment of five blood samples was performed. WBCs were performed directly after blood collection and after being refrigerated for one day. A correlation factor of 0.99 was found.

3.2.5.2 Laboratory parameters

To monitor the patients' health before and after elective surgery, a number of laboratory tests were performed (Table 5). For reference intervals, the ranges specified by the respective laboratory or manufacturer were applied and are listed together with the respective results.

Table 5: Laboratory investigations

	Day				
	-1	**0**	**1**	**3**	**5**
Haematology[1]	X				X
Creatinine[1]	X		X	X	X
SAA[2]	X	X	X	X	X
Iron[2]	X	X	X	X	X
Haptoglobin[2]	X	X	X	X	X
Albumin[2]	X	X	X	X	X
Globulin[2]	X	X	X	X	X
Total Serum Protein[2]	X	X	X	X	X

[1]: Tierklinik Lüsche GmbH
[2]: Central Laboratory, Department of Veterinary Medicine, Justus-Liebig University, Gießen

Haematology was performed on the day prior to surgery and on the fifth day after surgery. For the first 46 probands, the LaserCyte® Dx Hematology Analyzer (IDEXX Europe B.V., Hoofddorp/The Netherlands) was used. After these 46 horses, the ProCyte Dx® Hematology Analyzer (IDEXX Europe B.V., Hoofddorp/The Netherlands) was utilised due to a laboratory machine change in the clinic. The LaserCyte® Dx Hematology Analyzer works with laser flow cytometry (Idexx 2015). The ProCyte Dx® Hematology Analyzer also is based on laser flow cytometry, but additionally utilises optical fluorescence and laminar flow impedance™ (Idexx 2015). The equality of both analysers was tested by comparing CBCs in sixteen blood samples from different horses. A correlation factor for WBC of 0.99 was found.

To monitor the health of the horses' kidneys, the creatinine was measured every second day (starting one day prior to surgery) until the fifth day after surgery. The creatinine was determined with the Catalyst Dx® Chemistry Analyzer (IDEXX Europe B.V., Hoofddorp/The Netherlands). Spectral analysis is the functional principle of the Catalyst Dx® Chemistry Analyzer for determining the concentrations of blood parameters (Idexx 2015).

The serum amyloid A, iron, haptoglobin, albumin, globulin, and total serum protein were measured at the central laboratory of the department of veterinary medicine of the Justus-Liebig-University, Gießen. The samples were transported frozen to the laboratory where they were stored at -18°C until analysed in batches. The automated analyser utilized was a Pentra 400 ABX (HORIBA ABX SAS, Montpellier Cedex 4/France). The SAA was determined using the latex agglutination assay “Eiken” serum amyloid A (Mast House, Bootle, Merseyside/United Kingdom) where SAA-antibodies bonded with latex react with the SAA possibly present in the sample. The SAA-concentration is determined by measuring the change in turbidity (Mast House 2015). For haptoglobin, the PHASE™ RANGE Haptoglobin kit (Second Generation; Tridelta Development Limited, Maynooth/Ireland) was used. This measurement is based on the preservation of the peroxidase activity of haptoglobin-haemoglobin complexes at a low pH, as the peroxidase activity of haemoglobin alone is inhibited under acidic conditions (Tridelta Development Limited 2015). The method used for determining the iron was the ABX Pentra Iron CP (HORIBA ABX SAS, Montpellier Cedex 4, France) was colourimetry. Under acidic conditions, the iron is separated from the transferrin. This is followed by a redox reaction with iron and ascorbic acid (reduction from Fe^{3+} to Fe^{2+}), then blue iron (II) – ferene complexes form and are quantitatively detected (Horiba 2015). Colourimetry is also the basic principle in the albumin measurement by the ABX Pentra Albumin CP kit (HORIBA ABX SAS, Montpellier Cedex 4, France). At pH 4.2, bromocresol green binds selectively to albumin, and a blue colouration results (Horiba 2010). The biuret test is used for determining the total serum protein (LT Sys® Labor + Technik, Eberhard Lehmann, Berlin, Germany). Bivalent copper and protein form purple complexes under alkaline conditions. The colour intensity is directly proportional to the protein concentration (Hallbach 2006). The concentration of globulins is determined by subtracting the albumin concentration from the total serum protein concentration. All measurements were performed in accordance with their manuals by experienced laboratory personnel.

3.2.6 Statistical analysis of the prospective study

The statistical assessment of the data was supported by Dr. Klaus Failing and Marion Sparenberg from the Unit for Biomathematics and Data Processing, Justus-Liebig University, Gießen/Germany.

The the data of the two independent groups – horses that underwent clean orthopaedic surgery and received antibiotics as a single shot (treated) versus horses that had no antimicrobial drugs administered (control) – were compared statistically. The main target parameters were the score points assorted to the surgical incisions.

Most of the statistical tests (ANOVA, Wald test, t-test, chi-square test, Wilcoxon-Mann-Whitney test) were conducted using BMDP/Dynamic, Release 8.1 (Dixon 1993) (Statistical Solutions Ltd., Cork/Ireland). For Fisher's exact test and the exact Wilcoxon-Mann-Whitney test, StatXact-9 (9.0.0, Cytel, Cambridge, Massachusetts/USA) (Cytel 2010) was additionally used. Data are presented as arithmetic mean ± standard deviation (minimum – maximum), if not mentioned otherwise and the significance level was set at 0.05 as usual.

Data were tested for normal distribution. If data were skewed (Serum Amyloid A, age), they were logarithmized before analysis and and delogarithmized for presentation. For these data, instead of comparison the arithmetic means and standard deviation, the geometric means and dispersion factors were used instead.

Treated and control group tested for equality before the intervention. Groups were tested for differences in age (Wilcoxon-Mann-Whitney test), weight (t-test), and gender (Fisher's exact test).

Also, the groups were tested for differences regarding surgery-related factors: for the main target parameters, namely the score points applied to the wounds, the exact Wilcoxon-Mann-Whitney test was used.

For the secondary objective parameters, Fisher's exact test, the t-test, and the Wilcoxon-Mann-Whitney test were performed.

The t-test was performed to detect differences in rectal body temperatures between the groups at the single measurements per day. Also, the t-test was used to determine the differences between the groups regarding WBC, creatinine, iron, haptoglobin, albumin, globulin, and total protein as well as rectal body temperatures. To test for differences in the SAA-concentrations, the Wilcoxon-Mann-Whitney test was performed.

Fisher's exact test was used to detect any differences regarding the type of surgery and the number of legs/joints operated on, the various analgesic drugs used, and the surgeon. The t-test was applied for testing for differences in duration of the surgery. The chi-square test was used for testing for differences in seasons and the Wilcoxon-Mann-Whitney test was used for testing for differences in hospitalisation time.

Additionally, a multifactorial ANOVA was performed to consider the common effect of the influencing variables daytime and/or day of measurement on clinical scores, laboratory parameters, and body temperatures. Since not all data sets were complete, the Wald test (method of maximum likelihood) was used for the laboratory parameters.

For the graphical presentation of the data, Microsoft® Excel® for Mac 2011 (Version 14.5.2) was used.

4 Results

4.1 Retrospective study

4.1.1 Study population

Of the 684 case files retrieved, 32 horses (4.7%) were not included in the statistical evaluation. Reasons for not considering these case files were a development of respiratory disease post-surgically (13), incomplete documentation (8), intra-operative euthanasia (4), surgical treatment of other diseases that required induction of antimicrobial therapy (4), development of severe gastrointestinal disease (2), and one horse in whose file arthroscopy was scheduled but was actually presented for a septic joint. In total, the case files of 652 horses contained complete documentation. For 560 (85.9%) of these, information on the breed was given: 524 were Warmbloods (93.6%), and only 36 patients belonged to other breeds. These included Quarter Horses (11), ponies (9), Friesians (3), Arabian horses (2), Icelandic horses (2), Pura Raza Española (1) as well as Haflinger (1), Norwegian Fjord horse (1), Trotter (1), Welsh (1), Thoroughbred (1), and Lipizzaner (1). Furthermore, one Arab-Icelandic cross and one Arab-pony cross were included in the study.

The gender was noted in 616/652 (94.5%) of horses included. The study population consisted of 154 stallions, 251 geldings, and 211 mares (Table 6). The frequency of applying the antibiotics significantly varied between the sexes ($p = 0,0001$): 54.5% of the stallions received antibiotics whereas only 34.1% of the mares and 36.3% of the geldings received perioperative antimicrobial treatment.

Table 6: Distribution of gender (known for 616/652)

	PAP		Control		Total	
	n	**%**	**n**	**%**	**n**	**%**
Geldings	91	36.8	160	43.4	251	40.7
Mares	72	29.1	139	37.7	211	34.2
Stallions	84	34.0	70	19.0	154	25.0
Total	247	100	369	100	616	100

PAP: perioperative antimicrobial prophylaxis

The horses' age, for which the details were available on 615/652 (94.3%) horses, ranged from 43 days (0.12 years) to twenty years. The horses that received prophylactic antimicrobials were significantly younger (5.0 ± 3.9 years) than the controls (7.1 ± 4.3 years; $p < 0.0001$). The body weight was recorded for 73/652 (11.2%) horses, which means that 579 horses were not weighed prior to surgery, but their body weight was estimated by experienced clinicians. Treated horses (593 ± 95 kg) were significantly heavier than the controls (532 ± 133 kg; $p=0.03$).

A white blood cell count (WBC) was performed in 302/652 (46.3%) cases prior to surgery. The average WBC was 7.90 ± 2.34 G/l (range: 2.90 – 16.65 G/l). The WBC in treated horses (8.57 ± 2.58 G/l; n=135) was significantly higher ($p < 0.0001$) than in controls (7.37 ± 1.98 G/l; n=167). Among the different kinds of surgery, the WBC was distributed significantly different ($p=0.012$). However, this was attributed mainly to the comparatively rare desmotomies and splint bone extractions in which surgeons preferred to administer antibiotics if they showed relatively high WBCs. In a total of only six combinations of surgical procedures, antibiotics were administered to cases with low WBC (Table 7).

Table 7: Kind of surgical intervention and respective WBC (mean; G/l)

Kind of surgery	PAP (n=135)		Control (n=167)		Total
	n	WBC	n	WBC	n
Arthroscopy	107	8.62	109	7.58	216
Fasciotomy	3	7.43	12	6.52	15
Splint bone extraction	11	9.04	12	7.31	23
Tenonotomy	6	10.71	12	7.40	18
Tendovaginoscopy/Bursoscopy	2	6.18	7	7.06	9
Neurectomy	3	7.43	12	6.47	15
Combination	3	4.67	3	7.50	6

PAP: perioperative antimicrobial prophylaxis

Furthermore, in the multivariate analysis of just the patients in which a preoperative WBC was evaluated, a significant interaction was detected between the perioperative antimicrobial prophylaxis and the number of surgical lesions. However, overall the WBC was not significantly different between patients with 1, 2, or ≥3 surgical lesions.

4.1.2 Perioperative antimicrobial prophylaxis

4.1.2.1 Antibiotic agents

Of the 652 patients included, 259 (39.7%) received perioperative antibiotic prophylaxis (PAP), whereas 393 (60.3%) horses did not (controls). 171 horses received penicillin-based antibiotics, and 36 were given trimethoprim and sulfadiazine. Combinations of gentamicin and penicillin-based antibiotics was administered in 43 cases. Seven horses were treated with cephalosporins. Other combinations (cefquinome and gentamicin; trimethoprim-sulfadiazine and gentamicin) were used in two patients undergoing arthroscopy (Table 8). For the statistical analysis, gentamicin and penicillin-based antibiotic were grouped together with cephalosporins since a quite similar broad spectrum efficacy was assumed. The antibiotic administration started in the evening prior to surgery and continued for 3-5 days. Each antibiotic agent was dosed as appropriate to its pharmacokinetics. The antibiotic substances varied according to the preference of the surgeon and over time.

Table 8: Antibiotic agents used in the retrospective study

	Pen-b		TMS		Pen-b + Genta		Other	
	n	%	n	%	n	%	n	%
Arthroscopy	130	76.0	28	77.8	37	74.0	2	100
Fasciotomy	6	3.5	0	0.0	2	4.0	0	0.0
Splint bone extraction	12	7.0	2	5.6	5	10.0	0	0.0
Tenotomy	12	7.0	2	5.6	2	4.0	0	0.0
Neurectomy	6	3.5	2	5.6	0	0.0	0	0.0
Tendovaginoscopy/ Bursoscopy	2	1.2	1	2.8	0	0.0	0	0.0
Combinations	3	1.8	1	2.8	4	8.0	0	0.0
Total	171	100	36	100	50	100	2	0.0

Pen-b: Penicillin-based antibiotics: Procaine-benzylpenicillin, procaine–benzylpenicillin plus dihydrostreptomycin, and amoxicillin;
TMS: Trimethoprim-Sulfadiazin;
Pen-b+Genta: Penicillin-based antibiotics and gentamicin

4.1.2.2 Types of surgery

The application of antibiotics significantly varied between the types of surgery (p=0.0007). The majority of the patients underwent arthroscopy (462/652). Fourteen patients had two different procedures done during one surgical session. Perioperative antimicrobial prophylaxis was most often initiated in combinations of different surgeries (57.1%) and least often administered in tendovaginoscopy/bursoscopy (Table 9).

Table 9: Types of surgery and administration of perioperative antimicrobial prophylaxis (PAP) in absolute numbers and percentages

	PAP (n=259)		**Control (n=393)**		**Total (n=652)**	
	n	**%**	**n**	**%**	**n**	**%**
Arthroscopy	197	42.6	265	57.4	462	100
Fasciotomy	8	18.6	35	81.4	43	100
Splint bone extraction	19	46.3	22	53.7	41	100
Tenotomy	16	43.2	21	56.8	37	100
Neurectomy	8	25.0	24	75.0	32	100
Tendovaginoscopy/ Bursoscopy	3	13.0	20	87.0	23	100
Combinations	8	57.1	6	42.9	14	100
Total	259	39.7	393	60.3	652	100

4.1.2.3 Number of surgical lesions

The more surgical lesions were placed in one surgical session, the more often perioperative antimicrobial prophylaxis was initiated (p= 0.03). In 479 horses, one joint/leg was operated, and in 180 (37.6%) of these patients, perioperative antibiotics were prescribed. Thirty horses had three or more surgical interventions in one session, and two-thirds of them were operated under antibiotic cover (66.7 %; Table 10).

Table 10: Perioperative antimicrobial prophylaxis (PAP) and number of surgical lesions

	PAP (n=259)		Control (n=393)		Total (n=652)	
	n	%	n	%	n	%
1 surgical lesion	180	37.6	299	62.4	479	100
2 surgical lesions	59	41.3	84	58.7	143	100
≥ 3 surgical lesions	20	66.7	10	33.3	30	100
Total	259	39.7	393	60.3	652	100

4.1.2.4 Surgeons

629 out of the 652 surgeries were performed by four surgeons (297, 136, 109, and 87 each). Twenty horses were operated on by four other surgeons. For three interventions, the surgeon could not be identified. The frequency of antibiotic prophylaxis significantly depended on the surgeon ($p<0.0001$). Three colleagues used antibiotics in 28.6% to 39.0% of their cases (n=520) whereas the fourth surgeon and the group "other surgeons" administered antibiotics to 65.0% and 73.4% of their patients (n=129, Table 11).

Table 11: Performing surgeon and application of perioperative antimicrobial prophylaxis (PAP; known for 649/652)

	PAP (n=259)		Control (n=393)		Total (n=649)	
	n	%	n	%	n	%
Surgeon A	85	28.6	212	71.4	297	100
Surgeon B	28	32.2	59	67.8	87	100
Surgeon C	53	39.0	83	61.0	136	100
Surgeon D	80	73.4	29	26.6	109	100
Other	13	65.0	7	35.0	20	100
Total	259	39.9	390	60.1	649	100

During the evaluation period, 42 veterinarians assisted the responsible surgeon. Six of them participated in more than 30 surgeries. For 51 procedures, the assisting surgeon could not be determined. Due to the high number of assisting veterinary surgeons and the even higher number of combinations with the responsible surgeons, an influence of the assisting veterinary surgeon was not assumed, and therefore, a further statistical analysis not pursued.

4.1.2.5 Year and season

The rate of antibiotic application varied significantly between years ($p < 0.0001$) and seasons ($p = 0.03$) of surgery. The annual distribution of the surgeries and the administration of perioperative prophylactic antibiotics during the period reviewed is shown in Table 12. Except for 2011, where the study started in the middle of the year, and 2015, which just included January, the number of surgeries ranged from 170 to 196 per year. Regarding the three full years of the study, the fewest horses were operated under antimicrobial cover in 2012.

Table 12: Annual distribution of surgeries and application of perioperative antimicrobial prophylaxis (PAP)

	PAP (n=259)		Control (n=393)	
	n	**%**	**n**	**%**
June to Dec. 2011	44	43.6	57	56.4
2012	50	27.5	132	72.5
2013	102	52.0	94	58.0
2014	61	35.9	109	64.1
Jan. 2015	2	66.7	1	33.3
Total	259	39.7	393	60.3

Table 12 shows the seasonal distribution of surgeries and the application of perioperative antimicrobial prophylaxis. The number of surgeries performed in the different seasons was almost equal. The fewest horses (150/652) were operated on in spring, and the most horses during winter (180/652). Perioperative antimicrobial prophylaxis was significantly applied differently between the seasons ($p = 0.03$). In winter and spring, percentage-wise more horses were operated on with antimicrobial prophylaxis than in summer and autumn.

Table 13: Seasonal distribution of surgeries and application of perioperative antimicrobial prophylaxis (PAP)

	PAP (n=259)		Control (n=393)	
	n	%	n	%
Dec.-Feb.	79	43.9	101	56.1
March-May	70	46.7	80	53.1
June-August	52	32.5	108	67.5
Sept.-Nov.	58	35.8	104	64.2
Total	259	39.7	393	60.3

4.1.3 Complications

Of the 652 surgical candidates, a total of 255 (39.1%) developed some kind of complications, whereas the healing was completely inconspicuous in 397 (60.9%) of the patients (Table 13). Overall, there was no significant difference in the development of post-surgical complications between the treated and the control horses (p=0.5). Entirely uncomplicated healing was observed in 162/259 (62.6%) antibiotic-receiving horses and in 235/393 (59.8%) controls. On the other hand, 97/259 (37.5%) horses that received antimicrobial prophylaxis and 158/393 (40.2%) controls developed complications during the healing process (Table 14).

Table 14: Development of complications and distribution of perioperative antimicrobial prophylaxis (PAP) in 255/652 patients in absolute numbers and percentages

	PAP (n=259)		Control (n=393)	
	n	%	n	%
Complication	97	37.5	158	40.2
Inconspicuous	162	52.5	235	59.8
Total	259	100	393	100

Looking into the kinds of complications in more detail, there were no significant differences between PAP and control horses as well.

4.1.3.1 Arthritis

The worst and potentially life-threatening complication, septic arthritis, occurred in 7/463 (1.51%) patients that underwent arthroscopy. One horse underwent arthroscopy and fasciotomy and was therefore classified into the “combination of surgical procedures” group. It developed septic arthritis 27 days after the procedure despite receiving perioperative penicillin. Looking at the patients that underwent only arthroscopy, the incidence in the controls was 2/265 (0.76%) and 4/197 (2.03%) in the group with perioperative antibiotics. This difference was not statistically significant ($p = 0.12$).

A total of 5 horses developing septic arthritis despite perioperative antibiotic administration received either penicillin (2), trimethoprim and sulfadiazine (2), or penicillin and gentamicin (1), starting on the day before surgery and ending five days after surgery. Septic arthritis occurred postoperatively on days 2 (3 horses), 3, 5, 6, and 27 respectively. One of the controls with septic arthritis was euthanized 9 days after surgery. More details on the outcome of horses with septic arthritis can be found in section 4.1.3.4 “Course and Outcome”.

4.1.3.2 Alterations at the incision site

Moderate and severe wound swelling was observed in 167/652 of the cases (25.6%). It was present in 99/393 controls (25.2%) and in 68/259 antibiotic-receiving horses (26.3%). Exudation was found in 75 patients. In 49 cases, this was the only postoperative complication. 34/49 (69.4%) did not receive antimicrobial prophylaxis, and 15 (30.6%) did. Purulent exudation was present in 12 patients (11 controls, 1 treated). Five controls with purulent discharge also developed suture dehiscence, three showed increased swelling too and one control concurrently developed a seroma (Table 15). Details on course and outcome can be found in section 4.1.3.5 “Course and outcome”.

Table 15: Complications after elective orthopedic surgery in equines with or without perioperative antimicrobial prophylaxis (PAP)

	PAP (n=259)		Control (n=393)		Total (n=652)	
	n	**%**	**n**	**%**	**n**	**%**
Dehiscence	4	1.5	8	2.0	12	1.8
Seroma	3	1.2	2	0.5	5	0.8
Exudation without incisional alterations	15	5.8	34	34.6	49	7.5
Exudation with incisional alterations	8	3.1	18	4.6	26	4.0
Moderate or severe Swelling	68	26.3	99	25.2	167	25.6
Fever without incisional alterations	2	0.8	13	3.3	15	2.3
Fever with incisional alterations	3	1.2	12	3.1	15	2.3
Individuals with at least one complication	97	37.5	158	40.2	255	39.1

4.1.3.3 Postsurgical fever

Postsurgical fever (≥ 38.6°C) occurred in 30/652 (4.60%) of the patients. Five of the 259 horses in the PAP group (1.93%) and 25/368 controls (6.79%) developed abnormally high body temperature. Statistically, there was no significant difference between the groups (p=0.2).

In 15 horses, fever was the only complication: 2/15 (13.3%) received perioperative antibiotics, and 13/15 (86.7%) controls developed just the high body temperature. Fourteen feverish horses showed an alteration at the incision site, too; two of these (14.3%) had been treated with antibiotics whereas the other 12/14 (85.7%) belonged to the control group.

One patient that received trimethoprim-sulfadiazine developed a fever and signs of septic arthritis on day six after surgery. One colt treated with penicillin developed a fever, seroma at the incision site, and self-limiting diarrhoea.

4.1.3.4 Time of occurrence

In the controls, complications occurred significantly earlier (3.5 ± 2.9 days; range 1-14 days) than in the antibiotic-receiving patients (4.9 ± 4.4 days; range 1-27 days; $p=0.01$).

Of all the 255 complications, 195 (76.5%) developed prior to the fifth day after surgery. Of these horses, 67 (34.3%) had been treated antibiotically, and 128 (65.6%) had not ($p = 0.13$). The interactions between the complications arising until day five and the antimicrobial prophylaxis varied according to season ($p = 0.005$).

The onset of two joint infections, one seroma/neuroma, seven cases of dehiscence, and nine cases of exudation occurred later than five days after surgery. The remaining 41 post-surgical complications setting in more than five days after surgeries were cases of increased swelling (Figure 2).

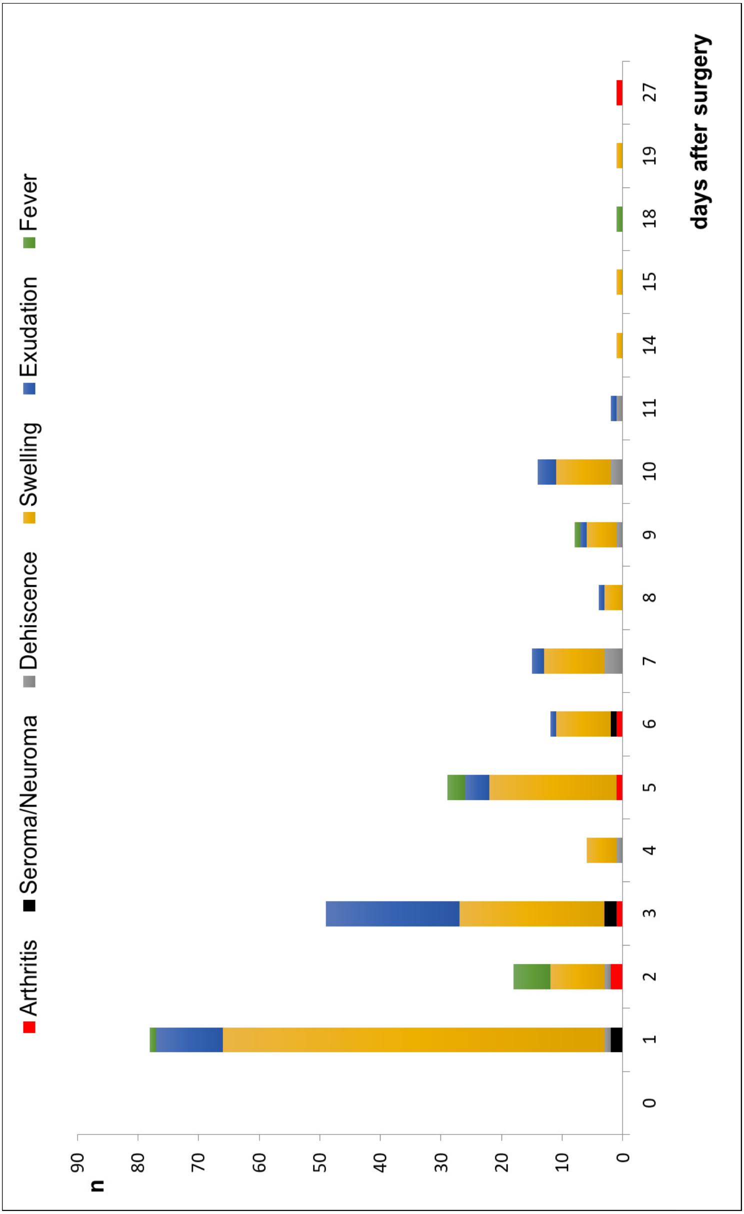

Figure 2: Onset of postsurgical complications in 255 treated and control horses

4.1.3.5 Course and outcome

As soon as a septic joint was diagnosed, which occurred in a total of seven patients that underwent arthroscopy (1.51%), systemic antibiotics, multiple joint lavages including the instilling of antibiotics, and intravenous regional limb perfusion were initiated. Horses with increased swelling were monitored carefully for other signs of surgical site infection. Alterations other than increased swelling were treated locally, and systemic antimicrobial therapy was initiated if necessary. In 14/25 control horses that developed fever, antimicrobial therapy was initiated. Of these 14 horses, eight did not show any alteration at the incision site.

Regarding the outcome, overall, no significant differences were found between the horses receiving antibiotics and the controls (p=0.3). Of all patients, 606/652 (92.9%) were discharged. Twenty-one antibiotically treated patients, as well as 21 controls, were operated on for at least a second time because more orthopaedic interventions were necessary. Two horses that were still unsuitable for riding remained in the clinic. Their hospitalization time was not included in the statistical analysis. One horse in the treatment group (penicillin) with no incisional alterations was humanely destroyed due to a severe lameness remaining despite prolonged treatment. One horse in the control group was euthanized due to complications in its septic joint after nine days. The other six horses were discharged. Information about a return to athletic performance was not available (Table 16).

Table 16: Outcome

	PAP (n=259)		**Control (n=393)**		**Total (n=652)**	
	n	**%**	**n**	**%**	**n**	**%**
Discharge	236	91.1	370	94.1	606	92.9
2nd surgery	21	8.1	21	5.3	42	6.4
Euthanasia	1	0.4	1	0.3	2	0.3
Stayed	1	0.4	1	0.3	2	0.3
Total	259	39.7	393	60.3	652	100

The median hospitalisation time for horses with antimicrobial prophylaxis was 11 days (range 2-179 days) and for controls 10 days (range 1-185 days, Table 17). The PAP horses nearly stayed significantly longer in the clinic than the control horses (p=0.055). The hospitalisation time was significantly affected by the surgeon (p=0.01) as well as by the type of surgery (p=0.001). The horses remaining at the clinic for a long time stayed either for rehabilitation under the supervision of a veterinary orthopaedic surgeon or due to a prolonged treatment of severe complications such as septic arthritis.

Table 17: Hospitalization time (known for 606/652)

	PAP (n=259)	**Control (n=393)**
Minimum (days)	2	1
1st quartile (days)	10	9
Median (days)	11	10
3rd quartile (days)	16	14
Maximum (days)	179	185

4.2 Prospective clinical study

4.2.1 Study population

4.2.1.1 Drop-outs

In total, 75 horses needed to be included in the randomized study in order to arrive at the aspired group size of 30 patients each. Sixteen patients (21.3%) dropped out due to one of the following reasons (Table 18):

Six horses were discharged after surgery without being hospitalized for five days. Five of these did not receive antibiotics.

Four horses were not examined exactly as scheduled. During their hospitalization, the emergency load at the clinic was so high that it was decided to change the bandages of these patients every third day instead of every second, as long as the patients were otherwise sound, and their vital parameters were within normal limits. Two of these patients received preoperative antibiotics, the other two did not. All wounds were inconspicuous, and the horses went home sound.

The antibiotic protocol of three horses was changed by the responsible surgeon. All three were randomized into the antibiotic receiving group. In two cases, the surgeon decided to administer additional gentamicin on the first two days after surgery. In the other case, additional antimicrobials were listed on the invoice but not in the patient file. Due to this discrepancy, it was decided to drop this patient from the study.

Three more horses were randomised without including their data in the statistical analysis: In Horse 42, the necessity of implants became evident intra-operatively. Horse 1 underwent a second surgery on day 4. This horse had OCD lesions in multiple joints, so that not all of them could be removed in one surgical session. Horse 33 developed a severe respiratory infection.

In total, nine drop-outs were randomised as controls whereas seven received preoperative antibiotics. Thirteen of the 16 drop-outs underwent arthroscopy, and tendovaginoscopy, fasciotomy, and fasciotomy/neurectomy were performed on one patient each.

Table 18: Characterization of drop-outs (n = 16)

Reason for drop-out	Patient Number	AB	Breed	Age	Gender	Type of Surgery
Early discharge	5	No	WB	2	G	Arthroscopy
	6	No	WB	2	G	Arthroscopy
	9	No	WB	2	S	Arthroscopy
	17	Yes	WB	2	G	Arthroscopy
	22	No	A	13	M	Arthroscopy
	35	No	WB	4	G	Arthroscopy
Change of examination protocol	46	No	WB	11	G	Arthroscopy
	47	Yes	WB	6	G	Fasciotomy
	48	Yes	WB	2	S	Arthroscopy
	49	No	Trotter	6	S	Arthroscopy
Change of antibiotic protocol	36	Yes	Pony	8	G	Tendovaginoscopy
	39	Yes	WB	5	M	Arthroscopy
	40	Yes	WB	15	G	Fasciotomy/ neurectomy
Implant	42	Yes	WB	11	G	Arthroscopy
2nd surgery	1	No	WB	2	G	Arthroscopy
Respiratory disease	33	No	WB	2	S	Arthroscopy

WB: Warmblood; A: Arabian; S: Stallion; G: Gelding; M: Mare; AB: Antibiotics

4.2.1.2 Characteristics of the study population

A total study population of 60 horses was planned with a group size of 30 patients each. However, Horse 36 completed the observation period but was excluded due to an inconsistency in its patient file. Therefore, 59 horses in total remained for statistical evaluation. Twenty-eight horses received preoperative antimicrobials, and 31 served as controls without antibiotic treatment. One horse did not receive antimicrobials due to a communication error, but stayed in the study as a control, because all its examinations took place as planned.

Fifty warmbloods, 3 ponies, 2 Arabian horses, 2 Quarter Horses, 1 Frisian as well as 1 Icelandic horse finished the study (Table 19). Of the 59 horses, eleven were stallions, 31 geldings, and 17 mares. Six stallions, 16 geldings, and 6 mares received prophylactic antimicrobial treatment. The genders were distributed evenly between the groups (p = 0.57).

The horses' average age was 5.3 ± 2.3 years. It ranged from four months to 23 years. The average age of horses with antimicrobial prophylaxis was 5.3 ± 2.8 years (0.3 – 23 years); the control group was aged 5.3 ± 1.9 years (2 – 16 years). The age was not significantly different between the groups (p = 0.97).

The horses weighed 534 ± 91.9 kg (190 – 698 kg). The treated horses weighed 525.6 ± 109.7 kg (range: 190 – 698 kg) and controls 541.7 ± 73.2 kg (range: 400 - 670 kg). There was no significant difference between the groups (p = 0.5).

Table 19: Patient data in treated and control group (no significant differences)

	Treated (n = 28)	**Control (n = 31)**	**Total (n = 59)**
Stallions	6	5	11
Geldings	16	15	31
Mares	6	11	17
Age (years)	5.3 ± 2.8	5.3 ± 1.9	5.3 ± 2.3
Weight (kg)	525.6 ± 109.7	541.7 ± 73.2	534 ± 91.9

Hospitalization time, too, was not significantly different between the groups (p = 0.27, Table 20). The horses in the control group stayed hospitalized for a median (min – max) of 11 days (6 – 43 days) and the treated horses for a median of 10 days (5 – 77 days).

Table 20: Comparison of hospitalization duration (days) between groups (no significant difference)

	Treated (n = 28)	**Control (n = 31)**	**Total (n = 59)**
Minimum	5.0	6.0	5.0
1st quartile	8.0	10.0	8.5
Median	10.0	11.0	10.0
3rd quartile	13.0	12.0	12.5
Maximum	77.0	43.0	77.0

As a non-steroidal anti-inflammatory drug (NSAID), 44 horses received phenylbutazone and 15 horses meloxicam (Table 21). The groups differed significantly (p = 0.03) as to the choice of NSAID: The patients in the treated group received meloxicam more often than the patients in the control group – which implies that phenylbutazone was used more often in the controls.

In three horses, phenylbutazone was discontinued on the day after surgery due to elevated creatinine levels (52: treated, 53: control) or anorexia (64: treated). After discontinuing the phenylbutazone, the three horses stayed weight-bearing and lame-free. The creatinine levels returned to normal limits. Horse 64 resumed to feeding normally. There were no elevated creatinine levels or anorectic horses noted in the 15 horses that received meloxicam.

Table 21: NSAID application according to group (p = 0.03)

	Treated (n=28)	**Control (n=31)**	**Total (n=59)**
Phenylbutazone	17 (38.6%)	27 (61.4%)	44
Meloxicam	11 (73.3%)	4 (26.7%)	15

4.2.2 Surgical procedures

Thirty-six (61%) of the 59 study participants underwent arthroscopy (Table 22). Of these, 19 were not treated with antimicrobials, whereas 17 received the amoxicillin-gentamicin combination once preoperatively.

Table 22: Joints (n=47) and patients (n=36) undergoing arthroscopy according to group

	Treated	Control	Total
1 fetlock joint	8	6	14
1 tarsal joint	2	4	6
2 fetlock joints	2	3	5
1 stifle	2	2	4
1 coffin joint	1	2	3
2 tarsal joints	1	0	1
1 fetlock and 1 coffin joint	0	1	1
2 fetlock joints and 1 tarsal joint	1	0	1
1 fetlock joint and 2 tarsal joints	0	1	1
Total horses	**17**	**19**	**36**
Total joints	**22**	**25**	**47**

The second most frequently performed surgeries were tendovaginoscopy, tenotomy, or their combination, which were summarized in one group for statistical evaluation (n=9). The types of surgeries were distributed evenly between the groups (p=0.5; Table 23). One horse underwent a tendovaginoscopy, tenotomy, and a splint bone extraction on the same leg. As the tendovaginoscopy/tenotomy took longer than the splint bone extraction, the horse was assigned to the tendovaginoscopy/tenotomy group. Nine horses underwent tendovaginoscopy, tenotomy, or combinations of these, and 5 of them received preoperative antimicrobial prophylaxis. One distal splint bone was extracted in 8 horses of which two were treated with antibiotics. Fasciotomy, neurectomy, and the combination of these surgeries were summarised in one group for statistical evaluation because in the hind limbs, these surgeries were generally performed simultaneously.

Table 23: Types of surgeries (not significantly different between groups)

	Treated (n=28)	Control (n=31)	Total (n=59)
Arthroscopy	17 (47.2%)	19 (52.8%)	36 (61.0%)
Tendovaginoscopy/Tenotomy	5 (55.6%)	4 (44.4%)	9 (15.3%)
Splint Bone Extraction	2 (25.0%)	6 (75.0%)	8 (13.6%)
Neurectomy/Fasciotomy	4 (66.7%)	2 (33.3%)	6 (10.2%)
Total	**28 (47.5%)**	**31 (52.5%)**	**59 (100%)**

Forty-six horses had one surgical intervention, and 22 of these received antibiotics. Eleven horses underwent two surgical interventions in their surgery session, and 5 of these were treated with antibiotics. Two horses (1 treated, 1 control) had three arthroscopies during their one surgery session. For statistical evaluation, horses with more than one surgical lesion were grouped together. Horses with one and patients with two or more surgical lesions were distributed evenly between the treatment and control groups (p = 0.6). In patients with more than one surgical intervention, the highest score reached in the evaluation of all wounds was filled in the evaluation sheet.

Five different surgeons performed the surgical interventions. Two of them only had one surgery, and one of them six, so that these three surgeons were summarised in the group “other”. There was no significant difference in the distribution of antimicrobial prophylaxis between the surgeons (p = 0.47, Table 24).

Table 24: Surgical interventions per surgeon (treated/control)

	Surgeon A	Surgeon B	Other
Arthroscopy	20 (9/11)	10 (4/6)	6 (4/2)
Tendovaginoscopy/Tenotomy	3 (1/2)	6 (4/2)	0
Splint bone extraction	4 (2/2)	3 (0/3)	1 (0/1)
Neurectomy/Fasciotomy	3 (1/2)	2 (2/0)	1 (1/0)
Total	**30 (13/17)**	**21 (10/11)**	**8 (5/3)**

The 16 assisting veterinarians were not considered in the statistical evaluation because an assisting surgeon mostly just passed the surgical instruments to the surgeon in charge and did not manipulate the wound himself.

Surgery time ranged from 24 to 115 minutes with an average of 57 ± 21 minutes. Horses without antimicrobial prophylaxis were operated on for 55 ± 19 minutes with a range of 25 - 105 minutes. The intervention in horses with antimicrobial prophylaxis lasted for 60 ± 23 minutes (24 - 115 minutes). A significant difference between the groups in the duration of the surgery was not detected ($p = 0.42$).

All horses recovered to a standing position between 30 – 60 minutes after the end of their procedure. The recovery time was not subjected to statistical analysis.

4.2.3 Clinical examinations

According to the results of the pre-anaesthetic examinations, all horses were clinically healthy from an internal medical point of view and only showed their orthopaedic problem. The horses had a body temperature of 37.5 ± 0.41 °C (36.5 - 38.4°C). The arithmetic means and standard deviations of the temperatures measured during the study are displayed in Table 25. In 10 patients, it was not possible to take the temperature on all occasions because they were uncooperative and/or behaving dangerously. In four controls a temperature measurement was not possible at all. In six horses (4 control, 2 treated), only 41 out of 66 measurements could be taken.

In the initial examination, three horses showed slightly elevated body temperatures (38.2 °C, 38.4 °C, 38.6 °C). This rise in body temperature was probably induced by the transport to the clinic. The temperatures of these horses were within normal limits at the next measurement. Temperatures below 37.0°C were observed 20 times in 11 patients during winter (Table 25).

Table 25: Temperatures (°C) during the study given as arithmetic mean ± standard deviation (Minimum - Maximum)

	Treated		Control	
Day	**T**	**n**	**T**	**n**
-1 evening	37.60 ± 0.44 (36.5 – 38.4)	26	37.45 ± 0.36 (36.5 - 38.0)	28
0 morning	37.49 ± 0.49 (36.0 – **38.7**)	25	37.53 ± 0.39 (36.6 - 38.0)	28
0 evening	37.80 ± 0.44 (37.0 – **38.6**)	26	37.94 ± 0.68 (37.0 - **40.2**)	28
1 morning	37.59 ± 0.45 (36.7 – 38.5)	24	37.51 ± 0.33 (36.8 – 38.1)	26
1 evening	37.51 ± 0.49 (36.7 – 38.5)	25	37.57 ± 0.28 (37.0 - 37.9)	27
2 morning	37.50 ± 0.41 (36.7 – 38.5)	26	37.77 ± 0.59 (37.0 – **39.7**)	27
2 evening	37.45 ± 0.39 (36.5 – 38.3)	25	37.66 ± 0.52 (37.0 – **39.5**)	27
3 morning	37.47 ± 0.44 (36.1 – 38.3)	26	37.72 ± 0.51 (36.5 – **38.9**)	27
3 evening	37.35 ± 0.45 (36.2 – 38.3)	25	37.54 ± 0.36 (36.7 – 38.2)	27
4 morning	37.45 ± 0.39 (36.1 – 38.0)	26	37.46 ± 0.28 (36.9 – 38.0)	28
5 morning	37.44 ± 0.33 (36.9 – 38.1)	25	37.59 ± 0.56 (36.9 – **39.6**)	28

Bold: Temperature > 38.5 °C

Five horses (1 treated, 4 controls) showed elevated body temperatures within 5 days postoperatively (Figure 9). The course of rectal body temperatures and specific treatment of the horses is explained in detail in section 4.2.7 “Inflammatory parameters in horses with post-surgical fever”.

The courses of morning temperatures from day 0 to day 5 of all horses were analysed statistically using a two-factorial Wald test. Statistically significant differences could neither be determined between the groups, nor between the days of measurement, nor for their interactions. A three-factorial Wald test was performed for the days with two temperature measurements (days 0 to 3). The considered factors were antibiotic treatment, day, and daytime of measurement. No significant differences between the groups were found. However, a statistically significant difference for the different days of measurement ($p = 0.02$) was detected as well as a significant influence of the interactions of day and daytime ($p < 0.0001$). The temperatures were furthermore analysed within each day with two measurements (day 0 to day 3). The analysed factors were antimicrobial prophylaxis and daytime of temperature determination. No significant differences were detected between the groups. On the day of surgery, body temperatures were significantly higher in the evening ($p < 0.0001$) than in the morning. On the third day after surgery, temperatures were significantly higher in the morning than in the evening ($p = 0.007$). Regarding the single temperature measurements on the day before and on the days 4 and 5 after surgery, no significant difference between the groups was determined.

In the evening after surgery, 3 control horses were detected with an elevated heart rate (Horse 3: 48 bpm; Horse 34: 72 bpm; Horse 56: 52 bpm). Two of these horses concurrently showed a slightly elevated body temperature (Horse 3: 38.4°C; Horse 56: 38.2 °C), whereas Horse 34 had a fever of 39.1°C. Since Horse 56 showed a very nervous behaviour during the examination, this was deemed to be the cause for the slightly elevated clinical parameters.

Horse 64 (treated) was anorectic on the day after surgery. The horse received phenylbutazone, and after discontinuing the NSAID, it restarted feeding normally.

4.2.4 Wound evaluations

Using a two-factorial ANOVA, globally significant differences were detected between the groups in total score, swelling, and skin temperature (Table 26) whereas exudation and dehiscence were neither different between the groups nor between days.

Table 26: Results of two-factorial ANOVA in wound evaluation scores

	Difference between groups	**Difference between days**	**Interaction between AB and day**
Total score	0.002	<0.0001	n.s.
Exudation	n.s.	n.s.	n.s.
Swelling	0.002	<0.0001	n.s.
Skin temperature	0.007	0.0002	n.s.
Dehiscence	n.s.	n.s.	n.s.

AB: 10 mg/kg amoxicillin and 6.6 mg/kg gentamicin once preoperatively
n.s.: not significant

4.2.4.1 Total score

During the five-day evaluation period, the total scores were significantly higher in the treated group than in the control group ($p = 0.002$). The total score decreased significantly over time ($p < 0.0001$). Also, in a post-hoc analysis (Wilcoxon-Mann-Whitney Test), the total score was significantly higher in horses with antimicrobial prophylaxis at the evaluations on day ($p = 0.03$) and 5 ($p = 0.01$). On day 3, the difference between the groups was almost significant ($p = 0.06$,Table 27).

Table 27: Total scores in treated in control horses

	Treated	**Control**	**p**
Day 1	2.23 ± 1.18	1.48 ± 1.12	0.03
Day 3	1.89 ± 1.29	1.24 ± 0.89	0.06
Day 5	1.46 ± 1.25	0.71 ± 0.85	0.01

However, in both groups, most horses only had an absolute total score of 3 points or less (Figure 3 - Figure 8). The highest scores were observed in the treated group on day one. Three horses had 7.5, 5, and 4 score points each (Figure 7). On the other hand, ten horses, of which eight were in the control group, had a total score of 0 points on the first day after surgery. Six horses in each group showed a total score of 0 points on day three. On day five, six horses in the treated and 15 horses in the control group received 0 points.

On day three after surgery, the highest score of 4 points was reached by three antimicrobial-treated patients as well as by one control. Four points were also given to two horses in the treated group on day five.

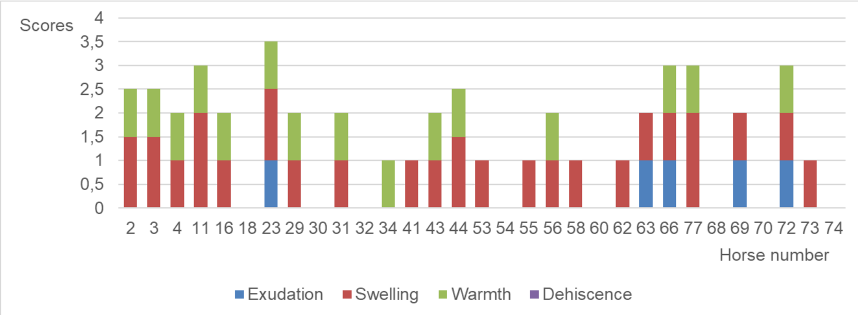

Figure 3: Individual wound scores in controls on day 1

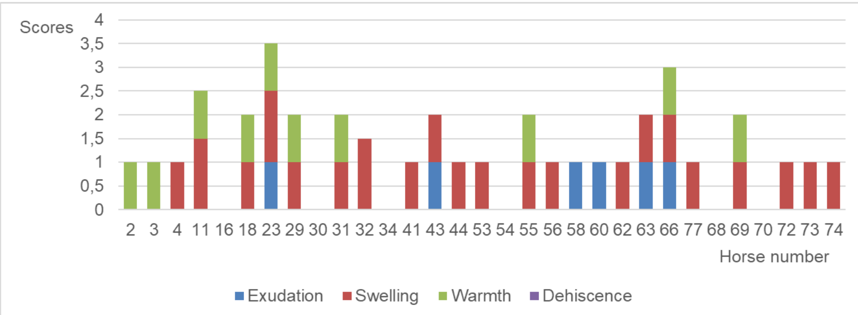

Figure 4: Individual wound scores in controls on day 3

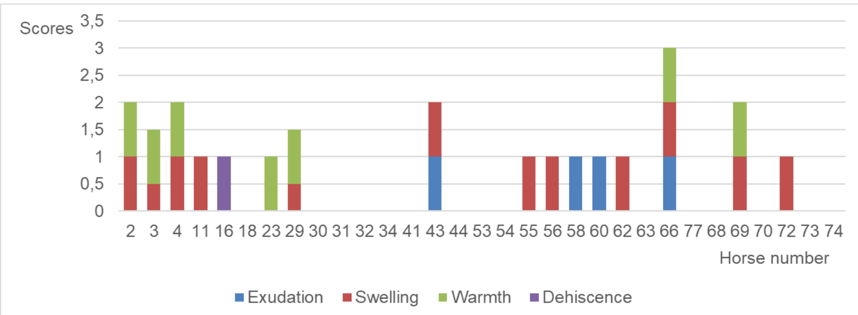

Figure 5: Individual wound scores in controls on day 5

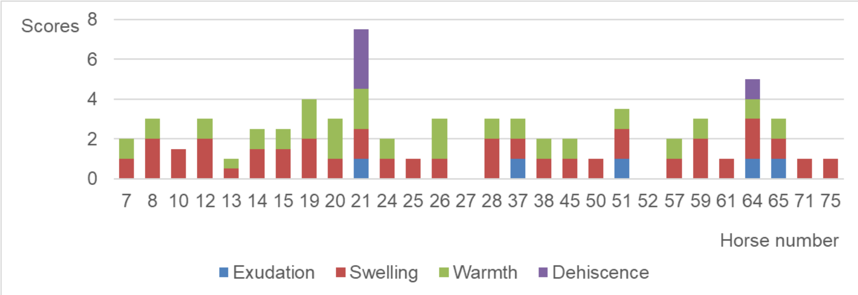

Figure 6: Individual wound scores in treated horses on day 1

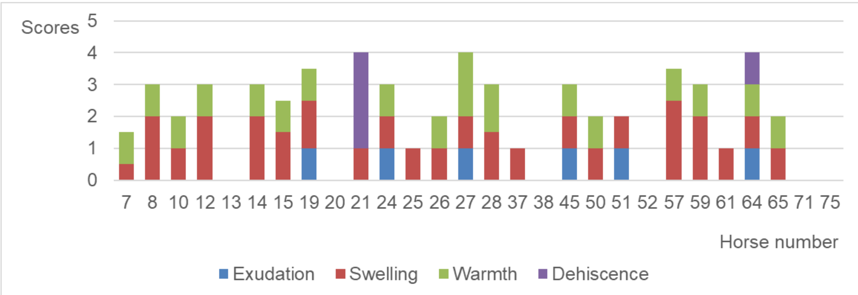

Figure 7: Individual wound scores in treated horses on day 3

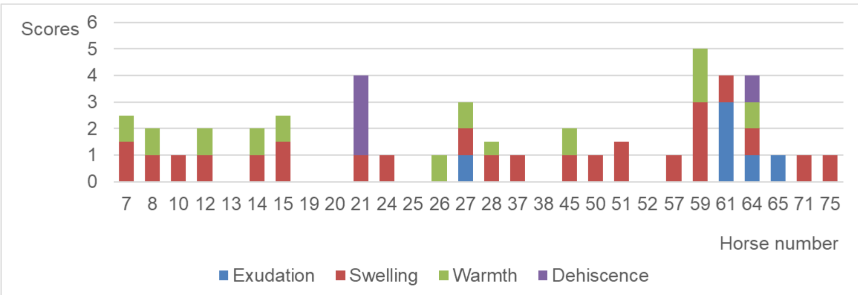

Figure 8: Individual wound scores in treated horses on day 5

4.2.4.2 Exudation

Regarding exudation, no significant differences were found, either between groups or between days. At each evaluation, more than 75% of all patients did not have any exudation from their incisions. Purulent wound discharge (3 score points) was only observed in one horse (horse 61) in the treated group on day 5. This patient underwent neurectomy. Like in the previous evaluations, the wound additionally showed a low-grade swelling (1 score point), no increase in skin temperature (0 score points), and no dehiscence. The patient responded well to local treatment. After developing the exudation, the wound was cleaned daily and disinfected with octenidine (Octenisept®**,** Schülke and Mayr GmbH, Norderstedt/Germany). After cleaning, an antibiotic ointment was applied**.** The score points for exudation are shown graphically in section 4.2.4.1 “Total Score” in Figures 4-9.

4.2.4.3 Post-surgical swelling

During the first five days after surgery, the horses receiving antibiotics showed significantly more swelling at the incision site than horses in the control group ($p = 0.002$). The swelling decreased significantly during the observation period ($p < 0.0001$). Horses in the treated group had significantly higher swelling scores on the first ($p = 0.02$) and fifth ($p = 0.002$) day after surgery than the controls had. However, there was no significant difference between groups on the third day ($p = 0.1$).

On the first day after surgery, the surgical wounds in eleven horses did not show any swelling at all (treated: 2; control: 9). On day one, the swelling around the incision in most horses (treated: 14; control: 16) was scored with one point. The highest score given on this day was 2 score points, which was attributed to 6 horses in the treated and to 2 horses in the control group. Only one treated patient developed a swelling scored with three points on the fifth day after surgery. Additional complications did not occur in this horse.

4.2.4.4 Skin temperature

During the study period, the antibiotic-receiving horses had statistically significant higher skin temperatures around their incisions than the horses in the control group ($p = 0.007$). The skin temperature differed significantly over time ($p = 0.0002$). Regarding each time point individually, the skin temperature around the incisions was significantly higher in horses that had received perioperative antimicrobials on the days 1 ($p = 0.04$) and 3 ($p = 0.03$) after surgery.

To the incisions in the control group, 0 or just 1 point was given during the study period. Four horses in the treated group received 2 points on day one due to their skin temperature around the incision. On days 3 and 5, it was one treated horse that received 2 points on each day.

4.2.4.5 Dehiscence

Dehiscence developed in just three of the 59 patients (2 treated, 1 control). On the first day after surgery, dehiscence was already present in two horses (horse 21 and horse 64) in the treated group. In Horse 21, none of the 2 sutures that should cover an arthroscopy portal wound were in place any more. A fractured splint bone was extracted in horse 64. Two sutures out of seven were dehiscent at the first wound evaluation. On day five, 1 control patient (horse 16) was given 1 point for dehiscence. In this patient, who had a splint bone removed, the lowest two sutures of the incision became dehiscent. All three patients responded to local treatment and were discharged in sound condition.

4.2.5 Laboratory parameters

During the course of the study, each of the 59 patients should have had his blood parameters checked 5 times (n=295). However, the following samples were lacking: In one control horse, haematology and creatinine were not performed on the day before surgery. Its leucocytes were counted manually (6.5 G/l) but due to the different method, not included in the statistical analysis. The creatinine value was not determined on this day, too, due to a problem in the laboratory. In four more horses (3 controls, 1 treated), the inflammatory parameters and creatinine values were not determined on the day prior to surgery due to a communication problem. On the fifth day after surgery, the leucocytes in one control were determined manually (8.3 G/l) and, therefore, not considered for statistical evaluation. As some of the serum samples were haemolytic, 4 iron concentrations could not be included in the statistical evaluation (see page 73).

Overall, for statistical purposes, the number of samples per parameter were deemed satisfactory. Due to the values sporadically lacking for statistical analysis, Wald tests were performed.

No statistically significant differences were detected in the inflammatory parameters between the groups, whereas mostly highly significant differences were calculated between the different days they were determined. Significant interactions of prophylactic antibiotic treatment and day of measurement were found for SAA. For iron, this difference was just not statistically significant (Table 28).

Table 28: Laboratory parameters – results of the two-factorial Wald-test

	Influence of AB	**Influence of day**	**Interaction between AB and day**
SAA (n=291)	n.s.	<0.0001	<0.0001
Iron (n=287)	n.s.	<0.0001	0.06
Haptoglobin (n=291)	n.s.	<0.0001	n.s.
WBC (n=116)	n.s.	0.01	n.s.
Albumin (n=291)	n.s.	<0.0001	n.s.
Globulin (n=291)	n.s.	<0.0001	n.s.
Total Protein (n=291)	n.s.	<0.0001	n.s.
Creatinine* (n=231)	n.s.	<0.0001	n.s.

AB: Amoxicillin and gentamicin once pre-operatively; n.s.: Not significant
*: Creatinine was checked 4 times (day prior to surgery, day 1, day 3, day 5)

4.2.5.1 Serum amyloid A (SAA)

The SAA values of four patients (3 controls; 1 treated) were not available prior to surgery. The cut-off value for healthy horses is an SAA concentration of less than 2.7 µg/ml. Eight patients (6 treated, 2 controls) showed elevated SAA concentrations prior to surgery. A more than 100-fold elevation was present in 3 horses (54, 29, and 64, Table 29).

Table 29: Patients with elevated SAA (µg/ml) prior to surgery

Patient	AB	Day -1	Day 0	Day 1	Day 3	Day 5
54	No	**1351.1**	**933.4**	**1086.3**	**411.8**	**133.2**
29	No	**953.5**	**1009.1**	**1132.5**	**2372.4**	**2187.8**
64	Yes	**802.5**	**740.8**	**499.5**	**289.7**	**17.6**
28	Yes	**40.3**	0.7	6.3	0.3	1.1
14	Yes	**29.5**	2.5	**651.3**	**440.3**	**36.7**
50	Yes	**21.4**	**48.3**	**498.8**	**307.8**	**9.5**
59	Yes	**11.8**	**51.5**	**277.2**	**32.5**	0.1
61	Yes	**3.4**	**4.0**	**297.5**	**209.4**	0.1

AB: 10 mg/kg amoxicillin and 6.6 mg/kg gentamicin once preoperatively.
Cut off: <2.7 µg/ml. **Bold**: Values above cut-off.

Horse 54 (control) showed its highest SAA (1351.1 µg/ml) on the day prior to surgery. The cause for this elevation could not be determined. The horse was randomised into the control group, and its SAA, directly after surgery on day 0, dropped to 933.4 µg/ml. The surgery (a splint bone extraction) only slightly elevated SAA in this horse to 1086.3 µg/ml on day 1, and the follow-up measurements on days 3 and 5 showed a dramatic normalisation. The post-surgical healing process in Horse 54 was completely inconspicuous with a total score of 0 points at every wound evaluation.

Horse 29 (control) which underwent arthroscopy and which had the second-highest pre-operative SAA of 953.5 µg/ml, was castrated a few days prior to the orthopaedic intervention. The SAA concentrations continued to rise in this horse until the third day after surgery (2372,4 µg//ml). Horse 29 received 1 score point each at the wound evaluations on day 1 and 3 after surgery for swelling and incisional skin temperature. On day 5, the incision was given 0.5 points for swelling and 1 point for incisional skin temperature, respectively. No other abnormalities were noted during the observation period. This horse was discharged 13 days after surgery without any complications.

In Horse 64 (treated), the initial SAA concentration (802.5 µg/ml) steadily declined despite the surgical intervention (splint bone extraction) to a less than tenfold elevation on day five. However, this horse developed a surgical site infection with suture dehiscence. It received phenylbutazone and temporarily developed elevated creatinine levels. More details concerning its healing process can be found in chapter 4.2.6 “Inflammatory parameters of horses with surgical site infections”.

Using the Wald test, no global significant difference in SAA levels was detected ($p = 0.18$) between the groups. There was a significant difference in the SAA concentrations between the days of sample collection ($p < 0.0001$). Also, a significant interaction was calculated between the day of sample collection and the antibiotic treatment ($p < 0.0001$). Analysing each day separately with the Wilcoxon-Mann-Whitney test, the SAA levels were significantly higher in the controls than in the treated group (Table 30) on the third ($p = 0.03$) and fifth ($p = 0.007$) day after surgery.

Equine patients in both groups already showed an increase in median SAA concentrations on the first day after surgery. In the controls, the highest median concentration was reached on day 3 (239.1 µg/ml, Table 30). A lower median with 19.2 µg/ml was seen on day 5, but the value was still higher than before the surgical intervention (0.2 µg/ml). In the treated group, the highest median was observed on day 1 after surgery with 155.8 µg/ml. The median was close to its initial value (0.5 µg/ml) on day 5 after surgery (Table 30).

Table 30: SAA (µg/ml) in treated and control patients before and after surgery.

	Day -1 n.s.		Day 0 n.s.		Day 1 n.s.		Day 3 p=0.03		Day 5 P=0.007	
	Treated (n=27)	Control (n=28)	Treated (n=28)	Control (n=31)	Treated (n=28)	Control (n=31)	Treated (n=28)	Control (n=31)	Treated (n=28)	Control (n=31)
Minimum	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1st quartile	0.1	0.1	0.1	0.1	0.1	12.6	1.7	7.0	0.1	0.2
Median	0.2	0.2	0.4	0.2	155.8	166.0	70.1	239.1	0.5	19.2
3rd quartile	1.5	0.3	2.4	1.2	292.4	385.0	208.9	649.2	6.3	399.0
Maximum	802.5	1351.0	740.8	2159.6	651.3	1195.0	440.3	2890.7	159.2	4692.0

Cut off <2.7µg/ml.

n.s.: not significant.

On day five after surgery, the highest SAA level in treated horses was 159,2 µg/ml. Four control horses, of which just one developed a surgical site infection, had severely elevated SAA concentrations (>1500 µg/ml) on day 5 (Table 31). Except horse 30, that developed septic arthritis on day 8, all horses showed inconspicuous healing with a total score of 0 points.

Table 31: Control horses (4/31) with elevated SAA (µg/ml) concentration on day 5 after surgery

Horse	**SAA -1**	**SAA 0**	**SAA 1**	**SAA 3**	**SAA 5**
3	0.1	1.2	**12.6**	**2079.1**	**4692.0**
29	**953.5**	**1009.1**	**1132.5**	**2372.4**	**2187.8**
30	1.0	0.2	**480.3**	**525.9**	**1565.6**
63	1.0	1.0	**363.5**	**2031.0**	**1968.2**

Cut off <2.7 µg/ml. **Bold**: Values above Cut off.

4.2.5.2 Iron

Forty-three of the 291 samples were macroscopically haemolytic. Of these, 27 (63%) were collected directly after surgery. Five were taken on each, the first and fifth day after surgery. Four were obtained on the day prior to surgery, and two on the third day after surgery.

Iron values in haemolytic samples were not included in the statistical evaluation if they were more than twice as high than the value measured directly before or after. This happened in 4 samples: directly after surgery in Horses 29 (control) and 64 (treated); on day 3 in Horse 30 (control), and on day 5 in Horse 16 (control).

In three patients (1 treated, 2 controls), initial iron concentrations were not within the reference interval (14.0 - 43.0 µmol/l, Table 32).

Table 32: Serum iron (µmol/l) concentrations outside the reference interval prior to surgery in patients undergoing elective surgery on day 0

Patient	AB	Day -1	Day 0	Day 1	Day 3	Day 5
30	No	**65.6**	**56.5***	14.4	**67.4**	**7.7**
29	No	**9.0**	**48.9***	16.2	**10.4**	17.9
59	Yes	**7.7**	**9.4**	**7.0**	25.7	28.6

AB: 10 mg/kg amoxicillin and 6.6 mg/kg gentamicin once preoperatively;
*: Value not considered for statistical evaluation due to haemolytic sample. Reference interval: 14.0 - 43.0 µmol/l. **Bold**: Values not within normal limits.

Horse 29, which was castrated a few days prior to the orthopaedic intervention and also showed elevated SAA levels prior to surgery, had iron levels below the reference interval on the day prior to surgery as well as on day 3. It received 1 score point for swelling and incisional skin temperature at the wound evaluations on day 1 and 3 after surgery. On day 5, the incision was given 0.5 points for swelling and 1 point for incisional skin temperature, respectively.

Horse 59 (arthroscopy) showed serum iron concentration below the reference interval prior to surgery as well as on day 0 and 1. On day 3 and 5, its iron values were within the reference interval. This horse received 2 score points for swelling on days 1 and 3. On day three, the swelling had increased to 3 score points. The incisional skin temperature decreased from 1 score point on days 1 and 3 to 0 points on day 5.

The serum iron concentrations in Horse 30 (arthroscopy) showed the biggest abnormalities: they were above the reference interval prior to surgery as well as on day 0 and 3. On day 5, the serum iron concentration was below the reference interval. On the 8th day after surgery, Horse 30 developed a septic arthritis of the coffin joint operated on. The disease process is described in detail in chapter 4.2.6 “Inflammatory parameters in horses with surgical site infections”.

Regarding all patients, no statistically significant differences in iron concentrations were found between the groups (p=0.76), but between the different days of sample collection (p<0.0001). The iron concentrations on day 1 were notably lower than at other points in time (Table 33).

Table 33: Serum iron concentrations (µmol/l) in treated and control patients before and after surgery

	Treated		**Control**	
Day	**concentration**	**n**	**concentration**	**n**
-1	24.3 ± 6.8 (**7.7** - 37.7)	27	26.4 ± 10.1 (**9.0 - 65.6**)	28
0	25.1 ± 6.1 (**9.4 - 43.2**)	27	28.3 ± 8.4 (**15.3 - 56.5**)	30
1	14.2 ± 6.7 (**5.7** – 37.0)	28	13.0 ± 6.5 (**4.2**- 35.8)	31
3	26.0 ± 5.6 (**9.8** - 33.1)	28	23.6 ± 11.9 (**4.9 - 68.2**)	30
5	25.5 ± 9.8 (**7.9 - 57.1)**	28	21.7 ± 8.6 (**7.7** - 42.1)	30

Mean ± standard deviation (minimum-maximum). Reference interval 14.0-43.0 µmol/l. **Bold:** Iron concentrations outside the reference interval.

4.2.5.3 Haptoglobin

Initial haptoglobin concentrations were elevated in 14 patients (Table 34). Four of them received preoperative antimicrobial treatment, and 10 did not.

Table 34: Patients with elevated haptoglobin concentrations prior to surgery

Patient	AB	Day -1	Day 0	Day 1	Day 3	Day 5
19	Yes	**4.04**	1.92	1.82	1.64	1.31
28	Yes	**4.50**	0.93	1.54	1.71	**3.85**
32	No	**3.86**	**4.16**	**4.22**	**5.71**	**6.37**
41	No	**2.68**	1.94	1.80	2.46	**2.84**
43	No	**3.18**	**2.96**	**3.02**	**4.20**	**4.08**
44	No	**4.38**	**3.72**	**2.70**	**7.62**	**8.32**
50	Yes	**3.04**	**3.24**	**3.36**	**3.46**	**3.06**
54	No	**4.32**	**3.60**	**4.54**	**4.56**	**4.90**
55	No	**3.16**	**2.88**	**2.52**	**2.86**	**2.54**
56	No	**3.24**	**3.20**	**3.16**	**3.44**	**3.82**
58	No	**2.50**	2.18	**3.06**	**3.40**	**3.04**
59	Yes	**2.82**	**3.42**	**3.04**	**4.72**	**4.78**
60	No	**2.68**	**2.48**	2.10	2.20	2.20
63	No	**2.60**	**2.60**	1.29	1.81	1.73

AB: 10 mg/kg amoxicillin and 6.6 mg/kg gentamicin once preoperatively.
Reference interval: 0.13-2.47 mg/ml. **Bold**: Values above upper limits.

In Horse 59, the initial SAA and iron concentrations were also not within the reference interval. In Horses 28, 50, and 54, the initial iron concentrations were not within normal limits as well.

In Horse 43, a serous exudation scored with 1 point developed on day five after surgery. In Horse 59, a swelling scored with 3 points developed on the fifth day after surgery.

In all other horses with initially elevated haptoglobin levels, the total wound score decreased continuously during the observation period.

There were no significant differences between the groups regarding haptoglobin concentration (p=0.85). However, as in SAA and iron, the haptoglobin concentration was significantly different between the days of sample collection ($p < 0.0001$, Table 35). Haptoglobin concentrations showed a continuous increase throughout the study period. On day 3, an elevation of the mean haptoglobin concentration above the reference interval was observed, which increased even further to day 5.

Table 35: Haptoglobin (mg/ml) in treated and control patients before and after surgery

Day	Control (n = 31)	Treated (n = 28)
-1	2.27 ± 1.05 (0.83 – **4.38**)	2.18 ± 0.90 (0.66 – **4.5**)
0	2.19 ± 1.02 (0.67 – **4.16**)	2.14 ± 0.99 (0.48 – **4.23**)
1	2.20 ± 1.02 (0.89 – **4.54**)	2.35 ± 1.0 (0.96 – **4.74**)
3	**2.89** ± 1.41 (1.24 – **7.62**)	**2.78** ± 1.31 (1.34 – **6.79**)
5	**3.05** ± 1.68 (1.04 – **8.32**)	**2.87** ± 1.25 (1.14 – **6.12**)

Data presented as the mean ± standard deviation (minimum-maximum).
Reference interval 0.13-2.47 mg/ml. **Bold**: Values above upper limits

4.2.5.4 Albumin, globulin, and total protein

For albumin, globulin, as well as total protein, no significant differences between the groups were found. The Wald test revealed a significant difference between the days of the sample collection for the concentrations of the protein fractions (Albumin: $p < 0.0001$; globulin: $p < 0.0001$, total protein: $p < 0.0001$).

It is obvious (Table 36) that the concentrations of the protein fractions did not differ much during the sampling period. A slight decline in the concentrations of both, albumin, and globulin and therefore also in the total serum protein was seen directly after surgery (day 0). All concentrations returned to the range of the initial levels already on the day after surgery.

Table 36: Albumin, globulin and total protein in treated and control patients before and after surgery

	Day	Control	Treated
Albumin (g/l) (27.4-35.7 g/l)	**-1**	32.7 ± 1.6 (29.7 – 34.9)	32.0 ± 3.0 (**26** – **37.9**)
	0	29.3 ± 2.2 (**25.3** – 33.5)	28.2 ± 2.7 (**20.6** – 31.9)
	1	33.3 ± 1.9 (30.2 – **38.0**)	33.0 ± 3.4 (**22.6** – **37.1**)
	3	32.0 ± 2.1 (28.7 – **38.0**)	31.1 ± 2.7 (**24.0** – **37.1**)
	5	31.7 ± 1.7 (28.6 – **35.9**)	30.8 ± 2.9 (**24.5** – **36.8**)
Globulin (g/l) (24.7-41.5 g/l)	**-1**	29.3 ± 4.9 (**16.5** – 40.7)	30.3 ± 3.7 (**22.6** – 37.7)
	0	26.9 ± 3.8 (**19.1** – 34.2)	26.3 ± 4.15 (**18.4** – 33.6)
	1	30.2 ± 3.9 (**23.4** – 39.8)	30.9 ± 4.5 (**23.1** – 38.3)
	3	29.7 ± 3.5 (**22.9** – 37.0)	29.5 ± 3.9 (**22.5** – 36.6)
	5	29.6 ± 3.6 (**24.0** – 38.8)	29.2 ± 3.3 (**22.2** – 38.3)
Total Protein (g/l) (57.7-72.9 g/l)	**-1**	61.9 ± 5.0 (**50** – **73.1**)	62.4 ± 4.8 (48.6 – 69.9)
	0	56.2 ± 4.5 (**47.0** – 63.5)	54.5 ± 5.4 (**41.1** – 63.1)
	1	63.5 ± 3.9 (**55.4** – 72.0)	63.9 ± 6.4 (**46.9** – **74.9**)
	3	61.7 ± 3.5 (**55.1** – 68.8)	60.7 ± 4.9 (**48.9** – 68.1)
	5	61.3 ± 3.2 (**54.7** – 68.0)	60.1 ± 4.4 (**48.0** – 68.2)

Data presented as mean ± standard deviation (minimum-maximum).

4.2.5.5 White blood cells

116 samples of 118 planned ones were available for statistical analysis. In one control horse prior to surgery and in a different control horse on the fifth day after surgery, the WBC was not included in the statistical evaluation due to a different determination method (manual WBC count). A significant difference between groups in leucocyte numbers was neither determined prior to surgery nor on day 5. At the day before surgery, the WBC was significantly higher than on day 5 ($p = 0.01$). However, from a clinical perspective, this mean decrease remained in an irrelevant dimension (Table 37). Patient 9 which underwent a splint bone extraction had a WBC of 2.67 G/l on day 5. This horse never showed any fever or other abnormalities, which could explain the leucopoenia. On day 5, 1.5 score points were given to swelling and 1 score point to skin temperature around the surgical incision.

Table 37: Blood WBC (G/l) in treated and control horses before and 5 days after surgery

Day	**Control**	**Treated**
-1	7.59 ± 2.30	7.92 ± 1.99
5	7.11 ± 2.01	6.64 ± 2.67

Mean ± standard deviation (minimum-maximum). Reference interval: 4.90 - 11.10 G/l. **Bold:** Value not within the reference interval.

4.2.5.6 Creatinine

There was no significant difference in the creatinine concentrations between the groups ($p = 0.58$). However, even in the creatinine, a statistically significant difference was calculated between the days of measurements. As listed in Table 38, on day 1 after surgery, the creatinine values in both groups showed a maximum.

Table 38: Serum creatinine concentration (µmol/l) in treated and control horses before and after surgery

Day	Control (n = 31; on day -1 n = 27)	Treated (n = 28; on day -1 n = 27)
-1	120.1 ± 22.4 (**79.6** - 168.0)	121.1 ± 24.7 (**79.6** - 168.0)
1	132.6 ± 26.4 (88.4 - **203.3**)	137.7 ± 28.0 (**79.6** - **194.5**)
3	123.5 ± 24.0 (88.4 - **194.5**)	128.8 ± 24.6 (88.4 - 168.0)
5	118.1 ± 25.2 (**79.6** - 176.8)	123.8 ± 28.7 (70.7 - **194.5**)

Mean ± standard deviation (minimum-maximum). Reference interval: 79.65 – 177.0 µmol/l. **Bold:** Value not within the reference interval. Values were converted from mg/dl to µmol/l.

A total of three individuals, all receiving phenylbutazone as post-surgical NSAID, developed increased creatinine levels. The creatinine concentrations in horse 52 and 53 exceeded the reference interval (79.65-177.0 µmol/l) at the day after surgery. After discontinuation of phenylbutazone, their creatinine levels returned to the reference interval within the observation period. Horse 75 showed an elevated creatinine level on day 5 after surgery. Its creatinine concentration also returned to normal limits within two days.

Table 39: Patients with elevated creatinine levels after surgery (all received phenylbutazone)

		Creatinine			
Patient	**AB**	**Day -1**	**Day 1**	**Day 3**	**Day 5**
52	Yes	132.6	**194.5**	168.0	150.3
53	No	132.6	**194.5**	141.4	123.8
59	Yes	141.4	168.0	159.1	**194.5**

AB: 10 mg/kg amoxicillin and 6.6 mg/kg gentamicin once preoperatively. Reference interval: 79.65 – 177.0 µmol/l. **Bold**: Values exceeding the reference interval. Values were converted from mg/dl to µmol/l.

4.2.6 Inflammatory parameters in horses with surgical site infections

Septic joint infection after arthroscopy was observed in two horses. However, in both cases, the arthritic signs developed after the study period, on day 8 (control Horse 30) and 9 (Horse 39), postoperatively. The latter was randomised into the treated group, but it had to be dropped because it received additional gentamicin already on the first and second day after surgery due to a personal decision of the surgeon.

During the study period, other surgical site infections (SSI) developed in 4 horses. Three of these received praeoperative antibiotics, whereas one did not. The surgical site infections (SSI) included dehiscence and purulent drainage (Table 40).

Table 40: Characterization of patients with SSI

Type of SSI	Patient	AB	Type of Surgery	Day
Septic Joint	30	No	Arthroscopy	8
	39*	Yes	Arthroscopy	9
Dehiscence	21	Yes	Arthroscopy	1
	64	Yes	Splint bone extraction	1
	16	No	Splint bone extraction	5
Purulent drainage	61	Yes	Neurectomy	5

Day: Day of SSI occurrence; AB: Amoxicillin gentamicin pre-operatively; M: Mare; G: Gelding; *: Horse was excluded from statistical evaluation.

The concentrations of the inflammatory parameters of all six horses developing either arthritis or other SSI are displayed in Table 41. The surgical wounds of these patients are described in detail afterwards.

Table 41: Inflammatory parameters in horses with SSI during the study period

	Day	Control		Treated			
		16	**30**	**21**	**39#**	**61**	**64**
SAA Cut-off <2.7 µg/ml	**-1**	0.1	1.0	0.1	*	**3.4**	**802.5**
	0	0.1	0.2	0.1	0.1	**4.0**	**740.8**
	1	**670.0**	**480.3**	**254.3**	**3.5**	**297.5**	**499.5**
	3	**2890.7**	**525.9**	**106.4**	0.1	**209.4**	**289.7**
	5	0.1	**1565.6**	0.1	0.1	0.1	**17.6**
Iron RI: 14.0-43.0 µmol/l	**-1**	26.0	**65.6**	37.7	*	25.2	27.2
	0	28.3	**56.5**	26.5	**12.6**	*	33.7
	1	**5.1**	14.4	14.4	**9.8**	17.9	**12.3**
	3	16.2	**56.5**$^{+}$	27.9	25.0	26.8	31.3
	5	42.1	**7.7**	31.0	22.4	23.5	42.3
Haptoglobin RI: 0.13-2.47 mg/ml	**-1**	1.26	1.70	1.96	*	2.22	1.90
	0	1.14	1.34	1.85	**2.48**	**2.85**	1.42
	1	2.09	1.56	2.07	**2.52**	1.92	1.38
	3	**3.38**	1.24	1.46	2.44	**2.49**	2.06
	5	1.04	1.52	1.32	**2.58**	2.19	1.98
WBC RI: 4.9- 11.1 G/l.	**-1**	8.38	**4.73**	7.96	6.62	9.95	6.23
	5	7.3	**11.88**	5.32	6.0	5.99	7.52

#: Horse dropped out of the study due to additional antibiotics from day 1 on; RI: reference interval; $^{+}$: value was not subjected to statistical analysis; *: Missing value. **Bold:** Values outside the reference interval. Highlighted in grey: Horses that developed SSI after the observation period.
Horse 16: Dehiscence on day 5;
Horse 21: Dehiscence on day 1;
Horse 30: Arthritis on day 8;
Horse 39: Arthritis on day 9;
Horse 61: Purulent discharge on day 5;
Horse 64: Dehiscence on day 1.

Horse 30 (control) showed the first clinical signs of a septic joint eight days after arthroscopy of the left front coffin joint; the horse was supposed to stay in the clinic until suture removal. During the study observation period of 5 days and afterwards, the mare showed no fever. The highest temperature in this mare was 38.2 °C on day 10 after surgery. No increased swelling, soreness (0 score points),

or other abnormalities of the wound were detected. However, her serum samples showed a steady augmentation of SAA from day 1 to 5. The highest value (1565.6 µg/ml) was measured on day 5 after surgery, the last day of the examination protocol. At the same time, the white blood cells were only slightly elevated (11.9 G/l, reference interval 4.9-11.1 G/l). The serum iron showed a decline throughout the sampling period, which was most obvious on day 5 after surgery. On day 3, the sample was macroscopically haemolytic and the iron value (56.5 µmol/l) more than 4 times higher than the values in the previous and following measurement. Therefore, this value was considered as type one error, probably due to poor sample handling and was excluded. Haptoglobin values in horse 30 remained in the reference interval at all times. In retrospect, in this horse, SAA and iron clearly indicated the development of a serious inflammation already on day 5.
Horse 39 (treated, drop-out) developed a joint infection on day 9 after arthroscopy of the right front coffin joint, despite receiving additional gentamicin (6.6 mg/kg; Belamox®, bela-pharm, Vechta, Germany) on days 1 and 2. Due to the additional antibiotic treatment, this horse was not included in the statistical evaluation; since standardised clinical and laboratory evaluations took place all the same, the results are presented nonetheless: Body temperatures and wound evaluations seemed inconspicuous; however, a relatively late – recognised on day 5 for the first time – mild swelling of both the incision and the joint developed (swelling and total score: 1 point). In this horse, SAA levels were consistently low with a maximum of 3.5 µg/ml in the morning after surgery. Directly after surgery, the serum iron value (12.6 µmol/l) was below its reference interval. A further decrease was seen on day 1 (9.8 µmol/l). On days 3 and 5, the iron levels were within the reference interval. The haptoglobin values varied slightly around 2.5 mg/ml at all times, which is just very slightly above the upper limit of the reference interval of 0.13 - 2.47 mg/ml. In summary, the laboratory parameters during the first five days after surgery were not indicative of the joint infection developing in this patient.

In the following, the wounds and inflammatory parameters of horses with less severe surgical site infections are described.

In Horse 16 (control), two sutures became dehiscent (1 score point) on the fifth day after a splint bone extraction. The incision was not swollen, and dry (swelling: 0 score points, exudation: 0 score points), the skin still adapted, and the temperature of the surrounding tissue was not increased (0 score points). In the morning of the second day after surgery, the patient developed a fever (38.9 °C) which resolved after the routine application of phenylbutazone. Afterwards, the horse showed no elevated temperature during its stay in the clinic. On day 3 after surgery, this patient showed a maximum SAA of 2890.7 µg/ml. On day 5, the dehiscence occurred but the SAA had returned to the base level. Iron was low on the day after surgery but returned to the reference interval two days later. Just on day three, a 2.7-fold elevation of haptoglobin was observed.

Horse 21 (treated) showed dehiscence on the day after arthroscopy in one out of two of the joints operated on (3 score points). The incision with dehiscent sutures also showed a slight serous discharge (1 score point), low- to middle-grade swelling (1.5 score points), and a moderate increase in skin temperature (2 score points). On the third day after surgery, the incision was dry and the swelling subsided (1 score point). The skin temperature was not increased (0 score points). Five days after surgery, there was no swelling (0 score points), the incision remained dry (0 score points), and the skin temperature was within normal limits. Fever was never present. Looking at the inflammatory parameters, a relatively mild peak in SAA (254.3 µg/ml) was seen in this horse on the day after surgery. On day five, the SAA returned to its basal concentration. The serum iron value was never below the reference interval. The serum iron level declined on day 1 and approached the base level in the following samples. On the third day after surgery, this elevation of iron might have been falsely high, as the sample was haemolytic. The haptoglobin level showed an increase after surgery, which did not exceed the reference interval. On day 3 and 5, this value declined.

In Horse 61 (treated), which underwent neurectomy on both of its front limbs and which received prophylactic antibiotic treatment, a haemopurulent discharge (3 score points) was observed on day five after surgery. Like at the evaluations before, the surgical wound showed a low-grade swelling (1 score point), no increased skin temperature (0 score points), and no dehiscence (0 score points). On day 5 when the discharge appeared, the SAA already had returned to the reference interval, the serum iron exceeded the initial level, and the haptoglobin remained within the reference interval. A decrease in the haptoglobin concentration was seen in this individual on the day of and the day after surgery. The highest haptoglobin concentration was observed after recovering from surgery.

Horse 64 (treated) had two dehiscent sutures out of 7 (1 score point) at the day after a splint bone extraction. The horse was part of the antibiotic-receiving group. A serosanguinous discharge (1 score point) and a middle-grade swelling (2 score points) were present as well. On the third and fifth day after surgery, the exudate was serous (1 score point). By day three, the swelling had declined to a low-grade swelling (1 score point) which remained throughout the following evaluation. The skin temperature at the incision site was mildly elevated at all evaluation times (1 score point). The horse’s body temperature never exceeded 38.5°C. The SAA levels were elevated at all times. With 802.5 µg/ml, the presurgical SAA level showed an almost 300-fold increase and was the highest measured SAA concentration in this patient. This horse never underwent any surgery nor experienced any obvious trauma before starting the study. There was no explanation found for its high initial SAA values. However, the SAA concentration declined steadily but did not return below the cut off of 2.7 µg/ml by the fifth day after surgery. As the serum sample that was obtained directly after surgery was haemolytic, the serum iron value was probably falsely high (more than twice as high than the following value) and, therefore, not considered for the statistics. The serum iron concentration declined to 12.3 µmol/l and returned to the reference interval on the third day after surgery (Table 41). The course of haptoglobin undulated; the highest value was detected directly after surgery, the lowest on the day after surgery. The dehiscent incision responded well to local treatment.

Overall, these less severe surgical site infections did not cause prominent changes in inflammatory parameters.

4.2.7 Inflammatory parameters in horses with post-surgical fever

For this study, fever was defined as a rectal body temperature of 38.6°C or higher. Five horses (1 treated, 4 controls) showed such elevated body temperatures postoperatively within 5 days (Figure 10). Interestingly, only one of these horses developed suture dehiscence on day 5 (Horse 16, Table 40). In the evening after surgery, two controls had a fever (Horse 32: 40.2 °C; horse 34: 39.1 °C). Horse 32 was febrile also in the morning of the third day after surgery (38.9 °C). Fever was also present in horse 34 on day 5 (39.6 °C). Its total wound score on this day was 0 points. On day six, rectal temperature normalised to 37.8 °C. The patient was bearing weight well and was discharged. A follow-up examination including suture removal was scheduled nine days after surgery at his home stable. The patient was clinically sound and the surgical wound looked inconspicuous.

Horse 3 (Control) and Horse 32 (Control) were treated with antibiotics for five days due to fever. Horse 3 received trimethoprim-sulfadiazine on day 2 and horse 32 penicillin, streptomycin, and gentamicin in the evening after surgery, respectively. Horse 32 also received Zylexis® ad us. vet. on the day after surgery to stimulate its immune system. Horse 14 (Treated) showed a slightly elevated temperature at 38.6°C, which was seen only once in the evening after surgery.

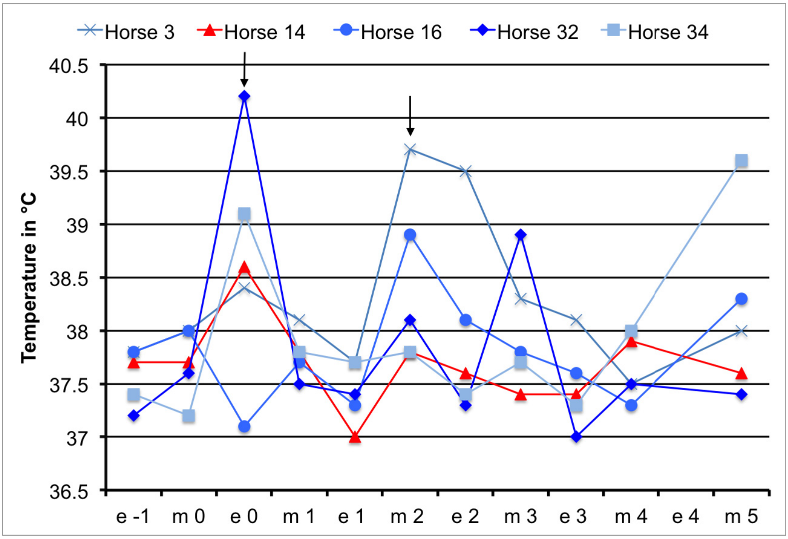

Figure 9: Temperatures in febrile patients.
Blue: Controls
Red: Treated,
m: Morning of the day of study,
e: Evening of the day of study.
Arrows mark the beginning of antimicrobial therapy for 5 days in horse 3 and 32.

In the following, the course of inflammatory parameters (Table 42) in horses with fever and their accompanying wound evaluation scores are specified.

Table 42: Inflammatory parameters in horses with post-surgical fever

		Treated	Controls			
Horse		**14**	**3#**	**16**	**32#**	**34**
Fever Occurrence*		e0	m2 e2	m2	e0 m3	e0 m5
Temperature peak (°C)		38.6	39.7 39.5	38.9	40.2 38.9	39.1 39.6
	Day					
SAA Cut-off <2.7 µg/ml	**-1**	**29.5**	0.1	0.1	0.3	0.1
	0	2.5	1.2	0.1	1.2	0.1
	1	**651.3**	**12.6**	**670.0**	**1195.0**	**385.0**
	3	**440.3**	**2079.1**	**2890.7**	**1601.3**	**255.9**
	5	**36.7**	**4692.0**	0.1	**399.0**	**143.5**
Iron RI: 14.0-43.0 µmol/l	**-1**	35.5	29.7	26.0	22.0	21.8
	0	24.0	34.1	28.3	24.5	24.4
	1	**12.2**	18.1	**5.1**	**4.9**	**6.7**
	3	16.9	**5.3**	16.2	21.9	19.6
	5	23.0	16.0	42.1	19.6	**7.7**
Haptoglobin RI: 0.13-2.47 mg/ml	**-1**	**3.11**	1.29	1.26	**3.86**	1.60
	0	**2.69**	1.21	1.14	**4.16**	1.59
	1	**2.70**	0.89	2.09	**4.22**	1.92
	3	**2.50**	**2.85**	3.38	**5.71**	**4.09**
	5	**3.28**	**4.84**	1.04	**6.37**	**4.86**
WBC RI: 4.9 – 11.1 G/l.	**-1**	6.15	5.36	8.38	8.95	6.54
	5	**12.35**	**4.44**	7.3	6.41	**11.34**

RI: reference interval; **Bold:** Value exceeding the reference interval * e0: evening day 0; e2: evening day 2; m2: morning day 2; m3: morning day 3; m5: morning day 5

#: Initiation of antibiotics

Horse 3 (control) was febrile on both temperature measurements on the second day after surgery. On the day after surgery, 1.5 points were given for swelling and 1 point for skin temperature around the incisions. The swelling subsided to 0 points on the third day after surgery. On day five, 0.5 points were given for swelling. The skin temperature around the incisions remained at 1 point. A minor increase in SAA, from 1.2 µg/ml to 12.6 µg/ml, was observed on the day after surgery. Its concentration massively increased to 2079.1 µg/ml on the third day after surgery and further to 4692 µg/ml on day five. The lowest iron value (5.3 µmol/l) was observed on the third day after surgery. The lowest haptoglobin concentration in Horse 3 was measured on the day after surgery (0.89 mg/ml). An increase in haptoglobin concentration was observed on day 3 (2.85 mg/ml) with a further increase on day 5 (4.84 mg/ml). The WBC decreased from 5.36 G/l prior to surgery to 4.44 G/l on day five. This value was just below the reference interval.

In Horse 14 (treated), which was febrile in the evening after surgery, 1.5 points were given for swelling on the day after surgery. Also, on day 1, the SAA reached its highest concentration (651.3 µg/ml), whereas the iron level showed its lowest (12.2 µmol/l). On day 3, the score for swelling was 2 points and on day 5, one point was given for swelling. On day 5, the highest haptoglobin concentration was measured (3.28 mg/ml). The WBC was approximately twice as high on the fifth day after surgery than prior to surgery (12.35 G/l). The skin temperature around the incision was consistently scored with one point.

In the morning of the second day after surgery, the horse 16 (control) developed a fever (38.9 °C) which resolved afterwards while receiving the regular application of phenylbutazone. Two distal sutures of the initial seven became dehiscent (score: 1) on the fifth day after a splint bone extraction. The incision was not swollen, and dry, the skin still adapted, and the temperature of the surrounding tissue was not increased (total score: 0 points). In this patient, the SAA reached its highest concentration on the third day after surgery (2890.7 µg/ml). On day 5, the dehiscence occurred, SAA had returned to the base level (0.1 µg/ml). Iron declined on the day after surgery and returned to the reference interval two days later. On day three, a 2.7-fold elevation of haptoglobin was observed.

Horse 32 (control) was febrile in the evening after surgery and in the morning of the third day after surgery. For its incision a total score of 0 points was given on days 1 and 5. On the third day after surgery, a swelling was present, which was scored with 1.5 points. Concurrently with the fever on day 3, the highest SAA concentration was measured on the third day after surgery, too (1601.3 µg/ml). The lowest iron concentration was measured on day 1 (4.9 µmol/ml), and the highest haptoglobin concentration on day 5 (6.37 mg/ml). WBC was lower on day 5 than prior to surgery. but within the reference interval in both measurements.

In Horse 34 (control), which was febrile in the evening after surgery and in the morning of the fifth day after surgery, a score of 1 point was given for skin temperature on the day after surgery. The total scores were 0 in the other two wound evaluations. The highest SAA concentration (385.0 µg/ml) was determined on the day after surgery, and the highest haptoglobin concentration on the fifth day (4.86 g/ml) after surgery. The lowest iron concentration was observed on day 1 (6.7 µmol/ml). On day 5, the iron concentration also was below the reference interval (7.7 µmol/ml). The WBC had almost doubled by the fifth day after surgery (11.34 G/l). The horse was discharged clinically healthy on day 6 after surgery.

Except in patients who were febrile on day 0, serum amyloid A concentrations were increased in patients during post-operative fever. The iron concentrations mostly decreased by the time the fever developed. Of the inflammatory parameters studied here, febrile horses showed the least prominent changes in haptoglobin concentration.

In conclusion, 5 of the 59 patients (8%) developed post-surgical temperatures above 38.6 °C. A clear relationship to the development of a surgical site was not seen. Both patients that developed a septic joint after arthroscopy showed normal body temperatures within the five-day study period. Due to the low number of horses correlations of febrile body temperatures with the inflammatory parameters were not calculated. However, from a descriptive basis, SAA often seemed to rise fast and high in fever, accompanied by a decline in iron, whereas haptoglobin changes were slight and often seemed to occur with a delay.

5 Discussion

5.1 Discussion of the retrospective study

5.1.1 Advantages of the retrospective study design

The retrospective cohort study included 652 equines undergoing elective orthopaedic surgical procedures from June 2011 to January 2015. 259 patients received perioperative antibiotics, and 393 did not and were used as controls. This comparatively large number of cases (MacDonald et al. 1994, Borg and Carmalt 2013) was compiled over a period of just 3.5 years. It offered the advantage that there were no major changes in surgeons, procedures, or drugs used compared to other publications whose data were compiled over a longer period of time, for example nine years (Olds et al. 2006). Treated and control horses were distributed quite evenly over this time. Significant differences between the treated and control group were found regarding age and WBC: the younger ones and horses with high WBC received antibiotics more often. Furthermore, individual surgeons used perioperative antibiotics significantly more often than others. Findings like these are typical confounders in retrospective studies. As long as they are detected, their influence on the validity of the results needs to be discussed. In the author's opinion, the fact that antibiotic-receiving horses were 5.0 ± 3.9 years old and significantly younger than controls with 7.1 ± 4.3 years, this difference does not seem big enough to be able to influence the results. Concerning the WBC (treated: 8.57 ± 2.58 G/l; control: 7.37 ± 1.98 G/l; $p < 0.0001$), the difference between the groups – though significant – seems not likely to have affected the results, especially since mean and standard deviation values of the treated group are just slightly above the reference interval (4.9-11.1 G/l). Overall, even in the subgroups, the relatively high number of cases could be assembled due to the retrospective nature of the study and assures reliable results.

5.1.2 Limitations of the retrospective study design

A typical difficulty in retrospective studies are the doubts about the accuracy of the record keeping. Since routine systematic and electronic documentation was introduced in the clinic years before the study period began, this aspect was not judged of being of high importance. Only eight cases could not be included due to a lack of information.

Non-systematic evaluation of clinical and laboratory examinations and non-controlled inter-observer agreement are typical disadvantages of retrospective studies, too. Nevertheless, it must be kept in mind that the timeframe of the study was comparatively short and that there were no major changes in the team during this time. Therefore, many wounds were evaluated by the same observers, so possible inter-observer differences seem negligible.

Probably, several patients were actually at increased risk to develop wound complications and, therefore, received perioperative antibiotics – this was statistically true for those with high WBC preoperatively. However, most horses in the treated group seem to have received antibiotic coverage due to the subjective concerns of the responsible surgeon which were not documented and, therefore, not available for retrospective analysis. In future clinical trials, surgeons should document their reasons for initiating the perioperative antimicrobial prophylaxis in detail so that these horses can still be included in the statistical analysis. Assuming that these decisions were correct and that the chosen antibiotic regime was efficient, the complication rate (1.5% septic arthritis, 39.1% total incisional complications) would have been higher in the treated group if these patients would have been left without an antibiotic cover. On the other hand, the fact that 393 patients did not get any antibiotic cover and did not show higher complication rates allows the conclusion that at least the routine use of antibiotics in all patients undergoing elective procedures does not seem justifiable. Future research should focus on parameters which could predict the need for antibiotic administration in such procedures.

5.2 Discussion of the prospective study

5.2.1 Advantages of the prospective study design

The prospective part of the study consisted of a randomised controlled cohort study. The aim was to detect a possible effect of amoxicillin and gentamicin, given once preoperatively, on the development of post-surgical complications in clean orthopaedic surgery in equines. Due to the prospective study design, all probands were examined and treated at the same time points and received the antibiotics in the same dosages, which makes the surgical wound evaluations directly comparable with the laboratory examinations. To the author's knowledge, this is the first prospective clinical study on preoperative antimicrobial prophylaxis in this kind of surgery.

Mostly, the author evaluated the wounds personally, and only 15 patients were examined by two colleagues. These two colleagues had the wounds of at least five horses at all examination time points evaluated in cooperation with the author. Therefore, these two were familiar with the scoring system and due to the exactly defined and relatively simple scoring procedure, it is assumed that they had judged the wounds like the author would have done it.

The surgical wounds were evaluated using a score sheet which included exudation, swelling, skin temperature, and dehiscence (see chapter 3.2.4). This wound evaluation system was developed for the prospective study according to the main remarks found in the retrospective analysis. It showed similarities to the scoring system that was used in Miller's dissertation (Miller 2006). She evaluated wounds and inflammatory parameters not just after elective orthopaedic surgical procedures (arthroscopy and splint bone extraction) but also after laparoscopy and castration. Therefore, the wound size and risk of complications in her study showed a far wider scope than seen in patients presented here. Miller scored exudation according to amount and character, whereas in this study, due to the small amounts of secretions absorbed by the wound coverage, the score was focused on the character of the exudate. Miller evaluated local pain (max. 3 points) and swelling (max. 3 points) separately whereas these two parameters were combined with a maximum of 3 score points in this study. The assessment of skin temperature in the incision area was alike. In Miller's study, dehiscence was not included in the score.

The laboratory parameters were measured on the day before surgery, within 30-60 minutes after the horse regained its standing position after anaesthesia (named "directly after surgery") as well as on the first, third, and fifth day after surgery. This is less frequent than in Miller's dissertation. She determined the inflammatory parameters in 24 horses undergoing arthroscopy directly before, 6, 12, 24, 48, and 72 hours after surgery, and if possible, also seven days after surgery (Miller 2006). Jacobsen et al. (2009) determined SAA, iron, and fibrinogen before, as well as 1, 2, 3, 5, 7, 9, and 11 days after surgery in 11 horses that had an arthroscopy. Due to the shorter hospitalization time and the intention to evaluate the inflammatory parameters together with the wounds, other time points of sample collection were chosen than in the previously mentioned studies, so that a direct comparison of the results is not possible (Miller 2006, Jacobsen et al. 2009). However, since the samples were taken at the same time as the wounds were evaluated, the concentration of the inflammatory parameters can be put in direct context with the wound healing. A retrospective study evaluating 26 horses with systemic inflammation, 114 horses with local inflammation, and 61 healthy horses/horses with non-inflammatory disease found significantly higher serum amyloid A concentrations and significantly lower iron concentrations in horses with systemic infection than in horses with local infections (Hooijberg et al. 2014).

5.2.2 Limitations of the prospective study design

In the early days of equine clean orthopaedic surgery, an overall complication rate of 8.1% was reported (MacDonald et al. 1994). The incidence of septic arthritis, one of the most severe complications following arthroscopy, is naturally lower and was reported to be close to 1% (Borg and Carmalt 2013, Olds et al. 2006). Due to this low post-arthroscopy arthritis rate, calculating the number of probands needed to be able to differentiate between treated and control animals was based on an assumed overall complication rate of 20%. This complication rate included increased swelling, increased skin temperature around the surgical incision, exudation, and dehiscence, which were assumed to be caused mainly by bacterial contamination. In order to calculate the number of cases needed to be able to differentiate between horses receiving antibiotic prophylaxis and controls, our hypothesis was that antibiotic prophylaxis should diminish these complications by at least by 12%. With 30 patients in each group, this study has a statistical power of 60% and an alpha value of 0.3, which seems acceptable for a first controlled study in this area. However, statistically, these values have to be seen critically, and the results certainly need assurance by evaluation of higher patient numbers. For a statistical power of 80% and an alpha of 0.05, 130 horses would have been needed per group. To reach this number, a much longer study period would have resulted, which would have included more changes in surgeons and more changes in procedures. In summary, despite their relatively low power, the results are considered reliable enough, to conclude that the overall complication rate in the control group was not higher than in the treated group. Certainly, minor possible advantages in the antibiotic-treated group may not have shown up due to the relatively low patient numbers.

Furthermore, after conducting the study, a critical point in its design needs to be pointed out: the typical onset of septic arthritis has been reported to be 15-17 days after surgery (Borg and Carmalt 2013), which indicates that our observation period may not have been long enough for detecting this severe form of complication. Nevertheless, during the first five days after surgery, other complications occur, such as increased swelling or increased skin temperature, which can be indicative of developing septic arthritis or other severe surgical site infections. Horses showing these signs were monitored closely even after finishing the study period. Furthermore, if there would have been any concern in the owner or the trainer about the healing of a discharged horse after surgery, due to overall intense contact with the clients, the clinic certainly would have been informed. Therefore, it seems very unlikely that any complications occurring after discharge were missed.

For a more objective wound evaluation, a blinded observer would have been optimal. As the author was responsible for the randomisation of horses and the wound evaluations, this was not possible. Furthermore, if antibiotics were administered, it was obvious in the patient file, which was open to the author.

However, using a clearly defined semiquantitative scoring system as strictly as possible should have diminished a bias due to the knowledge which horse was treated antibiotically or not. Furthermore, in concurrently examining the horses clinically every day and measuring the rectal body temperature, these wound evaluations were seen as an important part but have never stood alone in assessing possible complications. This supporting information clearly pointed out which horses needed to be extensively monitored in particular. In addition to randomization and blinding the study, the application of a placebo would have completed an optimal study design. Since the antibiotics were applied less than one minute prior to inducing general anaesthesia, effects of any manipulation or application of a comparable volume, e.g., of 0.9% NaCl were not expected, and therefore not pursued.

5.2.3 Methods of the prospective study

The number of drop-outs was almost equal in both groups. 9/45 horses in the control group (22.5%) and 7/35 horses in the antimicrobial-treated group (20%) were randomised but did not finish the planned observation period, and therefore, their data were not included. However, these relatively high drop-out rates were not considered likely to have diminished the validity of the study results as the reasons for any drop-out were unrelated to the surgery and group assignment. The surgical incisions of the dropped-out horses were scored using the same protocol as for the horses that remained in the study. Extraordinarily high scores were not observed in these horses. Furthermore, in horses discharged before the end of the 5-day postoperative study period and in case of complicated wound healing, it was assumed, that the clinic was contacted by the owner, trainer, or even referring veterinarian. Therefore, it seems very unlikely that any form of complicated wound healing or even arthritis was missed. The outcome regarding the ability of preoperative antibiotics to prohibit post-surgical complications was most likely not altered.

Penicillin and gentamicin are commonly used as prophylactic antimicrobials in equine surgery (Southwood 2014). During the study period, due to a production shortage, no approved preparation of penicillin for horses was available on the German market, so amoxicillin as the sodium salt (Belamox®, bela-pharm, Vechta, Germany) was chosen as an alternative antibiotic with an even broader spectrum efficiency. Though penicillin was used as a beta-lactam antibiotic in studies comparing different antibiotics in castrates (Haucke et al. 2017) and in colic surgeries (Durward-Akhurst et al. 2013), respectively, the use of amoxicillin in horses has been described for more than 30 years (Carter et al. 1986, Brumbaugh et al. 1999). In this study, as recommended by the manufacturer, the dose of amoxicillin (10 mg/kg) in combination with gentamicin in the commonly used dosage (6.6 mg/kg) was administered as a single shot immediately before inducing general anaesthesia in order to generate high tissue levels at the time of the first incision.

Determining the WBC using the same machine throughout the study would have been optimal. However, for the first 46 probands, the LaserCyte® Dx Hematology Analyzer (IDEXX Europe B.V., Hoofddorp/The Netherlands) was used. After these horses, due to a laboratory machine change in the clinic, the ProCyte Dx® Hematology Analyzer (IDEXX Europe B.V., Hoofddorp/The Netherlands) was used. The equality of both analysers was tested by comparing sixteen blood samples from different horses. A CBC was performed with both machines. A correlation factor for WBC of 0.99 was found. Therefore, it was assumed that the device change did not affect the WBC data.

Also, five horses were not operated in Tierklinik Lüsche GmbH but at a different location called Sanakena, and their haematology was performed on the day after blood collection. To prove that there were no valid differences between the haematology values in the same blood sample, a storage experiment of five blood samples was performed for which these values were compared by a CBC performed directly after blood collection and after having been refrigerated for one day. A correlation factor for WBC of 0.99 was found. Presumably, storing the blood samples for 24 h at 4 °C did not affect the WBC.

Laboratory evaluations were missing in six horses due to missing samples. In one control horse, haematology and creatinine were not performed on the day before surgery. In four horses (3 controls, 1 treated), the inflammatory parameters and creatinine values were not determined on the day prior to surgery due to a communication problem. On the fifth day after surgery, the leucocytes of one control were determined manually and therefore not considered for statistical evaluation. Overall, an influence of these few missing values was not assumed, and for statistical purposes, the Wald test was used, which allowed an analysis of all available data despite the lacking values.

Suboptimal preanalytic sample handling might have caused haemolysis in 43/291 blood samples. Of these, 27 were collected directly after surgery. Due to the hectic work environment, the blood was often transferred from the syringe into the sample tubes with an attached cannula, what might have caused preanalytic haemolysis. The results of four of the samples were not included in the statistical analysis as the iron concentration was more than twice as high as previously determined. Nevertheless, the results of the iron concentration as determined by the other measurements of these horses were included in the statistical analysis, and the method of maximum likelihood (Wald) test was applied.

Unfortunately, a significant difference between the treated and control groups was detected regarding the NSAID applied. The treated horses received significantly more often meloxicam than the controls (p=0.03). Forty-four horses did not receive meloxicam because of the higher costs. Fifteen horses were intended for human consumption, so an administration of phenylbutazone was not an option due to legal reasons. A recent study compared the efficacy of meloxicam and phenylbutazone in two experimental pain models in horses. In a lipopolysaccharide (LPS)-induced synovitis model, in which synovitis was induced in 16 horses by injecting 50 ng of LPS in 0.5 ml Ringer's solution into one intercarpal joint, both meloxicam and phenylbutazone reduced the joint temperature compared to a placebo. The inflammatory pain was measured by scoring pawing the floor or pointing a foot, movement, position in the stall, ear position, and orbital tightening. Regarding lameness, there was no significant difference between meloxicam and phenylbutazone, but meloxicam was more effective than phenylbutazone in reducing synovitis-associated changes in head movement (Banse and Cribb 2017). In the study presented here, neither lameness after surgery nor pain on palpation were observed in any of the patients. As a systemic inflammatory response, fever was present both in 3 horses that received meloxicam and in 2 patients receiving phenylbutazone. The total scores were highly variable both in horses treated with meloxicam and horses receiving phenylbutazone so that an influence of the NSAID on the score points in each group was not assumed.

Furthermore, in three of the phenylbutazone-receiving horses, suspected adverse reactions were seen: two patients showed elevated creatinine levels, and one horse was anorectic. After discontinuing the NSAID on the day after surgery, the creatinine levels returned to normal limits, and the third horse started eating again. In these patients, excessive elevations of the inflammatory parameters were not observed, so an effect of the absent non-inflammatory medication on wound healing seems implausible. Therefore, it seems reasonable to critically evaluate the type and the duration of postoperative analgesia with NSAIDs.

5.3 Advantages of the combination of studies

The main advantage of the retrospective study is the relatively high number of cases that were available. In the prospective study, the main advantage is the randomized allocation of the patients to either the application of antibiotics or the control group. Further advantages are the standardized medications and wound evaluations after surgery. The main disadvantage of the prospective part is the limited number of cases, which lowers the statistical power of the study. In the retrospective part, the main disadvantages are the inconsistent medications and wound evaluations. By performing these two study designs consecutively, an attempt was made to find those kinds of complications that could be influenced by antibiotics and seen in sufficient numbers to allow a prospective study with sufficient statistical power within a reasonable timeframe.

5.4 Septic arthritis – discussion of results

5.4.1 Septic arthritis in the retrospective study

Weese and Cruz (2009) retrospectively reviewed ninety-seven case files of horses undergoing an arthroscopy, of which 95 (98%) received antibiotics. They did not report any infectious complications, only one horse showed serous fluid dripping from the surgical site (Weese and Cruz 2009). Borg and Carmalt (2013) analysed 444 horses undergoing 636 arthroscopies from 2008-2010 without antimicrobial prophylaxis. Three joints (0.5%) became septic. This is even lower than the rate of 1.5% (7/463) in our total retrospective material. An overall joint infection rate of 1.5% was also reported by Olds et al. (2006), who reviewed 461 patient charts after arthroscopies with available follow-up information. In their study, 188 horses received perioperative antimicrobials, and 273 did not. One patient developed septic arthritis despite getting these drugs (0.5%), and six joint infections occurred in the group without antibiotics (1.5%). At first glance, these rates seem to be reversed by our findings: 2.53% of infected joints occurred in the antimicrobial-receiving group (5/198) and just 0.76% in the controls (2/265). Statistically, these differences were not significant. However, since the total number of joint infections is low, the meaning of the statistics-based statements on the benefits of perioperative antibiotics in arthroscopies remains questionable. Our study is the first evaluating mainly Warmbloods (93.6%), whereas Olds et al. (2006) have seen mainly Standardbreds (72%) and Thoroughbreds (15%). To the author’s knowledge, these two are the only publications evaluating perioperative antibiotic use in reasonably large controlled populations. If we put together the data of both studies, the results cover a total of 924 horses undergoing arthroscopies. Septic arthritis occurred in 1.55% of the patients receiving perioperative antibiotics and in 1.49% of the patients who did not. To improve the statistical power of such studies, it would be helpful to assemble comparable data from many more clinics, even retrospectively. However, these data may be biased due to an increased use of antibiotics in horses that are considered to be at a higher risk by the responsible surgeon. These reasons might not be documented, and therefore, a retrospective analysis might not be able to detect them. In conclusion, the rate of arthritic complications might be falsely low in the patients which received antibiotics. On the other hand, the retrospectively-assembled data in the controls definitely do not show an increased risk of arthritis after arthroscopies in patients which did not receive perioperative antibiotic prophylaxis. Therefore, to the best of the author's knowledge, the routine use of antibiotics in uncomplicated elective, non-suspicious arthroscopies in horses cannot be justified by the available data.

Readmission due to septic joints after arthroscopy on day 2, 15, and 17 was reported by Borg and Carmalt (2013). Furthermore, Olds et al. (2006) reported a median time of 20 days of operated horses presented with septic arthritis. The first signs of septic arthritis were only observed several days to weeks after surgery (Olds et al. 2006). In our retrospective material, three horses developed septic arthritis on day 2, and one each on day 2, 5, 6, and 27 postoperatively. The delayed onset of joint infection suggests a contamination of the joint at some time after surgery (Smith et al. 1982, Bertone 1996). That a pre-/perioperative antibiotic treatment can prevent infection from a contamination days after surgery is not probable. However, side effects, such as the development of colitis and/or bacterial resistance (Weese and Cruz 2009), clearly weigh out the benefits of a prolonged antibiotic treatment. Also, in humans undergoing coronary artery bypass surgery, a prolonged antibiotic prophylaxis (>48 h) was not only ineffective in reducing surgical site infections compared to a shorter protocol (<48 h) but also increased the antimicrobial resistance (Harbarth et al. 2000). As antibiotic resistance is considered to be a worldwide emerging health concern, the application of prophylactic antimicrobials in equine clean orthopaedic surgery should be reserved for patients at risk of developing a surgical site infection.

5.4.2 Septic arthritis in the prospective study

In the prospective part of this study on 47 arthroscopies in 36 horses, Horse 30 in the control group developed a septic coffin joint on day 8 after surgery, after the end of the study observation period (1/47 joints: 2.1%; 1/36 horses: 2.8%). Regarding only the controls, the rate of septic joint development would be 4%

(1/25 arthroscopies). With an antibiotic cover, none of the 22 joints in the 17 horses involved showed such a complication. However, the excluded Horse 39, initially randomised into the treatment group, developed a septic coffin joint despite receiving the routine amoxicillin/gentamicin combination preoperatively and additional gentamicin for two days after the arthroscopy. This dropped-out horse emphasizes the importance of including a sufficient number of horses in order to produce valid recommendations. The number of horses undergoing arthroscopies in the prospective part of this study is definitely not sufficient to allow conclusions on the usefulness of antibiotics in preventing septic joints. The aim of this study was to compare the incidence of all wound complications, which – based on the retrospective data – are estimated to occur in 39.1% of cases. Furthermore, it must be mentioned that Horse 39 succeeded several times in biting off its bandage despite the distal fixation with an adhesive bandage and a cover with “unpalatable” paste (AKS, Horse fitform AKS Verbiss-Stop, flüssig 1l, PHARMAKA GmbH & Co. KG, Essen, Germany). Therefore, the arthroscopy portals to the coffin joint were exposed to the environmental and possibly faecal germs. Typically, the pathogens found in equine surgical incisions are β-haemolytic *streptococci*, *staphylococci*, *enterobacteriaceae*, and *Pseudomonas* spp. (Ahern and Richardson 2011). These are saprophytes, and in the case of *enterobacteriaceae*, also faecal bacteria (Bauerfeind 2010, Wieler et al. 2010) which are ubiquitous in the horses’ surroundings. Gram-positive cocci are regularly found on the skin and the mucous membranes of healthy individuals (Valentin-Weigand 2013). As the onset of the septic arthritis was 8 or 9 days after surgery, respectively, in our study material, the observation period of five days was probably not long enough in this aspect, which is underlined by other retrospective studies (Olds et al. 2006, Borg and Carmalt 2013) and the retrospective material of this project. Nevertheless, the horses were continuously monitored either by the clinic or the owner/trainer, who with a very high probability would have contacted the clinic in any case of complications. Therefore, it is unlikely, that septic arthritis was missed in any of the patients. Furthermore, surgical site infections are defined to occur within 30 days after the surgical procedure if no implant was placed and within a year when an implant remains in the body (Waguespack et al. 2006). It was already hypothesized that the contamination of operated joints can occur at some point after surgery (Smith et al. 1982). Therefore it seems very unlikely that preoperative application of antibiotics as a single shot will prevent any infections occurring at some point after surgery. It needs to be emphasized, that after discharge after arthroscopy, a joint may still develop an infection and then requires intensive therapy immediately (Orsini 2017).

5.4.3 Conclusion

In neither the retrospective nor the prospective part of this project, nor in other retrospective studies (Olds et al. 2006), a reduction of the incidence of post-operative septic arthritis after equine arthroscopy by perioperative antimicrobial prophylaxis was demonstrated. Borg and Carmalt (2013) report post-operative septic joints in 0.5% of the joints and in 0.7% of the horses after 444 arthroscopic procedures without perioperative antimicrobial prophylaxis. To the best of the author's knowledge, no other controlled studies examining the efficacy of pre-/perioperative antimicrobial prophylaxis exist. Even if the arthritis rate in the antibiotic-receiving horses might be falsely low, the overall low incidence in controls should lead to the recommendation to not use perioperative antibiotics out of habit. The conscious decision to use or not use antibiotics in each case should become routine. Surprisingly, even in horses treated with antibiotics, a late onset of septic arthritis is sometimes seen. This warrants the careful evaluation of the patient (by the informed owner) in all cases for about four weeks postoperatively.

5.5 Incisional abnormalities - discussion of results

5.5.1 Incisional complications in the retrospective study

MacDonald et al. (1994) published a retrospective study on the occurrence of wound infections in 452 equine orthopaedic surgical cases. They described a complication as an infected wound, which meant purulent discharge, spontaneous dehiscence, erythema as well as pain and swelling in the "immediate" post-operative period (which was not clearly defined). An overall wound infection rate of 10.0% was found in their material. If just the clean surgeries were considered, the rate was reduced to 8.1%. Surprisingly, even after a multivariate analysis, they found a 4.6 times higher risk of developing wound infections in patients which were treated with preoperative antibiotics. The authors explain this phenomenon with the ability of surgeons to correctly identify the risk factors, e.g. the difficulty and amount of trauma associated with the procedure. This might have resulted in the decision to cover high-risk patients with antimicrobials preoperatively (MacDonald et al. 1994). In the retrospective study part presented here, complications were listed in more detail and – besides joint infections – comprised seroma, any kind of wound discharge, moderate and severe swelling as well as a body temperature higher than 38.5°C. This explains our high overall complication rate of 39.1% in 652 patients with clean surgical procedures, which exceeds the above-mentioned 8.1% by far. Despite this more detailed data collection, including increased wound swelling – and therefore probably higher percentages in our data – there was no clinically or statistically relevant decline in complication rates in patients that received perioperative antibiotics compared to controls that did not (p=0.5).

5.5.2 Incisional complications in the prospective study

In the prospective part of the project, the total wound evaluation score was significantly higher in the treated than the control patients on day one (p=0.03) and day five (p=0.01). Randomly allocated, antibiotic-receiving horses showed significantly higher scores for local swelling (on days one and five) and skin temperature (on days one and three). Admittedly, overall and even initial wound scores were quite low, and scores for swelling as well as skin temperature decreased significantly during the observation period in both groups. Furthermore, there were no significant differences in exudation and dehiscence between the treated and control patients. At this point, it seemed to be important to mention that the study was not blinded, and therefore, a bias of the examiner cannot completely be excluded. However, a systematic influence was avoided to the best of the author’s knowledge by defining the strict criteria for score point assignment before the start of the study and by enhancing the awareness of a possible bias.

MacDonald et al. (1994) assumed in their retrospective analysis that the significantly higher risk of developing surgical site infections under antibiotic cover was caused by the identification of additional risk factors by the responsible surgeon. Despite the antibiotic cover, those high-risk patients remained to have an increased risk of complications – which would even be higher if they had not received antibiotics. However, the prospective study presented here was randomised. Therefore, the horses in both groups should have had the same risk of developing surgical site infections. To the knowledge of the author, this is the first prospective controlled study that evaluated local reactions after performing elective orthopaedic surgical procedures in equines. The results show that the healing process in the control group was certainly not inferior in comparison to the group receiving "prophylactic" antibiotics.

5.5.3 Conclusion

Taken together, the results of MacDonald et al. (1994) and the retrospective and prospective studies presented here, show that there are no advantages evident in patients that received perioperative antimicrobial prophylaxis compared to controls that did not. The general application of these drugs in equine uncomplicated elective, clean orthopaedic surgery is not justifiable by the available data. In our prospective study, the "one-shot" preoperative amoxicillin and gentamicin failed to reduce post-surgical complications. A routine preoperative application of antimicrobials in this type of surgery does not seem necessary. The administration of antimicrobial pro- or metaphylaxis in equine uncomplicated, clean elective surgery requires an individual assessment of risks and benefits, which should include surgical and postoperative findings.

Additionally, these findings substantiate the German guidelines for antibiotic use in animals, which recommend the antibiotic prophylaxis for horses only in long and complicated surgeries (Bundestierärztekammer 2015).

5.6 Fever – discussion of results

In a retrospective study of human orthopaedic trauma patients suffering from closed (88%) or open (4%) fractures, and soft tissue injuries (8%), 106/582 (18.2%) developed a fever in the early postoperative period which was defined as five days after surgery. All patients received perioperative antibiotics for 24 hours, commencing at the time of surgery. It was not precisely specified when the last dose of antibiotics was given. Median onset of fever after surgery was 14 hours (range 8-32 hours) and the mean duration was 9 hours with a standard deviation of 11 hours. In 59/106 febrile patients, 110 diagnostic tests were performed, including bacterial culture of urine (n=31), blood (n=30), or wound exudate (n=1) as well as urine biochemical analyses (n=28) and chest radiographs (n=20). Bacterial causes of the fever were identified in 12% of the tests (1 blood and 3 urine cultures, 7 urine analyses, and 2 radiographs). It was assumed in almost all cases that the fever was not caused by a bacterial infection, but by an elevation of inflammatory parameters after trauma and surgery (Petretta et al. 2013).

In our retrospective study of elective orthopaedic surgical cases, 30/652 horses (4.6%) developed a fever during their hospitalization. Of these, 8/30 (26.7%) already showed temperatures above 38.5°C on the first day after surgery, and nine were febrile at the second day. Only one patient with fever (on day 6) developed septic arthritis on the same day. However, none of the patients in the retrospective part of the project with fever in the early post-operative period (2-5 days) developed further signs of a systemic infection. Nonetheless, due to the fear that an elevated body temperature might be caused by bacteria, antibiotic treatment was initiated in 14 of our feverish 25 control horses of the retrospective study. Knowing nowadays that a rise in body temperature might just be caused by a postsurgical increase in inflammatory parameters, the antibiotic use is questionable at the least. Certainly, horses with a postoperative fever should be particularly carefully checked for signs of infection as it is done in the prospective part of the study. However, to the author's knowledge, no systematic studies exist on the development or causes of postoperative fever after clean orthopaedic surgery in equines. A prospective study regarding the duration and degrees of body temperature after these procedures could help in differentiating between purely inflammatory and septic causes.

In the prospective part, a well-comparable percentage of horses (5/59; 5.5%) developed such an elevated body temperature within the five observation days.

Four belonged to the control group (n=31; 12.9%) of which only one (Horse 16) also developed a surgical site infection (2 dehiscent sutures at the distal edge of the incision). Only one patient in the treatment group (n=28; 3.6%) developed a fever. Two control horses (Horse 3, horse 32) received antibiotic treatment due to the fever and recovered without further complications. A post-operative monitoring of the horses and antibiotic treatment of patients identified to be at risk seems to be sufficient. Four controls and just one treated horse developed fever in the prospective study; the number of patients, however, does not seem large enough to evaluate this difference statistically. However, in our retrospective material, 25 controls and just 5 treated horses showed postoperative fever. Therefore, a close observation of the body temperature for at least 28 days postoperatively is strongly recommended.

5.7 Inflammatory parameters

5.7.1 Inflammatory parameters after clean elective orthopaedic surgery in horses

In our study, Serum Amyloid A, iron, haptoglobin, albumin and globulin as well as the WBC were determined as inflammatory parameters in the horse. Miller (2006) evaluated inflammatory parameters in equines after different types of surgery. In a subgroup of 24 horses undergoing an elective arthroscopy, the SAA peaked 48 hours after the procedure (210.4 ± 147.7 µg/ml). After seven days, the SAA concentrations were back in the reference interval (Miller 2006). Another study confirmed an inflammatory response after arthroscopy in 11 equines. Already on the first day after surgery, significantly higher SAA levels were found than preoperatively. Initial levels were reached five days after surgery (Jacobsen et al. 2009). Neither of these publications specifies the perioperative prophylactic treatment with antimicrobials. In our study, the SAA concentration was not measured as frequently as in the other studies. The SAA peak seems to occur around the same time as in Miller`s study: In our treated group, the individual concentrations peaked between day 1 and 3, in the control group the peak occurred at a later timepoint, between day 3 and 5. To the best of the author's knowledge, the study presented here is the first to compare post-surgical inflammatory parameters with and without antimicrobial prophylaxis in horses. Comparing the treated and the control patients, in the antibiotic-receiving group, the SAA levels returned to their initial concentrations within the observation period of 5 days, whereas in the control group, the decline of the SAA concentration started on day 3 but did not reach the initial levels even by day 5. Also, the SAA concentration seems relatively, though not significantly, higher in the control than in the treated group. This could be either caused by an additional anti-inflammatory effect of the antibiotics as described for tilmicosin, a macrolide antibiotic (Buret 2010). However, to the best of the author's knowledge, such effects were not described for either amoxicillin nor gentamicin so far. On the other hand, it is possible that the controls had higher SAA concentrations than the treated horses because of bacterial contamination during the surgery which was not prevented by antibiotics. This seems nonetheless unlikely, as more treated than control horses developed a surgical site infection. An influence of the NSAID cannot be completely excluded, as 11/28 treated and only 4/31 control horses received meloxicam instead of phenylbutazone. A recent study compared the efficacy of phenylbutazone and meloxicam in reducing inflammation-induced pain. The only difference between the two agents was that meloxicam was more effective reducing synovitis-induced head movement (Banse and Cribb 2017).

A significant decrease in the serum iron concentration was seen in 11 horses undergoing arthroscopy ($p<0.0001$), however, it returned to baseline on day two (Jacobsen et al. 2009). During our 5-day-long observation period, there was no significant difference between treated and control horses in serum iron concentration ($p = 0.76$), but there was a significant change in the iron concentration. Furthermore, similar to Jacobsen's study, a low serum iron concentration was determined directly after surgery, which went back to the initial concentrations already on day 1 after surgery. For using iron as a marker for inflammation/infection after surgery, more horses with such sequelae need to be examined – possibly they could be detected by continuously low iron levels.

In Miller's study, 48 hours after arthroscopy (n=24), there was a slight increase in the haptoglobin concentration (2.9 ± 1.5 mg/ml), which developed into a decisive augmentation after 72 hours (3.2 ± 1.6 mg/ml, Miller 2006). In our study, the serum haptoglobin concentration changed significantly over time ($p<0.0001$). The haptoglobin values on day three and five were generally, although not significantly, higher than at the previous measurements prior to, directly after, and on the day after surgery. However, in 14/59 horses, the initial haptoglobin concentrations were already above the reference interval before surgery. Therefore, it remains unclear whether an increase in serum haptoglobin concentration postoperatively can be a valuable indicator of inflammation/infection in a horse.

Jacobsen et al. (2009) reported a significantly higher WBC on day 1 than the preoperative baseline levels ($p<0.01$) and significantly lower levels on day 5 after surgery ($p<0.01$). From day 6 on, the WBC was back at the baseline level. In our study, the WBC was also significantly lower on day 5 than before surgery, but not to a clinically relevant extent. Neither of the horses with a WBC below 4.0 G/l developed a surgical site infection. However, 1/5 horses with a WBC >11.0 G/l developed a septic joint. Of course, there were not enough horses included into the study to make a statistically valid statement. However, according to the results, WBC changes are not seen regularly with SSI.

5.7.2 Inflammatory parameters in horses with surgical site infections

In the prospective study, decisive changes in inflammatory parameters were observed just in horse 30 that developed septic arthritis on day eight. In this horse, the course of serum amyloid A and iron suggested the onset of an inflammation. In this patient, the SAA concentration increased steadily until day five to 1565.6 µg/ml, and the serum iron concentration declined continuously. Retrospectively, the intense and prolonged post-surgical inflammatory response in horse 30 may have suggested the beginning inflammation of the joint. In order to detect imminent danger of a severe surgical site infection, the findings in this horse suggest that a control of SAA five days postoperatively might be useful, since in patients with uncomplicated healing after surgery this parameter should have returned to normal limits by then.

In Horse 39 which received additional gentamicin (excluded) and developed post-surgical septic arthritis nine days after surgery, however, the serum amyloid A concentration did not show a pronounced increase. On the day after surgery, it hardly exceeded the reference interval and returned to baseline levels on day three. On the day of surgery and on day one, the iron was below the reference interval in this horse. On the one hand, this mild change in inflammatory parameters could be a laboratory measurement error. On the other hand, it could also underline the theory that the infection of the joint often occurs at some point after the surgery (Smith et al. 1982, Bertone 1996). This is also supported by the observation that this horse repeatedly removed its bandage despite all precautions.

The presurgical SAA levels of Horse 64 (two dehiscent sutures on day one) showed an almost 300-fold elevation. This concentration then declined steadily. The lowest iron concentration in this patient was measured on the day after surgery. Its highest haptoglobin concentration was determined directly after surgery. An infection in a remote site of the body was defined as a patient-associated risk factor of the development of SSI in humans (World Health Organization 2009, Wacha et al. 2010, Bratzler et al. 2013). In Horse 64, the elevated serum amyloid A concentration prior to surgery suggested the presence of a clinically inapparent inflammation - possibly caused by an infection. Therefore, this patient was at risk of developing a surgical site infection. A preoperative assessment of the inflammatory parameters can reveal subclinical infections and may help minimize the development of surgical site infections. The pre- and postoperative monitoring of serum amyloid A, for which a portable test is available (Stablelab SAA test, exact results with the Stablelab EQ-1 Handheld Reader, Stablelab, Sligo, Ireland), seems for a preoperative risk assessment, even though just 2/8 horses with elevated preoperative SAA concentration developed a surgical site infection in the study presented here. Therefore further research seems warranted to identify and describe risk factors for SSI development in horses.

Except for the joint infections in horses 30 and 39, all patients responded well to local treatment. Significant differences in serum amyloid A have been reported as well as in the serum iron levels between horses with local and systemic infections (Hooijberg et al. 2014), which explains why the other four horses might not have shown major changes in the inflammatory parameters. Except for horse 39, the inflammatory response in our patients with surgical site infections also reflected its severity. The inflammatory parameters in horse 39, however, did not suggest the development of a septic joint during the first five days after surgery. Nevertheless, inflammatory parameters alone are no reliable assessment for differentiation between systemic and local inflammation (Hooijberg et al. 2014). Surgical patients with suspicious clinical findings and/or whose SAA levels are constantly rising and have declining serum iron levels, require further intense, precise, and frequent clinical and laboratory examinations in order to initiate an appropriate therapy if necessary. The regular additional monitoring of inflammatory parameters in post-surgical patients can be used complementarily in horses after clean, elective orthopaedic surgery to detect surgery-associated complications early.

5.7.3 Inflammatory parameters in horses with post-surgical fever

In a recent study on 582 human orthopaedic trauma patients suffering from closed (88%) and open (4%) fractures, or soft tissue injuries (8%), 106/582 (18.2%) developed post-operative fever. In these patients, a preoperative increase in inflammatory cytokines, which was initiated by the trauma and was further increased by the surgical procedures, was assumed to be the cause of the fever in the study of human orthopaedic trauma patients (Petretta et al. 2013). A similar phenomenon was also observed in the five (4 controls, 1 treated) febrile patients, of which just one control developed a surgical site infection, in the prospective study: the temperature peaks were seen at the same time when the serum amyloid A concentration increased and the serum iron concentration declined. In febrile patients. already directly after surgery and at all measurements following, the SAA level was higher than the median SAA concentration. From day 1 after surgery onwards, the serum iron concentration in febrile patients was below the median iron concentration, except in horse 3 which was febrile on both temperature measurements on day 2 and therefore received trimethoprim-sulfadiazine. In horse 3, the iron concentration was below the median iron concentration from day 3 onwards. Therefore, it is very well possible that the fever resulted from an increased inflammatory response due to surgery.

High haptoglobin concentrations, however, were not associated with increasing temperatures. It is therefore questionable whether haptoglobin is a valid parameter for measuring the inflammatory response in surgical patients.

6 Summary

This dissertation comprises a retrospective analysis of patient files and a randomised, prospective clinical study of postoperative complications in equines after clean orthopaedic surgical procedures. The main aim of these studies was to evaluate possible differences between horses pre-treated with antibiotics and those not having received these drugs.

For the retrospective part, 652 out of the 684 case files were suitable for statistical analysis. Details were compiled on the patients, the surgical procedure, and the surgery-associated complications. Antibiotic-receiving patients (n=259) were statistically compared to controls (n=393) by employing the Fisher-Freeman-Halton test or an ANOVA. The main parameter was the development of postoperative complications. For the post hoc analysis, Fisher's exact test and the Wilcoxon-Mann-Whitney-test were used. The overall complication rate in the retrospective study was 39.1%. An increased incisional swelling was observed most often (25.6%), followed by exudation (7.5%), fever (2.3%), dehiscence (1.8%), and seroma in five cases (0.8%). Septic arthritis was documented in 7/463 horses (1.5%) undergoing arthroscopies. There were no significant differences detected in the development of postoperative complications between the 97/259 (37.5%) antibiotic-receiving patients and in the 158/393 (40.2%) controls. The application of perioperative antibiotics was significantly influenced by the surgeon ($p<0.0001$), type of surgery ($p=0.0007$), and increased with the number of surgical lesions ($p=0.03$). Antibiotic-receiving patients were treated for 3-5 days after surgery and most of them received penicillin-based antibiotics. Patients with three or more interventions per session (n=30) received antibiotics significantly more often. In patients undergoing tendovaginoscopy/bursoscopy, fasciotomy, and neurectomy (n=98), antibiotic prophylaxis was initiated less frequently than in the other surgeries, e.g., combinations of surgeries, splint bone extraction, tenotomy, and arthroscopy (n=554).

In the prospective clinical study, 75 horses were assigned randomly to either the antibiotic-receiving (treated) or the control group. Treated horses received 10 mg/kg amoxicillin and 6.6 mg/kg gentamicin once directly before inducing general

anaesthesia. These horses were intensely monitored for five days. Using a semiquantitative scoring system for swelling, skin temperature, exudation, and dehiscence, the surgical wounds were assessed on the days one, three, and five after surgery. In addition, the inflammatory parameters SAA, iron, and haptoglobin were determined on the day before, directly after, and on days one three and five after the surgery. Differences between the groups in score points, rectal body temperature, concentrations of inflammatory parameters, and patient characteristics were tested by the Fisher-Freeman-Halton test or the ANOVA/Wald test. For the post hoc analysis, Fisher's exact test and the Wilcoxon-Mann-Whitney test were used.

Total score (p=0.002), the scores for swelling (p=0.002), and skin temperature (p=0.007) were significantly higher in the treated group than in controls. The post hoc analysis identified a significantly higher total score for the treated horses on day 1 (p=0.02), and 5 (p=0.01), which was mainly due to swelling with significantly higher scores on days 1 (p=0.02) and 5 (p=0.002), too. On day 1 (p=0.04) and 3 (p=0.03), the score points for skin temperature were significantly higher in the treated horses. Four horses in the control group and just one treated developed fever. There was no significant difference between the groups in SAA (p=0.18), serum iron (p=0.76), and haptoglobin (p=0.85) concentrations. Two horses (1 control, 1 treated drop-out), of which the treated one dropped out of the study because of postoperative additional gentamicin administration, developed a septic joint eight and nine days after surgery, respectively. Such severe complications in equine clean orthopaedic surgery are rare, whether having received perioperative antibiotics or not. In summary, the use of perioperative antibiotic prophylaxis in uncomplicated, elective orthopaedic surgical procedures in equines cannot be justified based on these data.

7 Zusammenfassung

Untersuchungen zur prä- oder perioperativen Antibiotika-Prophylaxe bei aseptischen, elektiven orthopädischen Eingriffen an Pferden

Dieses Dissertationsprojekt beinhaltet eine retrospektive Analyse und eine randomisierte, prospektive klinische Studie zu postoperativen Komplikationen bei Pferden nach sauberen, elektiven orthopädischen Eingriffen. Ziel beider Studien war es zu untersuchen, ob Unterschiede zwischen antibiotisch (vor-)behandelten und nicht mit Antibiotika behandelten Tieren (Kontrolle) hinsichtlich der Entwicklung postoperativer Komplikationen bestehen. Für den retrospektiven Teil konnten aus 684 Patientenakten 652 Fälle eingeschlossen werden. Einzelheiten zum Signalement, chirurgischen Eingriffen und OP-assoziierten Komplikationen wurden extrahiert. Mittels Fisher-Freeman-Halton-Test oder ANOVA wurde statistisch geprüft, ob Unterschiede zwischen mit Antibiotika behandelten Patienten (n=259) und Kontrollen (n=393) bestanden. Als post-hoc Tests wurden Fisher's Exact Test und der Wilcoxon-Mann-Whitney Test verwendet. Insgesamt traten bei 39,1% der Pferde in der retrospektiven Studie postoperative Komplikationen auf. Vermehrte Schwellung (25,6%) war die häufigste Komplikation, gefolgt von Exsudation (7,5%), Fieber (2,3%), Dehiszens (1,8%), septischer Arthritis [bei 463 arthroskopierten Patienten (1,5%)] und einem Serom, das bei fünf Patienten auftrat (0,8%). Signifikante Unterschiede in den OP-assoziierten Komplikationen zwischen antibiotisch behandelten Patienten (97/259) und Kontrollen (158/393) wurden nicht nachgewiesen. Die Gabe von perioperativen Antibiotika wurde signifikant vom Chirurgen (p>0,0001), der Art der Operation (p=0,0007) und der Anzahl chirurgischer Eingriffe pro Sitzung beeinflusst (p=0,03). Mit Antibiotika behandelte Pferde erhielten 3-5 Tage lang Antibiotika. Am häufigsten wurden Penicillin-basierte Wirkstoffe eingesetzt. Patienten mit drei oder mehr Eingriffen (n=30) erhielten signifikant häufiger Antibiotika. Perioperative Antibiotika wurden seltener bei Tendovaginoskopie/Bursoskopie, Fasziotomie und Neurektomie (n=98) als bei Kombinationen von Eingriffen, Griffelbeinextraktionen, Desmotomie und Arthroskopie (n=554) gegeben.

Für die prospektive Studie wurden 75 Pferde, die für einen elektiven orthopädischen EIngriff in der Klinik vorgestellt wurden, randomisiert entweder der Antibiotika-Gruppe oder der Kontroll-Gruppe zugeordnet. Antibiotisch behandelte Pferde erhielten einmalig 10 mg/kg Amoxicillin und 6,6 mg/kg Gentamicin vor der Einleitung der Narkose. Diese 75 Pferde wurden über 5 Tage intensiv überwacht. Mittels eines Scores wurden Schwellung, Hauttemperatur, Exsudation und Dehiszens am ersten, dritten und fünften Tag nach dem Eingriff beurteilt. Zusätzlich wurden am Tag vor, direkt nach sowie einen, drei und fünf Tage nach der Operation die Entzündungsparameter SAA, Eisen und Haptoglobin bestimmt. Die beiden Patientengruppen wurden auf Unterschiede in den Scorepunkten, Körpertemperatur, Konzentrationen der Entzündungsparameter und Patientencharakteristika mit dem Freeman-Halton-Test oder ANOVA/Wald-Test geprüft. Als post-hoc-Tests wurden Fisher's Exact Test und der Wilcoxon-Mann-Whitney-Test verwendet. Der Gesamtscore (p=0.002), die Scores für Schwellung (p=0.002) und Hauttemperatur (p=0.007) der antibiotisch behandelten Tiere waren signifikant höher als bei den Kontrollen. In der Post-hoc Analyse war der Gesamtscore bei behandelten Tieren an Tag 1 (p=0.02) und fünf (p=0.01) signifikant höher als bei den Kontrollen, ebenso wie der Score für Schwellung an Tag 1 (p=0.02) und 5 (p=0.002), sowie der Score für Hauttemperatur an Tag 1 (p=0.04) und 3 (p=0.03). Weder in der SAA- (p=0.18), noch in der Eisen- (p=0.76) oder Haptoglobin-Konzentration (p=0.85) gab es signifikante Unterschiede zwischen den Gruppen. Zwei Pferde (1 Kontrolle, 1 Drop-out) entwickelten ein septisches Hufgelenk. Das ausgeschlossene antibiotisch behandelte Pferd entwickelte an Tag 9 nach dem Eingriff ein septisches Hufgelenk trotz einer zusätzlichen Behandlung mit Gentamicin an Tag 1 und 2. Bei dem Kontrollpatienten trat die septische Arthritis an Tag 8 auf. Schwere postoperative Komplikationen bei sauberen orthopädischen Eingriffen beim Pferd sind selten. Die Inzidenzen schwerer Komplikationen bei Patienten mit und ohne perioperative Antibiose unterschieden sich nicht statistisch signifikant. Zusammenfassend lässt sich anhand der vorliegenden Daten ein grundsätzlicher Einsatz von Antibiotika bei unkomplizierten, elektiven orthopädischen Eingriffen beim Pferd nicht rechtfertigen.

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Appendix

Minimum, 1st and 3rd quartile, median, and maximum for score points in treated and control horses

Scores for exudation

	Treated (n=28)			Control (n=31)		
	Day 1	**Day 3**	**Day 5**	**Day 1**	**Day 3**	**Day 5**
Minimum	0	0	0	0	0	0
1st quartile	0	0	0	0	0	0
Median	0	0	0	0	0	0
3rd quartile	0	0	0	0	0	0
Maximum	1	1	3	1	1	1

Scores for swelling

	Treated (n=28)			Control (n=31)		
	Day 1	**Day 3**	**Day 5**	**Day 1**	**Day 3**	**Day 5**
Minimum	0.0	0.0	0.0	0.0	0.0	0.0
1st quartile	1.0	1.0	0.0	0.0	0.0	0.0
Median	1.0	1.0	1.0	1.0	1.0	0.0
3rd quartile	1.5	1.5	1.0	1.0	1.0	1.0
Maximum	2.0	2.5	3.0	2.0	1.0	1.0

Scores for skin temperature

	Treated (n=28)			Control (n=31)		
	Day 1	**Day 3**	**Day 5**	**Day 1**	**Day 3**	**Day 5**
Minimum	0	0	0	0	0	0
1st quartile	0	0	0	0	0	0
Median	1	1	0	0	0	0
3rd quartile	1	1	1	1	1	0
Maximum	2	2	2	1	1	1

Scores for dehiscence

	Treated			Control		
	Day 1	**Day 3**	**Day 5**	**Day 1**	**Day 3**	**Day 5**
Minimum	0	0	0	0	0	0
1st quartile	0	0	0	0	0	0
Median	0	0	0	0	0	0
3rd quartile	0	0	0	0	0	0
Maximum	3	3	3	0	0	1

P-values for the wound scores on day 1, 3, and 5

Parameter	Day	P
Exudation	1	0.9
	3	0.8
	5	0.8
Swelling	1	0.02
	3	0.1
	5	0.002
Skin temperature	1	0.04
	3	0.03
	5	0.2
Dehiscence	1	0.2
	3	0.2
	5	0.5
Total Score	1	0.02
	2	0.02
	5	0.01

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