Responsible antimicrobial use in critically ill adult horses

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Summary

Due to increasing microbial resistance, pressure on veterinarians is mounting to adhere to responsible use of antimicrobial drugs. Antimicrobials are frequently included in the treatment of systemically ill horses due to the strong likelihood of an infection and the innate difficulties in differentiating systemic inflammation secondary to non-infectious from infectious causes. In light of increasing antimicrobial drug resistance and the potential negative impact of antimicrobials on equine patients, every attempt should be made to identify non-infectious disease, choose first line antimicrobials and discontinue treatment as soon as possible. In most cases, a short duration of antimicrobial therapy ranging from a single dose (for example pre-operatively) to 24-72h might be sufficient with long-term treatment being rarely required. The article aims to provide practical guidelines for antimicrobial drug usage in critically ill adult horses by describing ancillary diagnostic aids that can help establishing whether or not an infection is present, discussing commonly encountered pathogens and their typical antimicrobial drug sensitivity patterns and providing some guidance how to safely shorten the duration of antimicrobial therapy.

Introduction

Critical or intensive care in people extends beyond internal medicine dealing with a subset of patients with immediate life-threatening conditions (Marshall, Bosco et al. 2017). In equine medicine, the term is used more loosely, often referring to animals that require more intense support than the average equine patient or simply animals with systemic and potentially life-threatening disease (Lascola, Vander Werf et al. 2017). Many critical illnesses in horses are associated with infection or with significant absorption of bacterial products and toxins causing activation of the inflammatory and coagulation system. Clinically, both scenarios are often indistinguishable. Although not effective against purely inflammatory conditions, antimicrobials are frequently included in the treatment of these animals due to the strong likelihood of an infection and the innate difficulties in differentiating systemic inflammation secondary to non-infectious causes from infections. However, due to increasing microbial resistance, pressure on veterinarians is mounting to adhere to responsible use of antimicrobial drugs. A recent study in a UK referral hospital over a ten-year period demonstrated an increase in prevalence of extended spectrum beta lactamase (ESBL)-producing *E. coli* as well as increased antimicrobial resistance to frequently used antimicrobials including doxycycline, gentamicin and 3rd generation cephalosporins (Isgren, Edwards et al. 2019); other studies have confirmed increasing resistance in bacteria isolated from horses, particularly after antimicrobial therapy (Maddox, Williams et al. 2011, Theelen, Wilson et al. 2020). Unnecessary or unnecessarily long use of antimicrobial drugs enhances development of resistance in pathogens, increases the cost of treatment and exposes the patient to possible side effects, most noticeably disruption of the intestinal microbiome and antimicrobial-induced diarrhoea (Gronvold, L'Abee-Lund et al. 2010, Johns, Verheyen et al. 2012, Barr, Waldridge et al. 2013, Costa, Stampfli et al. 2015). Every attempt should therefore be made to limit their use. This can be achieved by making every effort to identify non-infectious

disease, choosing first line antimicrobials in the first instance and discontinuing treatment as quickly as possible. The use of drugs of veterinary and human medical importance including 3rd-, 4th- and 5th-generation cephalosporins, glycopeptides (vancomycin), quinolones (enrofloxacin, marbofloxacin), macrolides (erythromycin, azithromycin, clarithromycin), newer, extended spectrum penicillins and carbapenems, should be avoided unless there is confirmed infection with a susceptible organism or for the treatment of life-threatening conditions unlikely to respond to first line choices (Raidal 2019). Many clinicians feel that some antimicrobials such as vancomycin and carbapenems should not be used in animals under any circumstances. Even drugs commonly used in horses and other veterinary species such as gentamicin, rifampicin, ampicillin and amoxicillin-clavulanic acid are now on the World Health Organisation list of critically important antimicrobials which could lead to restrictions of their use in veterinary medicine in the future (https://www.who.int/foodsafety/publications/antimicrobials-sixth/en/). In most cases, a short duration of antimicrobial therapy ranging from a single dose (for example pre-operatively) to 24-72h might be sufficient with long-term treatment being rarely required. Recent studies in human medicine have shown impressive reductions in antimicrobial resistance with a reduction of not only overall use but also with a decrease in days of antimicrobial therapy highlighting the importance of shortening treatment (Dona, Barbieri et al. 2020).

Unfortunately, in everyday life clinicians' efforts are often impeded by the inability to differentiate infectious from non-infectious conditions due to non-specific clinical signs, difficulties of culturing relevant pathogens, financial limitations impeding repeated testing and pressure from the owners, yard managers or trainers to use antimicrobial drugs. To further compound the issue, prospective studies investigating responsible antimicrobial drug use in horses are lacking and most guidelines are based either on information gained from

human research or, more commonly, clinical impressions, which are subjective and unreliable at best.

Identifying the need for antimicrobial treatment

Antimicrobial drugs are unlikely to have a significant beneficial effect against anything but infectious diseases, usually bacterial or rarely fungal or protozoal in origin. Although some antimicrobials such as tetracyclines also have anti-inflammatory effects (Pradhan, Madke et al. 2016), their use for purely inflammatory conditions is controversial and use of classic anti-inflammatories such as non-steroidal anti-inflammatory drugs is far more appropriate. Unfortunately, it is extremely difficult to reliably rule out an infectious process. Clinical examination findings are non-specific and rarely help with the differentiation. Heart and respiratory rates are greatly influenced by pain, cardiovascular compromise and systemic inflammation making them much more useful in judging disease severity rather than an infectious or non-infectious nature. While the presence of a fever increases the clinical suspicion of an infection, it is by no means conclusive as inflammation, hyperthermia, neoplasia or significant tissue trauma can also lead to an increase in rectal temperature. Equally, the absence of a fever does not rule out even severe infection. Haematology and acute phase protein concentrations such as fibrinogen or serum amyloid A (SAA) can be difficult to interpret as increases in concentrations can be triggered by infectious and non-infectious inflammatory conditions alike (Westerman, Tornquist et al. 2015, Long and Nolen-Walston 2020). Although a statistical difference in SAA concentrations between infectious and non-infectious airway disease has been reported, there was significant overlap between groups and in some horses with infection, SAA concentrations remained low or at 0mg/L (Viner, Mazan et al. 2017). In adult horses, leucopaenia is commonly observed with significant inflammation, often originating from the gastrointestinal system, and caused by

margination and extravasation of leucocytes at the site of inflammation. It does not necessarily indicate the presence of infection or the need for antimicrobial treatment. Leucocytosis in mature horses can be observed with inflammatory, infectious and neoplastic conditions or following administration of corticosteroids and is therefore also of limited use when trying to differentiate infectious from non-infectious conditions (Targowski 1975, White, Affolter et al. 2009, Meichner, Kraszeski et al. 2017). As immediate treatment is usually required, the clinician needs to make an educated guess whether or not infection is likely and antimicrobial drugs are needed. Many equine viral and some bacterial diseases can be diagnosed by polymerase chain reaction (PCR) with results often being available the following day. Cytological samples, submitted in addition to samples for culture and sensitivity, can be of great value when trying to rule out an infectious aetiology as results are much quicker available compared to culture and are not compounded by difficult culturing processes. In cases where clinicians have already initiated antimicrobial treatment but PCR or cytological results do not support an infection or identify a viral cause for the disease, antimicrobial treatment should be discontinued immediately. The old concept that “a course of antibiotics needs to be finished to avoid development of resistance” is incorrect and obsolete. The perception that stopping antibiotic treatment early encourages antibiotic resistance is not supported by evidence, while increasing length of antimicrobial use undoubtedly increases the risk of resistance development (Llewelyn, Fitzpatrick et al. 2017). In contrast, reducing the length of treatment has a significant effect on decreasing previously existing resistance (Dona, Barbieri et al. 2020). Submitting samples for culture and sensitivity remains essential as results will provide guidance for further antimicrobial choices if the initial treatment fails to resolve the infection. Over time, results also provide invaluable insight into regional pathogens and their antimicrobial sensitivity patterns (Johns 2017, Raidal 2019). Reports on commonly identified bacteria, the organ system they were cultured

from and their sensitivity patterns can often be obtained from regional laboratories providing insight into local resistance patterns.

Limiting the duration of antimicrobial treatment in proven infections can be challenging. Fear of negating a treatment success often leads clinicians to prolong use of antimicrobial drugs in clinically apparently recovered patients. Unfortunately, studies to identify the minimum effective treatment duration have rarely been performed, even in people, leave alone in horses. Current guidelines are therefore often purely based on absence of data for efficacy of shorter courses rather than the explicit need for long therapies (Llewelyn, Fitzpatrick et al. 2017). In the past, many clinicians have used return to normal haematologic parameters or normal concentrations of acute phase proteins, mainly fibrinogen, as a marker to safely discontinue antimicrobial treatment. Plasma fibrinogen has a relatively long half-life of 4.1-5.2 days in horses and awaiting normal concentrations likely results in over-treatment (Coyne, Hornof et al. 1985). Return to normothermia, improved appetite and return of normal demeanour might be better indicators that further treatment is not necessary. It is also common practice to initially treat systemically ill horses with injectable antimicrobial drugs for 48-72h, often in a hospital setting, followed by continued oral treatment at home. In most cases, this continuation of antimicrobial treatment is not necessary. An alternative approach is stopping antimicrobial treatment after 48-72h and monitoring the patient for another 24h whilst still in the hospital (or close monitoring by the owner at home). The additional cost for hospitalisation are at least partially offset by saving cost for drugs. Should signs of infection re-occur, such as recurrence of a fever, a decrease in appetite or change in demeanour, treatment can easily be re-initiated. If not, the patient can be discharged off all medications.

Responsible use of antimicrobials in gastrointestinal diseases

Horses with a primary complaint of colic rarely require antimicrobial therapy. The examination is focused on establishing the nature of the problem and deciding whether medical or surgical options should be pursued. Horses with fever and vague colic signs with or without diarrhoea often suffer from intestinal inflammation such as enteritis and colitis or from peritonitis. There is no evidence that antimicrobial therapy is beneficial in cases of intestinal inflammation. Some clinicians even feel that their use is contraindicated, considering the negative impact of antimicrobials on the microbiota, adding further insult to an already disturbed microbial environment (Harlow, Lawrence et al. 2013, Shaw and Stampfli 2018). Exceptions include colitis caused by *Neorickettsia risticii* (Potomac horse fever) or rare cases of *Lawsonia intracellularis* in adult horses (Page, Slovis et al. 2014) where treatment with oxytetracycline is indicated. Antimicrobial treatment has also been considered for clostridia-associated diarrhoea. An association between metronidazole treatment and survival was identified in horses diagnosed with clostridial diarrhoea but metronidazole had no effect on survival of horses with non-clostridia associated diarrhoea (Weese, Toxopeus et al. 2006). However, administration of metronidazole has also been linked with identification of metronidazole-resistant *Clostridium difficile* strains. These strains are suspected to be more virulent and carried an increased risk of mortality compared to horses infected with metronidazole-susceptible strains (Magdesian, Dujowich et al. 2006, Schoster and Staempfli 2016). Considering the controversial evidence and pressure of building resistance refraining from use of metronidazole in horses with diarrhoea might be preferable. Bacteraemia has been reported in adult horses with colitis and is sometimes considered as a reason for use of antimicrobials in these patients. However, prior treatment or treatment during hospitalization with antimicrobial drugs did not protect horses from development of bacteraemia arguing against their use (Johns, Tennent-Brown et al. 2008).

Peritonitis, a less common but potentially life life-threatening disease, is also often associated with fever and vague intestinal signs. Peritoneal fluid analysis can quickly rule peritonitis in or out and is a procedure that can be easily performed in the field. The gross appearance of the sample can be misleading and an accurate cell count and protein concentration should always be obtained to avoid misinterpretation. An increased cell count (>20-50x10^9/L) and protein concentration (>25g/L) in a horse with compatible clinical signs is sufficient to make a diagnosis of peritonitis, usually bacterial in origin. However, intestinal necrosis and infarction, for example secondary to migrating *Strongylus vulgaris* larvae can also result in peritonitis (Pihl, Nielsen et al. 2018). Best treatment options and prognosis for peritonitis depend highly on the underlying cause and presenting signs. Horses with *Actinobacillus equuli*-associated or so called idiopathic peritonitis (defined as peritonitis without identifiable cause such as trauma, abdominal surgery, intestinal necrosis, infarction or rupture or neoplasia) with little or no systemic compromise have a much better prognosis and might respond to monotherapy with first line antimicrobials (Henderson, Mair et al. 2008, Odelros, Kendall et al. 2019). In contrast, surgical exploration should be strongly considered in any horse with a history of previous trauma or abdominal surgery, a palpable or ultrasonographically visible mass or suspicion of foreign body ingestion. In a recent study of horses with wire ingestion all survivors underwent exploratory laparotomy highlighting the fact early surgical intervention can be life-saving (Marley, Soffler et al. 2018). Surgical exploration might also be indicated in horses non-responsive to medical treatment within 48h-72h to exclude the presence of significant intestinal compromise or other primary disease process (Pihl, Nielsen et al. 2018). The benefits of surgery include identification and possible correction of an underlying cause and recognition of cases with a poor prognosis, decreasing the need for prolonged or ineffective use of antimicrobials.

Intra- or extracellular bacteria can be identified in 17-53% of peritoneal fluid samples (Hawkins, Bowman et al. 1993, Matthews, Dart et al. 2001, Odelros, Kendall et al. 2019) and pleomorphic gram-negative rods might be indicative of *Actinobacillus equuli* peritonitis (Matthews, Dart et al. 2001). In an older study, the presence of bacteria in peritoneal samples was associated with non-survival (Hawkins, Bowman et al. 1993) but this is likely different for idiopathic cases and cases of *A. equuli* peritonitis. For these horses, the prognosis is usually good and microscopic presence of bacteria should not be interpreted as a worse prognostic indicator (Matthews, Dart et al. 2001, Odelros, Kendall et al. 2019). Bacterial peritonitis secondary to release of bacteria from the intestine usually results in mixed infections with anaerobes and *Enterobacteriaceae* predominating, most commonly *E. coli*. (van den Bogaard 1990, Davis 2003, Henderson, Mair et al. 2008). Gram positive bacteria might also be present and this should be considered when choosing antimicrobial therapy (Hawkins, Bowman et al. 1993). Penicillin remains a good first line choice for gram positive infections and most anaerobes. Susceptibility patterns of gram negative bacteria are more difficult to predict. Although increasing resistance can be problematic (Reuss and Giguere 2015) aminoglycosides remain a good initial choice. Early data indicated that penicillin-resistant *Bacteroides* spp. were isolated from 10-20% of equine peritonitis cases. A combination of penicillin, gentamicin and metronidazole has therefore traditionally been recommended while awaiting culture and sensitivity findings and use of this combination resulted in a reported survival rate of 86% in horses with peritonitis (Davis 2003, Henderson, Mair et al. 2008, Nogradi, Toth et al. 2011). However, in a more recent study, 91% of horses with idiopathic peritonitis without signs of systemic inflammation responded to penicillin alone. The remaining cases were predominately treated with penicillin and gentamicin and only 1% (n=2) received a combination of penicillin, gentamicin and metronidazole with an overall survival rate of 94% (Odelros, Kendall et al. 2019). Similarly, 61% of horses with

Actinobacillus equuli infection respond to penicillin alone; the reminder to penicillin and gentamicin (Matthews, Dart et al. 2001). In light of these findings, it might be appropriate to use penicillin as monotherapy in horses with suspected *Actinobacillus equuli* or idiopathic peritonitis that show little evidence of systemic inflammation or cardiovascular compromise. Should the clinical condition fail to improve within 24h, addition of gentamicin might be indicated. In horses with predisposing factors or systemic compromise, a combination of penicillin, gentamicin and metronidazole and consideration of surgical exploration if the condition fails to improve would be appropriate. The use of abdominal lavage with or without closed-suction abdominal drains has been described in horses with peritonitis (Nieto, Snyder et al. 2003, Nogradi, Toth et al. 2011). Further studies are necessary to determine whether both techniques improve the outcome or decrease the length for antimicrobial therapy.

Clinical signs and repeated peritoneal fluid analysis are probably the most useful tools when trying to establish whether therapy is successful and when treatment can be discontinued. Repeated abdominocenteses have no effect on peritoneal cell counts (Schumacher, Spano et al. 1985) and can be performed every 24-48h, or more frequently, as required by the case. The cell count should be substantially reduced but does not need to be normal before antimicrobial drugs can be discontinued as inflammation is likely to persist longer than infection. The author uses an arbitrary cut of point of a nucleated cell count of <10-20 $x10^9$/L before discontinuing antimicrobial drugs which appears to be clinically safe. Enterocentesis can increase the cell count significantly, up to 113±88 $x10^9$/L, and will make interpretation of samples impossible for the next 3-4 days (Schumacher, Spano et al. 1985). Intraperitoneal antimicrobial treatment administered via an intraperitoneal catheter has experimentally achieved higher peritoneal fluid concentrations than intravenous administration; however, the clinical usefulness and negative side effects still need to be fully evaluated before this can be recommended (Alonso, Peccinini et al. 2018).

Responsible use of antimicrobials in respiratory diseases

The history and general examination can help differentiate non-infectious from infectious respiratory disease. A horse with a chronic cough that is bright, alert and still performing, although not quite as well as usual, is unlikely to have an infectious pneumonia. Adventitious lung sounds are mainly caused by bronchoconstriction and mucus and exudate accumulation and can be present in infectious and non-infectious diseases alike. In contrast, a horse with fever and adventitious lung sounds that has recently travelled or suffered from choke should be suspected of having an infectious pneumonia or pleuropneumonia. Even if a horse with such a history is normothermic and has normal thoracic auscultatory findings significant intrathoracic disease cannot be ruled out and further diagnostics are indicated. Thoracic ultrasonography is a quick and easy way to rapidly identify infectious pneumonia and pleuropneumonia. If no areas of consolidation, free pleural fluid or large amounts of comet tails are visualised, a significant intrathoracic infection is highly unlikely. Cytological examination of a tracheal lavage or pleural fluid sample are very helpful in differentiating infectious from non-infectious respiratory conditions and add invaluable information. While often marked neutrophilic inflammation is common in infectious and non-infectious conditions, number and location of bacteria (intra- or extracellular), morphology and gram stain are helpful in identifying infections and essential for correct interpretation of culture results. Cases of bacterial pneumonia usually show an abundance of intra- and extracellular bacteria and profuse bacterial growth. A positive bacterial culture from a sample with neutrophilic inflammation but no or very few visible bacteria is in the vast majority of cases indicative of non-infectious airway inflammation such as equine asthma, particularly if growth is scant. These cases will likely respond to environmental management and/or anti-inflammatory treatment alone without the need for any antimicrobial treatment.

In respiratory tract infections, *Streptococcus equi* subsp. *zooepidemicus* is one of the most commonly isolated gram positive pathogens (Arroyo, Slovis et al. 2017, Carvallo, Uzal et al. 2017). *Streptococcus* spp. are almost always sensitive to penicillin which is therefore one of the cornerstones in treatment of respiratory infections in horses (Reuss and Giguere 2015). Other penicillin-susceptible organisms include most gram-positive and gram-negative anaerobic bacteria with the noticeable exception again being *Bacteroides fragilis*. Penicillin-resistant *Bacteroides* spp. were isolated from approximately 8% of pleuropneumonia cases (Hirsh and Jang 1987, Tomlinson, Reef et al. 2015). A foul smell, as it is often noted when draining pleural effusions, has been associated with anaerobic bacterial involvement even if no anaerobic organisms are isolated (Popp 1977, Ashford, Plant et al. 1984, Brook 2008, O'Brien 2012). Inadequate or delayed sample handling significantly reduces chances of culturing anaerobic organisms as exposure to oxygen for any length of time can damage or kill anaerobic bacteria (Brook 2008, Strobel 2009). Many clinicians therefore include metronidazole in their treatment regime if chances of an anaerobic infection are high. Gram negative bacteria involved in equine pleuropneumonia are variable but *E. coli, Klebsiella, Pseudomonas* and *Actinobacillus* have been isolated (Arroyo, Slovis et al. 2017). Either penicillin and gentamicin (pneumonia and pleuropneumonia without overt evidence of anaerobic infection) or a combination of penicillin, gentamicin and metronidazole (strong suspicion of anaerobic involvement) is typically recommended while awaiting culture and sensitivity findings (Davis 2003, Henderson, Mair et al. 2008, Nogradi, Toth et al. 2011). In a recent study, 92% of respiratory samples from ambulatory practice submitted to a laboratory in the South of England were sensitive to the combination of penicillin and gentamicin and 87% to trimethoprim-sulfamethoxazole. Sensitivities of respiratory samples submitted from a referral hospital to the same laboratory were slightly lower but still very acceptable with 83%

being susceptible to penicillin and gentamicin and 75% to trimethoprim-sulfamethoxazole (Potier and Durham 2019).

Although available information is limited and highly dependent on the geographical region, it is questionable whether 3rd or 4th generation cephalosporins would offer a significant treatment advantage over the combination of penicillin and gentamicin (Toombs-Ruane, Riley et al. 2015, Awosile, Heider et al. 2018, Potier and Durham 2019). Considering their reserved status, 3rd or 4th generation cephalosporins should only be used if indicated by culture and sensitivity when first line choices are not available. Enrofloxacin should not be used as stand-alone or first line therapy as it has no activity against *Streptococcus* spp. and anaerobes and, as a fluorquinolon, is a reserved antimicrobial drug. If first line choices do not improve the condition within 48-72h enrofloxacin could be used as a substitute for gentamicin if indicated by culture and sensitivity results in life-threatening disease due to its greater activity against *Enterobacteriaceae*, better penetration into phagocytic cells and tissues, and better activity in purulent material (Reuss and Giguere 2015). In the recent UK based study, no predictable efficacious second choice antimicrobial was identified for respiratory isolates resistant to the first-line antimicrobials highlighting the importance of obtaining a culture and sensitivity results early in the disease process (Potier and Durham 2019).

Pneumonia cases without significant tissue damage often only require a short course (2-4 days) of antimicrobials. Resolution of clinical signs or, ideally, repeat cytological evaluation of a repeated tracheal lavage can be used for guidance. Determining a safe point for discontinuation of antimicrobial treatment in pleuropneumonia cases is much more difficult as tissue damage is often extensive and abscess formation is common. Treatment for a minimum of 10 days or until clinical signs and diagnostic imaging findings indicate resolution has been recommended (Reuss and Giguere 2015, Raidal 2019). Although this is

not supported by any scientific evidence, it offers a reference point, keeping in mind that discontinuation of treatment after 2-4 days should be considered in cases with minimal tissue damage.

In summary, the desire to protect individual patients often leads veterinarians to use antimicrobial drugs “just in case” and to extend antimicrobial treatment for longer than necessary. Using easily available diagnostic tools such as cytology and ultrasonography can help identifying animals with a high chance of bacterial disease. Restricting the use of antimicrobial drugs to these horses and decreasing treatment duration, even if only by a couple of days, will reduce resistance development and benefit patients and the profession.

References

Alonso, J. M., R. G. Peccinini, M. L. Campos, T. Y. Nitta, T. Y. M. Akutagawa, A. P. Crescencio, A. L. G. Alves, C. A. Rodrigues, M. J. Watanabe and C. A. Hussni (2018). "Plasma and peritoneal fluid concentrations of ceftriaxone after intravenous and intraperitoneal administration in horses." Vet J **234**: 72-76.

Arroyo, M. G., N. M. Slovis, G. E. Moore and S. D. Taylor (2017). "Factors Associated with Survival in 97 Horses with Septic Pleuropneumonia." J Vet Intern Med **31**(3): 894-900.

Ashford, R., G. Plant, J. Maher and L. Teare (1984). "Double-blind trial of metronidazole in malodorous ulcerating tumours." Lancet **1**(8388): 1232-1233.

Awosile, B. B., L. C. Heider, M. E. Saab and J. T. McClure (2018). "Antimicrobial resistance in bacteria isolated from horses from the Atlantic Provinces, Canada (1994 to 2013)." Can Vet J **59**(9): 951-957.

Barr, B. S., B. M. Waldridge, P. R. Morresey, S. M. Reed, C. Clark, R. Belgrave, J. M. Donecker and D. J. Weigel (2013). "Antimicrobial-associated diarrhoea in three equine referral practices." Equine Vet J **45**(2): 154-158.

Brook, I. (2008). "The role of anaerobic bacteria in chronic suppurative otitis media in children: implications for medical therapy." Anaerobe **14**(6): 297-300.

Carvallo, F. R., F. A. Uzal, S. S. Diab, A. E. Hill and R. M. Arthur (2017). "Retrospective study of fatal pneumonia in racehorses." J Vet Diagn Invest **29**(4): 450-456.

Costa, M. C., H. R. Stampfli, L. G. Arroyo, E. Allen-Vercoe, R. G. Gomes and J. S. Weese (2015). "Changes in the equine fecal microbiota associated with the use of systemic antimicrobial drugs." BMC Vet Res **11**: 19.

Coyne, C. P., W. J. Hornof, A. B. Kelly, T. R. O'Brien and S. J. DeNardo (1985). "Extraction, radioiodination, and in vivo catabolism of equine fibrinogen." Am J Vet Res **46**(12): 2572-2577.

Davis, J. L. (2003). "Treatment of peritonitis." Vet Clin North Am Equine Pract **19**(3): 765-778.

Dona, D., E. Barbieri, M. Daverio, R. Lundin, C. Giaquinto, T. Zaoutis and M. Sharland (2020). "Implementation and impact of pediatric antimicrobial stewardship programs: a systematic scoping review." Antimicrob Resist Infect Control **9**: 3.

Gronvold, A. M., T. M. L'Abee-Lund, E. Strand, H. Sorum, A. C. Yannarell and R. I. Mackie (2010). "Fecal microbiota of horses in the clinical setting: potential effects of penicillin and general anesthesia." Vet Microbiol **145**(3-4): 366-372.

Harlow, B. E., L. M. Lawrence and M. D. Flythe (2013). "Diarrhea-associated pathogens, lactobacilli and cellulolytic bacteria in equine feces: responses to antibiotic challenge." Vet Microbiol **166**(1-2): 225-232.

Hawkins, J. F., K. F. Bowman, M. C. Roberts and P. Cowen (1993). "Peritonitis in horses: 67 cases (1985-1990)." J Am Vet Med Assoc **203**(2): 284-288.

Henderson, I. S., T. S. Mair, J. A. Keen, D. J. Shaw and B. C. McGorum (2008). "Study of the short- and long-term outcomes of 65 horses with peritonitis." Vet Rec **163**(10): 293-297.

Hirsh, D. C. and S. S. Jang (1987). "Antimicrobic susceptibility of bacterial pathogens from horses." Vet Clin North Am Equine Pract **3**(1): 181-190.

Isgren, C. M., T. Edwards, G. L. Pinchbeck, E. Winward, E. R. Adams, P. Norton, D. Timofte, T. W. Maddox, P. D. Clegg and N. J. Williams (2019). "Emergence of carriage of CTX-M-15 in faecal Escherichia coli in horses at an equine hospital in the UK; increasing prevalence over a decade (2008-2017)." BMC Vet Res **15**(1): 268.

Johns, I. (2017). "Antimicrobial stewardship in the treatment of equine bacterial infections." Vet J **219**: 4-5.

Johns, I., B. Tennent-Brown, B. Dallap Schaer, L. Southwood, R. Boston and P. Wilkins (2008). "Blood culture status in mature horses with diarrhoea: A possible association with survival." Equine Vet J **41**(2): 160-164.
Johns, I., K. Verheyen, L. Good and A. Rycroft (2012). "Antimicrobial resistance in faecal Escherichia coli isolates from horses treated with antimicrobials: a longitudinal study in hospitalised and non-hospitalised horses." Vet Microbiol **159**(3-4): 381-389.
Lascola, K. M., K. Vander Werf, S. Freese, A. Morgera, D. J. Schaeffer and P. Wilkins (2017). "Comparison of jugular and transverse facial venous sinus blood analytes in healthy and critically ill adult horses." J Vet Emerg Crit Care (San Antonio) **27**(2): 198-205.
Llewelyn, M. J., J. M. Fitzpatrick, E. Darwin, C. SarahTonkin, C. Gorton, J. Paul, T. E. A. Peto, L. Yardley, S. Hopkins and A. S. Walker (2017). "The antibiotic course has had its day." BMJ **358**: j3418.
Long, A. and R. Nolen-Walston (2020). "Equine Inflammatory Markers in the Twenty-First Century: A Focus on Serum Amyloid A." Vet Clin North Am Equine Pract **36**(1): 147-160.
Maddox, T. W., N. J. Williams, P. D. Clegg, A. J. O'Donnell, S. Dawson and G. L. Pinchbeck (2011). "Longitudinal study of antimicrobial-resistant commensal Escherichia coli in the faeces of horses in an equine hospital." Prev Vet Med **100**(2): 134-145.
Magdesian, K. G., M. Dujowich, J. E. Madigan, L. M. Hansen, D. C. Hirsh and S. S. Jang (2006). "Molecular characterization of Clostridium difficile isolates from horses in an intensive care unit and association of disease severity with strain type." J Am Vet Med Assoc **228**(5): 751-755.
Marley, L. K., C. Soffler and E. S. Hackett (2018). "Clinical features, diagnostic methods, treatments, and outcomes associated with ingested wires in the abdomen of horses: 16 cases (2002-2013)." J Am Vet Med Assoc **253**(6): 781-787.
Marshall, J. C., L. Bosco, N. K. Adhikari, B. Connolly, J. V. Diaz, T. Dorman, R. A. Fowler, G. Meyfroidt, S. Nakagawa, P. Pelosi, J. L. Vincent, K. Vollman and J. Zimmerman (2017). "What is an intensive care unit? A report of the task force of the World Federation of Societies of Intensive and Critical Care Medicine." J Crit Care **37**: 270-276.
Matthews, S., A. J. Dart, B. A. Dowling, J. L. Hodgson and D. R. Hodgson (2001). "Peritonitis associated with Actinobacillus equuli in horses: 51 cases." Aust Vet J **79**(8): 536-539.
Meichner, K., B. H. Kraszeski, J. R. Durrant, C. B. Grindem, B. A. Breuhaus, P. F. Moore, J. A. Neel, K. E. Linder, L. B. Borst, J. E. Fogle and J. L. Tarigo (2017). "Extreme lymphocytosis with myelomonocytic morphology in a horse with diffuse large B-cell lymphoma." Vet Clin Pathol **46**(1): 64-71.
Nieto, J. E., J. R. Snyder, N. J. Vatistas, S. J. Spier and L. Van Hoogmoed (2003). "Use of an active intra-abdominal drain in 67 horses." Vet Surg **32**(1): 1-7.
Nogradi, N., B. Toth and K. C. Macgillivray (2011). "Peritonitis in horses: 55 cases (2004-2007)." Acta Vet Hung **59**(2): 181-193.
O'Brien, C. (2012). "Malignant wounds: managing odour." Can Fam Physician **58**(3): 272-274; e141-273.
Odelros, E., A. Kendall, Y. Hedberg-Alm and J. Pringle (2019). "Idiopathic peritonitis in horses: a retrospective study of 130 cases in Sweden (2002-2017)." Acta Vet Scand **61**(1): 18.
Page, A. E., N. M. Slovis and D. W. Horohov (2014). "Lawsonia intracellularis and equine proliferative enteropathy." Vet Clin North Am Equine Pract **30**(3): 641-658.
Pihl, T. H., M. K. Nielsen, S. N. Olsen, P. S. Leifsson and S. Jacobsen (2018). "Nonstrangulating intestinal infarctions associated with Strongylus vulgaris: Clinical presentation and treatment outcomes of 30 horses (2008-2016)." Equine Vet J **50**(4): 474-480.

Popp, W. (1977). "[The diagnosis and treatment of mixed anaerobic vaginal dischargs (authors' transl)]." Geburtshilfe Frauenheilkd **37**(5): 432-437.
Potier, J. F. N. and A. E. Durham (2019). "Antimicrobial susceptibility of bacterial isolates from ambulatory practice and from a referral hospital." J Vet Intern Med.
Pradhan, S., B. Madke, P. Kabra and A. L. Singh (2016). "Anti-inflammatory and Immunomodulatory Effects of Antibiotics and Their Use in Dermatology." Indian J Dermatol **61**(5): 469-481.
Raidal, S. L. (2019). "Antimicrobial stewardship in equine practice." Aust Vet J **97**(7): 238-242.
Reuss, S. M. and S. Giguere (2015). "Update on bacterial pneumonia and pleuropneumonia in the adult horse." Vet Clin North Am Equine Pract **31**(1): 105-120.
Schoster, A. and H. Staempfli (2016). "Epidemiology and Antimicrobial Resistance in Clostridium difficile With Special Reference to the Horse." Current Clinical Microbiology Reports **3**(1): 32-41.
Schumacher, J., J. S. Spano and H. D. Moll (1985). "Effects of enterocentesis on peritoneal fluid constituents in the horse." J Am Vet Med Assoc **186**(12): 1301-1303.
Shaw, S. D. and H. Stampfli (2018). "Diagnosis and Treatment of Undifferentiated and Infectious Acute Diarrhea in the Adult Horse." Vet Clin North Am Equine Pract **34**(1): 39-53.
Strobel, H. J. (2009). Basic laboratory culture methods for anaerobic bacteria.
Targowski, S. P. (1975). "Effect of prednisolone on the leukocyte counts of ponies and on the reactivity of lymphocytes in vitro and in vivo." Infect Immun **11**(2): 252-256.
Theelen, M. J. P., W. D. Wilson, B. A. Byrne, J. M. Edman, P. H. Kass, L. Mughini-Gras and K. G. Magdesian (2020). "Differences in isolation rate and antimicrobial susceptibility of bacteria isolated from foals with sepsis at admission and after >/=48 hours of hospitalization." J Vet Intern Med.
Tomlinson, J. E., V. B. Reef, R. C. Boston and A. L. Johnson (2015). "The Association of Fibrinous Pleural Effusion with Survival and Complications in Horses with Pleuropneumonia (2002-2012): 74 Cases." J Vet Intern Med **29**(5): 1410-1417.
Toombs-Ruane, L. J., C. B. Riley, A. T. Kendall, C. F. Bolwell, J. Benschop and S. M. Rosanowski (2015). "Antimicrobial Susceptibilities of Aerobic Isolates from Respiratory Samples of Young New Zealand Horses." J Vet Intern Med **29**(6): 1700-1706.
van den Bogaard, A. E. (1990). "Peritonitis in adult horses." Vet Rec **127**(15): 387-388.
Viner, M., M. Mazan, D. Bedenice, S. Mapes and N. Pusterla (2017). "Comparison of serum amyloid A in horses with infectious and noninfectious respiratory disease." Journal of Equine Veterinary Science **49**: 11 - 13.
Weese, J. S., L. Toxopeus and L. Arroyo (2006). "Clostridium difficile associated diarrhoea in horses within the community: predictors, clinical presentation and outcome." Equine Vet J **38**(2): 185-188.
Westerman, T. L., S. J. Tornquist, C. M. Foster and K. P. Poulsen (2015). "Evaluation of serum amyloid A and haptoglobin concentrations as prognostic indicators for horses with inflammatory disease examined at a tertiary care hospital." Am J Vet Res **76**(10): 882-888.
White, S. D., V. K. Affolter, J. Dewey, P. H. Kass, C. Outerbridge and P. J. Ihrke (2009). "Cutaneous vasculitis in equines: a retrospective study of 72 cases." Vet Dermatol **20**(5-6): 600-606.