http://dx.doi.org/10.1590/1678-4162-11212

Arq. Bras. Med. Vet. Zootec., v.72, n.1, p.49-55, 2020

P.A. Canola
https://orcid.org/0000-0003-4354-6487
A.P.P.A. Faria
https://orcid.org/0000-0003-4213-0754
V.B. Paula
https://orcid.org/0000-0001-7489-5028
F.A. Ávila
https://orcid.org/0000-0002-9779-2213
M.V. Cardozo
https://orcid.org/0000-0003-3972-0198
G.G. Rivera
https://orcid.org/0000-0003-1416-8720

Antisepsys and profilatic antimicrobial therapy in prevention of surgical site infection of horses

[*Antissepsia e terapia antimicrobiana profilática no controle infeccioso do foco cirúrgico em equinos*]

P.A. *Canola*[1], A.P.P.A. *Faria*[2], V.B. *Paula*[3], F.A. *Ávila*[1], M.V. *Cardozo*[4], G.G. *Rivera*[3]

[1]Universidade Estadual Paulista - Jaboticabal, SP
[2]Aluno de pós-graduação – Universidade Estadual Paulista – Araçatuba, SP
[3]Aluno de pós-graduação – Universidade Estadual Paulista – Jaboticabal, SP
[4]Aluno de pós-doutorado – Universidade Estadual Paulista – Jaboticabal, SP

ABSTRACT

The efficacy of an antisepsis protocol comprising chlorhexidine gluconate and ethyl alcohol in combination with prophylactic antimicrobial therapy in controlling surgical site infection in horses was studied. To that purpose, seven mixed breed horses received potassium penicillin and gentamicin at least 30 minutes prior to surgery. The surgical site was scrubbed with chlorhexidine gluconate and rinsed with ethyl alcohol. Samples were collected at four time points: (A) - before and (B) - immediately following shaving of the hair coat, (C) – at the end of antisepsis procedures, and (D) – at the end of the surgical procedure. Duration of surgery was recorded. Samples were cultured in three different culture mediums: Mitis Salivarus (*Streptococcus sp*.), *Staphylococcus* 110 (*Staphylococcus sp*.), and Mac Conkey (Enterobacteria). A high level of bacterial growth was observed in all culture mediums at (A) and (B), with no bacterial growth in (C). *Staphylococcus sp*. growth was observed in (D) in a single patient whose surgical procedure lasted for 120 minutes. Shaving of the hair coat reduced microbial flora on the surface of the skin. Antisepsis in combination with prophylactic antimicrobial therapy was effective in controlling surgical site infection in elective procedures with an average duration of 90 minutes.

Keywords: chlorhexidine gluconate, culture media, potassium penicillin, *Staphylococcus sp*, SSIs

RESUMO

Objetivou-se averiguar a eficácia do protocolo de antissepsia com clorexidina degermante e álcool etílico hidratado 70%, em associação com terapia antimicrobiana profilática, no controle microbiano do foco cirúrgico de equinos submetidos a procedimentos cirúrgicos. Foram utilizados 07 cavalos adultos de raças variadas, onde ambos receberam o mesmo tratamento (terapia antimicrobiana profilática e antissepsia com clorexidina degermante 2% e álcool etílico hidratado 70%), coletando-se amostras em quatro tempos distintos [(A – antes da tricotomia), (B – imediatamente após tricotomia), (C – ao término da antissepsia), (D – ao término do procedimento cirúrgico)]. O tempo de cada procedimento cirúrgico foi contabilizado. Foram utilizados três meios de cultura diferentes, cada um com especificidade para um tipo de crescimento bacteriano. Constatou-se alta incidência de crescimento bacteriano nos três meios utilizados nos tempos de coleta A e B. Para o tempo C, não foi observado crescimento bacteriano. No tempo D averiguou-se crescimento bacteriano do tipo Staphylococcus *sp. em um único paciente, cujo tempo cirúrgico foi de 120 minutos de duração. Desta forma, a tricotomia reduziu a carga microbiana na superfície da pele. A antissepsia associada à terapia antimicrobiana profilática mostrou-se eficaz no controle microbiano do foco cirúrgico em procedimentos eletivos, com duração média de 90 minutos.*

Palavras-chave: infecção do foco cirúrgico, gluconato de clorexidina, meio de cultura, penicilina potássica, Staphylococcus sp

Recebido em 12 de dezembro de 2018
Aceito em 22 de março de 2019
E-mail: paulo.canola@unesp.br

INTRODUCTION

Preparation of the surgical site is an important part of the principles of surgical asepsis proposed by Koch and Halsted (Verwilghen, 2015). A rigorous antisepsis is essential in controlling wound infection. In human patients, infectious foci originating from the skin or mucosal surface (endogenous) are often identified in the subcutaneous tissue and bone structures at the surgical site (Stubbs *et al*., 1996; Anderson *et al*., 2013). However, antisepsis alone cannot completely prevent bacterial growth at the surgical site since 20% of the bacterial population is located in deeper cutaneous structures and is thus inaccessible to any type of antisepsis (Bernis Filho *et al*., 1998; Shmon, 2007). The primary defense mechanism against bacterial contamination is the physical skin barrier (epithelium and endothelium) but this is usually breached during surgery. Several risk factors for surgical site infection have been identified (Dunn, 1996), including virulence of the initial inoculum, the effectiveness of the body's defense mechanism, and the nature of the surgical procedure (Schirmer, 2007).

The age of the patient, duration of surgery and the decision on whether to clip or shave the hair coat preceding surgery have been proposed as predisposing factors for surgical site infection in horses. It has been shown in both human and equine patients that routine clipping of the hair coat minimizes the occurrence of surgical site infection (Fuller, 2000; Pitrez and Pioner, 2003). Roush (1999); Shmon (2007) stated that the longer the interval between hair removal and surgical procedure, the greater the incidence of post-surgical site infection. Thus, the recommendation is that hair removal should not be performed more than two hours before surgery (Fuller, 2000; Pitrez and Pioner, 2003).

Poor hand antisepsis and failure of aseptic preparation of the surgical site are still regularly implicated in surgical site infections (Ahern and Richardson, 2012; Anderson *et al*., 2013). Thus, for surgical interventions in which there is a high probability of infection, rigorous antisepsis of the hands and thorough preparation of the surgical site is recommended. Ideally the surgical site should be scrubbed for at least two minutes with chlorhexidine gluconate soap sponges. Prophylactic antimicrobial therapy has also been advised (Roush, 1999; Verwilghen and Singh, 2015), especially during surgery were the gastrointestinal, genitourinary or respiratory tract are incised, where there are contaminated wounds or during procedures that might last for more than ninety minutes (Silva, 2000). Its purpose is to reduce the concentration of inoculum below a level at which tissue infection occurs (Verwilghen and Singh, 2015).

Prophylactic antimicrobial therapy should always be given before surgery to allow the antibiotics to reach therapeutic levels within the tissues during surgery (Marques, 2005a, 2005b). Antibiotics should be administered at least 30 minutes before the initial incision (Canabrava and Rezende, 2000).

In addition, reduced duration of surgery and smaller incisions have been proposed to reduce the risk of surgical site infection (Tanner *et al*., 2006, 2007; Ahern and Richardson, 2012). Age, although associated with compromise of the immune system in older animals and failure of passive immunity transfer in newborns, has not been directly correlated with a higher prevalence of surgical infection in horses (Waguespack *et al*., 2006; Ahern and Richardson, 2012). Ahern, Richardson (2012) suggested that complication rates related to surgical site infection in young horses (less than 1 year of age and greater than 1 month of age - 15%) are lower than those in adults (more than 1 year of age - 43%).

Therefore, the purpose of this study was to investigate the efficacy of an antisepsis protocol using 2% chlorhexidine gluconate soap and 70% ethyl alcohol in combination with prophylactic antimicrobial therapy in controlling growth of microorganisms at the surgical site in horses undergoing a variety of surgical procedures.

MATERIALS AND METHODS

The study was approved and supervised by the institution's animal use and care committee (021611/14). Seven animals (01-07) admitted for surgical treatment for a variety of conditions were included in the study. Patients received prophylactic antimicrobial therapy comprised of potassium penicillin (β-lactam) and gentamicin (aminoglycoside) at least 30 minutes prior to surgery.

Microbiological material was sampled over the incision site. This first sampling (A) was performed over the animal's hair coat, following anesthetic induction and positioning of the patient on the operating table. The second sample (B) was collected immediately following shaving of the hair coat at the surgical site. Before shaving, the hair coat was wetted with tap water. Then the surgical site was antiseptically prepared as follows: scrubbed twice with a surgical sponge containing 2% chlorhexidine gluconate soap: initially for five minutes followed by rinsing with copious quantities of 70% hydrous ethyl alcohol and then the procedure was repeated for seven minutes. At the end of antisepsis and preceding the incision the third sample (C) was collected. The last sample (D) was obtained at the end of the surgical procedure immediately after skin closure. The duration of the surgical procedure was also recorded.

The study was conducted in an experimental design with one treatment (combination of prophylactic antimicrobial therapy and antisepsis with 2% chlorhexidine gluconate soap and 70% ethyl alcohol), four sampling of microbiological material ([A], [B], [C], [D]) and seven repetitions (equine patients).

All samples were collected with a sterile swab and stored in sterile test tubes containing pre-prepared peptone media (10ml, 10^{-1}). The collected material was sent to the institution's microbiology laboratory for further processing and analysis. Prior to use, peptone media was pre-diluted in distilled water and autoclaved (1.0 atm, 120 °C, 15 minutes). Based on previous test results, sample (A) was preserved in peptone at a concentration of 10^{-2} to facilitate counting of bacteria colony forming units (CFUs) following the incubation period. Samples (B), (C) and (D) were maintained at a concentration of 10^{-1}.

Subsequently, samples were seeded into petri dishes containing specific media for each bacterial species (Enterobacteria, *Staphylococcus sp*., *Streptococcus sp*.). The following culture media were used: Mac Conkey (MC), specific for Enterobacteria, such as *E. coli*; Mitis Salivarus (MS), specific for *Streptococcus sp*.; *Staphylococcus* 110 (S.110) specific for *Staphylococcus sp*. These specific media were also chosen based on previous test results. Culture media were prepared by dilution in distilled water and autoclaved at 1.0 atm, 120°C, for 15 minutes prior to use. Each petri dish contained approximately 20ml of each medium.

For the seeding, 0.1ml of peptone from each sample was spread over the culture media using a Drigalski's loop. Plates containing Mitis Salivarus media were completely sealed to provide anaerobiosis. All plates were incubated at 37°C for 24h. After this period, the number of CFUs were counted and the results were expressed in CFU / ml.

RESULTS

A high incidence of bacterial growth was observed in the three media used (McConkey, *Staphylococcus* S.110 and Mitis Salivarus) in samples (A) and (B). Numbers of *Staphylococcus sp*. colonies were greatest in (A), followed by *Streptococcus sp*. (Table. 1), and in (B) enterobacteria growth exceeded that of all other microorganisms.

No bacterial growth was observed in any culture media in (C) (Figure1). In (D), bacterial growth was only seen in the S.110 media (*Staphylococcus sp*. population), in a single patient with the second longest surgical duration (120 minutes; group mean of 90.7±28.3 minutes). This patient underwent cryptorchidectomy by exploratory parainguinal celiotomy. The surgical procedures performed in each patient and duration of the surgical procedures are shown in Table. 2.

Table 1. Surgical procedure, patient's age (years) and colony forming units (CFU / ml) of Enterobacteria, *Staphylococcus* and *Streptococcus* at the surgical site of seven horses undergoing different surgical procedures before shaving of the hair coat and antisepsis (A)

Patient	Surgical Procedure	Age (years)	Total Enterobacteria	Total *Staphylococcus*	Total *Streptococcus*
1	Umbilical Herniorraphy	0.3	4×10^3	3.0×10^4	2.9×10^4
2	Cryptorchidectomy	6	1.5×10^4	2.2×10^4	3.0×10^4
3	Umbilical Herniorraphy	2	3.8×10^4	1.1×10^5	2.6×10^3
4	Exploratory Celiotomy	0.6	2.3×10^4	2.6×10^4	1.0×10^3
5	Umbilical Herniorraphy	1	2.9×10^4	1.6×10^4	1.0×10^3
6	Tibial osteosynthesis	5	1.8×10^3	4.2×10^4	9.6×10^3
7	Cryptorchidectomy	10	1.0×10^3	1.7×10^4	2.0×10^4
$\bar{X}\pm$ DP		3.55±3.6	$1.6 \times 10^{4\pm}1.46 \times 10^4$	$3.76 \times 10^4 \pm 3.31 \times 10^4$	$1.33 \times 10^4 \pm 1.29 \times 10^4$

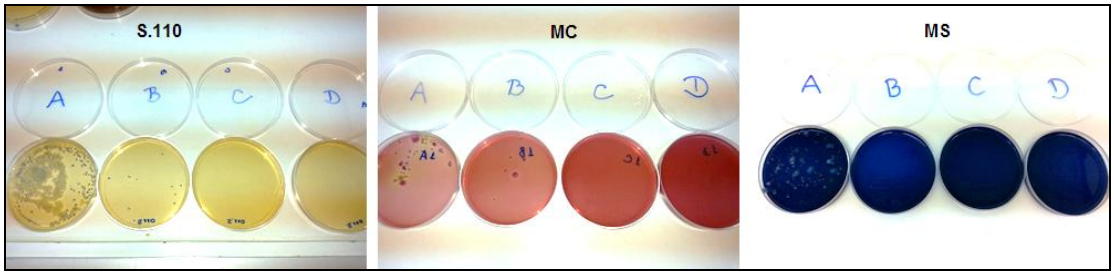

Figure 1. Microorganism growth from patient's skin with surgical procedure lasting for 1h20min. (S.110) *Staphylococcus* 110; (MC) Mac Conkey; (MS) Mitis Salivarus. (A) before and (B) following clipping of the hair coat; (C) at the end of antisepsis; (D) at the end of the surgical procedure.

Table 2. Surgical procedure and duration of surgery for the seven equine patients included in the study

Patient	Surgical Procedure	Duration of Surgery (minutes)
1	Umbilical Herniorraphy	80
2	Cryptorchidectomy	120
3	Umbilical Herniorraphy / Orchiectomy	90
4	Exploratory Celiotomy	135
5	Umbilical Herniorraphy	60
6	Tibial osteosynthesis	60
7	Cryptorchidectomy	90
$\bar{X}$±DP		90.7±28.3

DISCUSSION

A high level of bacterial growth was observed in all three media used for samples (A) and (B). This shows that prior to antisepsis the predominant microbial population on the patients' dermis consisted of enterobacteria, such as *E. coli*, and *Staphylococcus sp*. and *Streptococcus sp*., corroborating findings of previous reports *(*Stubbs *et al*., 1996; Anderson *et al*., 2013). These microorganisms are present on the surface of the equine dermis in high numbers and their concentration may vary according to the sampling location and age of the animal (Bernis Filho *et al*., 1998; Shmon, 2007). The higher prevalence of *Staphylococcus sp*. and *Streptococcus sp*. in (A) and the higher growth of enterobacteriaceae in (B) imply that *Staphylococcus sp*. and *Streptococcus sp*. microorganisms are located more superficially in

the hair coat while enterobacteriaceae are generally found on the epidermis itself.

Compared to adult horses, foals spend considerably longer time lying down. The constant contact of their skin and epidermal attachments with the ground surface predisposes to a greater skin contamination. Thus, age has been inversely related to the number of microorganisms present on the skin (Tanner *et al*., 2006, 2007). This finding was seen in the younger animals (less than 12 months) included in this study which had the highest CFU counts in (A). It has been suggested that the nearer the distal limbs are to the soil the greater the tissue microbial concentration. Patient (6) in our study had the highest concentrations of microorganisms in (A) (Table. 2), despite its age (five years) and, in this patient, the surgical procedure was performed in the pelvic limb at the level of the proximal tibia.

Bacterial growth was practically absent in samples (C) and (D). This confirmed the efficacy of antisepsis and antimicrobial therapy in controlling bacterial growth, and thus surgical site infection, in our patients. This information reinforces the principle that microbial contamination of the surgery site in elective procedures with an average duration of approximately 90 minutes, are reduced by strict antisepsis and effective prophylactic antimicrobial therapy (Verwilghen and Singh, 2015).

In a single patient (6), bacterial growth was observed in S.110 media (population of *Staphylococcus sp*.) in sample (D). This was one of the longest procedures being 120 minutes in duration (mean group 90.7±28.3 minutes), microbial proliferation could have been the result of decreased plasma concentration of the antimicrobials (Verwilghen and Singh, 2015), and/or in antisepsis efficacy, as a result of the duration of the surgery. It is recommended that in surgical procedures that last more than one or two half-lives (T ½) of the antimicrobial the drug should be administered again during surgery to maintain plasma concentrations. The half-life of potassium penicillin used in the study is approximately 40 minutes. Therefore, administration of a second dose is indicated in procedures lasting more than 80 minutes (Tanner *et al*., 2007; Ahern and Richardson, 2012).

According to previous studies, prophylactic antimicrobial therapy is most effective in surgical procedures up to 90 minutes in duration. However, elective surgical procedures with surgical times up to 90 minutes are considered clean, thus do not necessarily require antimicrobial prophylaxis. In these, antisepsis alone should be sufficient to inhibit local contamination (Tanner *et al*., 2006, 2007; Ahern and Richardson, 2012), since chlorhexidine gluconate has been shown to be minimally inhibited by organic material, with a residual persistence of approximately six hours (Rippingale and Fisk, 2013; Southwood, 2015; Lane, 2016).

The removal of the hair coat rapidly reduced the number of *Staphylococcus sp*. and *Streptococcus sp*. CFUs on the surface of patients' skin at the surgical site before antisepsis. In patients (2) and (3), *Streptococcus sp*. growth was not observed following the shaving of the hair coat (sample B). Possibly, the mechanical action of hair scraping was enough to remove the microorganism population located more superficially at the level of the hair coat. Therefore, clipping or shaving of the hair coat during surgical preparation is of fundamental importance in the control of surgical site infection in horses.

The incidence of postoperative infection of surgical wounds has been directly related to the interval between the shaving of the hair coat and the surgery itself (Roush, 1999; Shmon, 2007). To minimize the risks of infection, it has been recommended that the hair coat should be shaved no more than two hours prior to the surgical procedure (Fuller, 2000; Pitrez and Pioner, 2003). Bowers (2012); Lane (2016); Tartari *et al.* (2017) also advise against shaving with razor blades because they are likely to predispose to microabrasions at the dermal surface. Moreover, according to the authors, the traction on the hair follicles caused by the mechanical act of the scraping by the razor blade might cause protrusion of the follicular contents onto the dermal surface, resulting in further contamination. According to the authors, scraping of the hair coat results in a tenfold increase in the risk of surgical site infection.

Thus, the use of clippers could minimize the risks arising from the use of razor blades. Hague

et al. (1997) stated that in distal regions of equine limbs the use of clippers with a 40-blade did not predispose to skin contamination. Likewise, the presence of the remaining hair coat did not inhibit the action of antiseptic. Although the presence of hair coat is not directly associated with increased risk of surgical site infection, it could hinder the aseptic preparation of the surgical field (Niël-Weise *et al*., 2005; Tanner *et al*., 2007; Ahern and Richardson, 2012), predisposing to infection. In that sense, clipping of the hair coat before arthrocentesis did predispose to articular infection in horses (Gillespie *et al*., 2016).

Antisepsis in combination with prophylactic antimicrobial therapy was effective in reducing the number of CFUs in this study. Furthermore, elective procedures with an average duration of 90 minutes were less predisposed to microorganism proliferation. Shaving of the hair coat decreased the number of CFUs of *Staphylococcus sp*. and *Streptococcus sp*. obtained from the skin surface at the surgical site in horses.

ACKNOWLEDGMENT

The authors would like to thank the institution's pro-rectory of research (PROPe) for support – process 32596.

REFERENCES

AHERN, B.J.; RICHARDSON, D.W. Surgical site infection and the use of antimicrobials. In: AUER, J.A.; STICK, J.A. *Equine surgery*, Philadelphia: WB Saunders, 2012. p.68-84.

ANDERSON, M.E.C.; FOSTER, B.A.; WEESE, J. Observational study of patient and surgeon preoperative preparation in ten companion animal clinics in Ontario, Canada. *BMC Vet. Res.*, v.9, p.194, 2013.

BERNIS FILHO, W.O.; REZENDE, C.M.F.; ABREU, V.L.V.; BERNIS, V.M.O. Infecções hospitalares em feridas cirúrgicas de pequenos animais. *Arq. Bras. Med. Vet. Zootec.*, v.2, p.127-132, 1998.

BOWERS, L. Aseptic skin preparation: reducing the risk of surgical site infection. *Vet. Nurse, v.3, p.544-551, 2012.*

*CANA*BRAVA, H.A.N.; REZENDE, C.M.F. Profilaxia antimicrobiana em cirurgia. *Vet. Notícias*, v.6, p.109-118, 2000.

DUNN, D.L. Princípios Infecções cirúrgicas e antibióticos. In: SABISTON Jr., D.C.; LYERLY, H.K. *Fundamentos de cirurgia*. 2.ed. Rio de Janeiro: Guanabara Koogan, 1996. p.91-104.

FULLER, J.R. Preparo do local da cirurgia. In: _____. *Tecnologia cirúrgica:* princípios e prática. 3.ed. Rio de Janeiro: Guanabara Koogan, 2000. p.90-100.

GILLESPIE, C.C.; ADAMS, S.B.; MOORE, G.E. Methods and variables associated with the risk of septic arthritis following intra-articular injections in horses: a survey of veterinarians. *Vet. Surg.*, v.45, p.1071-1076, 2016.

HAGUE, B.A.; HONNAS, C.M.; SIMPSON, R.B. *et al*. Evaluation of skin bacterial flora before and after aseptic preparation of clipped and nonclipped arthrocentesis sites in horses. *Vet. Surg.*, v.26, p.121-125, 1997.

LANE, C. Preventing surgical site infections: equine surgical site preparation. *Vet. Nurse*, v.7, p.151-155, 2016.

MARQUES, R.G. *Infecção em cirurgia*. Rio de Janeiro: Guanabara Koogan, 2005a. p.41-53.

MARQUES, R.G. *Preparação para o ato operatório III* – equipe cirúrgica. Rio de Janeiro: Guanabara Koogan, 2005b. p.269-283.

NIËL-WEISE, B.S.; WILLE, J.C.; VAN DEN BROEK, P.J. Hair removal policies in clean surgery: systematic review of randomized, controlled trials. *Infec. Control Hosp. Epidemiol.*, v.26, p.923-928, 2005.

PITREZ, F.A.B.; PIONER, S.R. Avaliação e preparo pré-operatórios. In: _____. *Pré e pós-operatório em cirurgia geral e especializada*. 2.ed. Porto Alegre: Artmed, 2003. p.31-41.

RIPPINGALE, M.; FISK, N. Factors causing thrombophlebitis in horses: methods of prevention 1. *Vet. Nurse*, v.4, p.202-207, 2013.

ROUSH, J.K. Controle de infecção. In: HARARI, J. *Cirurgias de pequenos animais.* Porto Alegre: Artmed, 1999. p.43-53.

SCHIRMER, B.D. Princípios do preparo pré-operatório do paciente cirúrgico. In: SLATTER, D. *Manual de cirurgia de pequenos animais*. 3.ed. São Paulo: Manole, 2007. v.1, cap.12, p.43.

SHMON, C. Avaliação e preparação do paciente e da equipe cirúrgica. In: SLATTER, D. *Manual de cirurgia de pequenos animais.* 3.ed. São Paulo: Manole, 2007. v.1, cap.12, p.162-170.

SILVA, A.R.C. Pontos (e cuidados) a ponderar. *Cães Gatos*, v.14, p.31-34, 2000.

SOUTHWOOD, L.L. Equine abdominal surgery-nursing theatre essentials. *Vet. Times*, v.36, p.1-7, 2015.

STUBBS, W.P. JAMIER,R. BELLAH, D.V.M. *et al*. Chlorhexidine gluconate versus chloroxylenol for preoperative skin preparation in dogs. *Vet. Surg.*, v.25, p.487-494, 1996.

TANNER, J.; MONCASTER, K.; WOODINGS, D. Preoperative hair removal: a systematic review. *J. Perioperative Pract.*, v.17, p.118-132, 2007.

TANNER, J.; WOODINGS, D.; MONCASTER, K. Preoperative hair removal to reduce surgical site infection (review). *Cochrane Database Systematic Rev.*, n.3, p.25, 2006.

TARTARI, E.; WETERINGS, V.; GASTMEIER, P.; BAÑO, J.R.; WIDMER, A.; KLUYTMANS, J.; VOSS, A. Patient engagement with surgical site infection prevention: an expert panel perspective. *Antimicrobial Resistance and Infection Control*, v.6, p.1-9, 2017.

VERWILGHEN, D. Surgical site infections: what do we know? *Equine Vet. J.*, v.47, p.753-755, 2015.

VERWILGHEN, D.; SINGH, A. Fighting surgical site infections in small animals. Are we getting anywhere? *Vet. Clin. N. Am. Small Anim. Pract.*, v.45, p.243-276, 2015.

WAGUESPACK, R.W.; BURBA, D.J.; MOORE, R.M. Surgical site infection and the use of antimicrobials. In: AUER, J.A., STICK, J.A. *Equine surgery*, Philadelphia: WB Saunders,2006. v.3, p.70-87.