

Rational antimicrobial therapy for sepsis in cattle in face of the new legislation on critically important antimicrobials

Rationele antimicrobiële therapie voor sepsis bij runderen in het licht van de nieuwe wetgeving over kritisch belangrijke antibiotica

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ABSTRACT

Sepsis is a life-threatening condition in cattle, as in humans. Successful sepsis treatment requires a fast initiation of antimicrobial therapy with a broad-spectrum, bactericidal drug, administered intravenously. However, the new legislation limits the use of critically important fluoroquinolones and cephalosporins in food animal medicine, drugs which are considered highly effective for sepsis treatment and were previously frequently used for this indication. This article aims at providing an overview of the current knowledge of sepsis in cattle to aid practitioners in adapting their decision making process to treat sepsis. Sepsis incidence in cattle is likely seriously underestimated. This disease is almost unexplored in cattle, which makes it particularly difficult to design species specific guidelines. Sepsis diagnosis by blood culture reaches sufficient accuracy with the current laboratory technology. When adapting this procedure to a field environment, difficulties might arise, and the availability of adapted incubators in veterinary laboratories may be limited. However, these difficulties are likely to be overcome. Severe sepsis and septic shock are likely the only indications where the 'urgency' criterion of the new legislation would apply, but it is important to realize that alternative antimicrobial treatments with possibly similar efficacy are available.

SAMENVATTING

Sepsis is een levensbedreigende aandoening bij rundvee, net zoals bij de mens. Het snel toedienen van een breedspectrum-, bactericide antibioticum via intraveneuze weg is cruciaal voor een succesvolle behandeling van sepsis. De nieuwe wetgeving limiteert echter het gebruik van de kritisch belangrijke fluoroquinolonen en cefalosporinen bij voedselproducerende dieren. Deze antimicrobiële middelen worden als zeer effectief ervaren voor de behandeling van sepsis en werden vroeger dan ook veelvuldig ingezet voor deze indicatie. Het doel van dit artikel is een overzicht te geven van de huidige kennis over sepsis bij runderen om praktijkdierenartsen te sturen in hun beslissingsproces voor de behandeling van sepsis. De incidentie van sepsis bij rundvee wordt hoogstwaarschijnlijk ernstig onderschat. De ziekte werd vrijwel niet onderzocht bij runderen, wat het opstellen van speciesspecifieke richtlijnen sterk bemoeilijkt. De diagnose van sepsis door middel van bloedonderzoek is met de huidige laboratoriumtechnologie voldoende accuraat. Mogelijke hinderpalen voor een meer veralgemeende toepassing zijn de minder hygiënische stalomgeving en de beperkte beschikbaarheid van aangepaste apparatuur in veterinaire labo's, maar deze lijken niet onoverkomelijk. Ernstige sepsis en septische shock zijn mogelijk de enige indicaties die in aanmerking komen voor het hoogdringendheids criterium zoals in de wetgeving weergegeven, maar het is belangrijk in te zien dat er ook antimicrobiële behandelingen met niet kritisch belangrijke moleculen bestaan met een mogelijk gelijkaardige effectiviteit.

INTRODUCTION

Sepsis is a life-threatening condition affecting humans and all animal species (Fecteau et al., 1997a; Rhodes et al., 2017). Sepsis refers to a dysregulated

systemic inflammatory response to the presence of bacteria in the bloodstream (Rhodes et al., 2017). To successfully treat sepsis, an early treatment with antimicrobials active against the involved bacteria is crucial. In practice, the critically important fluoroqui-

nolones and cephalosporins are popular molecules for this indication, because of their bactericidal effects, broad-spectrum activity and relatively low resistance levels. Antimicrobial (multi)resistance in bacteria associated with sepsis is a problem in cattle and in humans (Marchese et al., 2012; Pardon et al., 2017). Current intensive antimicrobial use in food producing animals is under pressure (Callens et al., 2012; Lava et al., 2016; Pardon et al., 2012) and therefore, a covenant has been negotiated between the federal government and the involved sectors agreeing on a reduction in total antimicrobial use of 50% and in the use of critically important antimicrobials of 75% by 2020 (AMCRA, 2016). In addition, a new legislation (KB July 21st, 2017) limits the use of critically important cephalosporins (third and fourth generation) and fluoroquinolones (first to third generation). Before these molecules can be used in food animals, the legislation requires (1) a clinical examination by the veterinarian, (2) a bacterial cause of the disease, (3) adequate sampling and bacteriological culture in an accredited laboratory, (4) identification of the bacterial strain which likely caused the infection and (5) comparison of the strains susceptibility with at least seven other not-critically important antimicrobials, belonging to at least five different antimicrobial classes. Further, the legislation allows the following exceptions in which critically important antimicrobials can be used without previous laboratory confirmation: (1) if no laboratory result is reached or sampling is impossible (in this case, the veterinarian can use the molecules based on recent scientific data); (2) if laboratory results fulfilling the first five requirements above are available for the same group of animals in the same farm (valid for six months in veal calves and one year for other cattle operations) and finally (3) for 'urgency' reasons to save the life of a single animal. In the last case, the veterinarian needs to treat the patient him/herself, after a clinical examination, obligatory sampling and culturing. As soon as an antimicrobial susceptibility test result is reached, re-assessment of antimicrobial therapy and de-escalation to a non-critically important antimicrobial is obliged, whenever possible.

In this article, an overview is provided of the current knowledge of sepsis in cattle to support practitioners in their decision making process to rationally use antimicrobials in sepsis suspected patients. During the literature search, the lack of information on sepsis in cattle became painfully clear, and the authors were partly obliged to extrapolate knowledge from human medicine in an attempt to supply sufficient guidance.

DEFINITIONS

In general, sepsis (septicemia) is a term to describe a systemic illness associated with the presence of microorganisms and/or their toxins in the bloodstream (Fecteau et al., 2009). Bacteremia often refers to the

presence of bacteria in the bloodstream, whereas sepsis also holds the systemic inflammatory response to these microorganisms. In sepsis with gram-negative bacteria, lipopolysaccharide (= endotoxin) is responsible for an important part of the more severe inflammatory response compared to most gram-positive sepsis cases. Endotoxemia includes the presence of endotoxins in the bloodstream, causing similar symptoms as when accompanied by the bacteria itself. In veterinary medicine, most papers limit the diagnosis of sepsis to detection of bacteria in blood by culture (Hollis et al., 2008), either with additional clinical signs required (Fecteau et al., 1997a) or even without any confirmation of bacterial infection at all (Trefz et al., 2016). In contrast, in human medicine, different definitions are used to cover the whole spectrum of systemic inflammation and sepsis. In 1992, definitions for systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, septic shock and multiple organ dysfunction syndrome (MODS) have been established (Bone et al., 1992). SIRS refers to any systemic reaction, which occurs when the body fails to recognize or control a non-specific insult of either infectious or non-infectious origin. SIRS is non-specific and can be caused by ischemia, inflammation, trauma, infection or any combination of these. SIRS is clinically defined in humans as having two of the following four clinical signs: (1) fever $>38^{\circ}\text{C}$ or $<36^{\circ}\text{C}$; (2) heart rate >90 beats pro minute; (3) respiratory rate >20 breaths pro minute or arterial partial pressure of carbon dioxide (PaCO_2) <32 mmHg and (4) abnormal white blood cell count ($>12\,000$ cells/ μL or <4000 cells/ μL or $>10\%$ band cells) (Bone et al., 1992). Sepsis is the systemic response to infection and is currently defined as the presence of SIRS in addition to a documented bloodstream infection. Severe sepsis is characterized by sepsis with organ dysfunction (kidney, liver, lung, heart, etc.) and is often accompanied by hypotension and hypoperfusion. Patients meet the criteria for septic shock if perfusion abnormalities and hypotension (systolic pressure <90 mmHg) persist despite adequate fluid resuscitation (vasopressors needed and lactate increased), and no signs of other types of shock. Septic shock is a combination of the three types of shock, namely cardiogenic, hypovolemic and distributive shock. Finally, MODS is a syndrome, in which multiple organs dysfunction.

The SIRS approach has been criticized a lot in the last decade, especially because often, persons with a simple respiratory infection already fulfill two criteria. In 2016, new definitions have been proposed in human medicine, which define sepsis as life-threatening organ failure caused by a dysregulated host response to infection (Singer, 2016). The sequential organ failure assessment score (SOFA) is the central concept, which evaluates breathing, circulation, liver and kidney function, coagulation and the neurological condition. In first line practice, a quick SOFA score is recommended to identify those patients requiring urgent

treatment and referral to hospital. The quick SOFA score only evaluates three criteria: low blood pressure (systolic blood pressure ≤ 100 mmHg), high respiratory rate (≥ 22 breaths/min.) and an altered mentation (Glasgow coma scale) in presence of a documented or assumed infection. The qSOFA score has predicted mortality in hospital better than the SOFA or SIRS approach and has better identified persons requiring urgent treatment and hospitalization (Freund et al., 2017).

In summary, only a handful of studies on sepsis in cattle are available, with highly variable definitions (Fecteau et al., 1997a; Lofstedt et al., 1999; Trefz et al., 2016). It is clear that progress on sepsis management and rational treatment in food animals can only be made if a universal terminology becomes available. To what extent the above mentioned human definitions can be extrapolated to animals needs to be determined. Practical difficulties certainly exist, as for example reliable blood pressure measurements in large animals in a field setting are not straightforward.

SEPSIS INCIDENCE, ETIOLOGY AND RISK FACTORS IN CATTLE

Only a handful of studies have determined the prevalence of sepsis in large animals, and mostly in a subset of critically ill animals being admitted to veterinary (teaching) hospitals. In calves admitted with diarrhea and/or depression, 24-31% is bacteremic (Fecteau et al., 1997b). This is likely an underestimation as these studies did not have the more sensitive, contemporary sampling and culturing techniques at their disposal (see section on diagnosis).

Sepsis is regarded as a sporadic disease, but massive epidemics of sepsis can occur with virulent bacteria like *Salmonella spp.* (especially host adapted species like *Salmonella Dublin*), *Histophilus somni*, *Mannhaemia haemolytica* or *Pasteurella multocida*, especially in young animals (Catry et al., 2005; Mahu et al., 2015; McFadden et al., 2011). In the immunocompromised host, sepsis is more likely to occur. Risk populations are periparturient animals and calves, especially those with failure of passive transfer of maternal immunity (Lofstedt et al., 1999). Neonates lack a normal competitive intestinal flora, making colonization with virulent pathogens from the environment more easy. The source of the pathogenic bacteria in the neonate is most likely the contaminated environment and particularly, materials used to supply colostrum, which can be heavily contaminated. Outbreaks of sepsis with identical *Escherichia coli* clones have been described (Marchese et al., 2012), but to what extent blood culture results of a single case can be extrapolated to the whole of the group for a long period of time is unknown. Calves might also be born septicemic after in utero infection or during parturition (prolonged parturition or vaginal passage in case

of vaginal infections). In humans, group B *Streptococci*, which colonize the vagina, are a well-known cause of neonatal sepsis (Singer et al., 2016). To the authors' knowledge, no such relationships between vaginal flora and sepsis have been substantiated in cattle. Also malnourishment, infection with immunosuppressive viruses (e.g. bovine viral diarrhea virus) and immune-mediated panmyelophthisis are risk factors (Pardon et al., 2010). In adult cattle, septicemic spread from ruminal bacteria after an acidosis episode or from a local site of infection (e.g. traumatic reticuloperitonitis, mastitis, arthritis, etc.) to other organs (causing liver abscesses, metastatic pneumonia or endocarditis) must not be underestimated. Especially in the North American feedlots, a liver abscesses prevalence of 60-63% has been reported, in cull cows even as high as 78% (Amachawadi and Nagaraja, 2016). Fear is that this prevalence will even increase, if the in-feed use of tylosin becomes restricted in the United States, as in Europe.

The general message is that a multitude of bacteria can cause septicemia in the neonatal calf and in adult cattle. Gram-negative sepsis is far more common than gram-positive sepsis, in contrast to humans. In calves, the most commonly isolated bacterial family is the *Enterobacteriaceae*, with *E. coli* (50-65% of all cases), *Salmonella spp.* and *Klebsiella spp.* being most common (Fecteau et al., 2009), followed by gram-positive cocci (*Staphylococci* and *Streptococci*) (12-14%), gram-positive rods (*Trueperella pyogenes*, *Listeria spp.*, *Bacillus spp.*) (0-8%) and gram-negative non-enteric rods (*Pseudomonas aeruginosa*, *Mannheimia haemolytica*, *Acinetobacter spp.*, etc.) (0-8%). Anaerobes (*Bacterioides spp.* and *Clostridium spp.*) account only for a minority (0-6%) of sepsis cases. Despite that, also fungi and yeasts are well-known causes of sepsis in humans; there is no information available on their importance in large animals (Lamy et al., 2016). An interesting finding is that both in healthy periparturient cows as in cows with acute metritis, a similar prevalence of *Bacillus licheniformis* bacteriemia has been found (56% vs. 53%, respectively) (Credille et al., 2014). More research is urgently needed to clarify this finding.

In humans, the main portals of entry are the intestinal tract, the oropharynx (tonsils), surgical sites, contaminated inhalators and catheter infections. In calves, the main portals of entry are the intestinal tract, the lungs and the umbilicus, whereas in adults, sepsis more frequently occurs in association with mastitis, metritis or ruminitis. Sepsis may also be iatrogenic, for example through injection. In a recent report, vaccine contamination with *Streptococcus infantarius* subsp. coli resulted in sepsis in 15% of the vaccinated animals of which 57% died (Clarke et al., 2016). Surgical wounds and catheter infections are common causes of sepsis in humans, but have been hardly documented in calves. A recent report has shown how a single, multiresistant *E. coli* strain caused catheter-



Figure 1. Clinical signs of *Listeria monocytogenes* septicemia and meningitis in a two-week-old Belgian blue calf.

associated phlebitis in multiple calves and fatal sepsis in a single case (Pardon et al., 2017).

PREDICTION OF SEPSIS BASED ON CLINICAL SIGNS

A crucial question is which symptoms need to be used to identify animals at risk for sepsis. This question is particularly difficult in young calves, because severe dehydration and D-lactic acidosis are common and demonstrate similar clinical signs as sepsis (Lorenz, 2009). Also comorbidities, namely enteric infection, D-lactic acidosis, pneumonia and sepsis, are frequent.

Two studies have attempted to design a clinical model to predict septicemia in calves (Fecteau et al., 1997a; Lofstedt et al., 1999). None have been found for adult cattle. The included calves in both studies were suffering from neonatal enteritis and/or depression arriving at a veterinary teaching hospital. Important limitations of these studies are the sampling and culture methodology used to confirm sepsis. In one study, two blood samples of a limited sampling volume (7 ml) were used (Fecteau et al., 1997a). In the other study, different diagnoses, like positive blood culture, culture of the same bacteria from two body sides or culture from a joint were used (Lofstedt et al., 1999). Additionally, the obtained predictive models were not validated on a new dataset, limiting their external validity. Nevertheless, some information on which calves are at an increased sepsis risk can be derived from these studies and may aid practitioners in selecting suspected cases.

The presence of a focal infection (omphalitis, arthritis, hypopyon, soft tissue abscess or mucopurulent nasal discharge) in the study by Fecteau et al. (1997a) and andomphalitis, joint effusion, hypopyon and neurological signs in the study by Lofstedt et al. (1999) remained a significant factor in the multivariable model in both studies (Figure 1). The final model of one study consisted of focal infection, age

(<5-7 days), posture (recumbent upon arrival 3.0 (1.3-6.7 times higher odds than standing)) and a weak or absent suckling reflex (odds ratio (OR)= 1.1 (1.1-8.3)). This clinical model had a sensitivity (Se) and specificity (Sp) of 39.4% and 90.6%, respectively (Lofstedt et al., 1999). With a sepsis prevalence of 30%, the negative predictive value was 78.8% for this model. In the study by Fecteau et al. (1997a), a sepsis score was designed, based on five clinical signs, namely fecal consistency, hydration, attitude, umbilicus and scleral vessels. The final model consisted of this sepsis score, age (> seven days of age in this case) and a focal site of infection. The disadvantage of a score is that it combines different variables, of which possibly only a single one explains the predictive effect. In the other study, hydration rate, attitude and scleral injections were univariably associated with sepsis, but did not remain significant in the final model (Figure 2). The final model of Fecteau's study had a Se and Sp of 75% and 71%, respectively. An interesting observation is that in the study of Fecteau et al., no differences in rectal temperature, respiratory rate and heart rate were found between septicemic and normal calves, whereas Lofstedt et al. did find significant lower temperature and higher respiratory rates (heart rate not recorded) in septicemic calves, albeit very small differences. The above mentioned studies only dealt with the high risk group of diarrheic calves. For older animals, which frequently encounter pneumonia, no data is available to identify high risk animals. Although these predictive models for bacteremia are a promising feature, apparently also in human medicine, few models have been prospectively validated or implemented in clinical practice (Lamy et al., 2016). The authors mention additional workload for data entry as the main reason of reluctance to use these models.

In summary, available evidence for using selected clinical signs or a sepsis score to predict septicemia in calves is very limited and hampered by several methodological aspects. From the available work, some empirical directions can be derived. Young calves and animals with focal infections should be considered at



Figure 2. Scleral injections are often regarded as highly suggestive of sepsis.

an increased risk. In the authors' opinion, blood culture sampling can be recommended in animals displaying depression, recumbency, tachycardia, tachypnea and an abnormal rectal temperature, especially when also a focal site of infection (e.g. neurological signs) or scleral injections are present. The combination of all these symptoms is a reflection of a more severe state of sepsis, rather than the earliest stage. Recommending the use of human SIRS and sepsis criteria to assure early detection is in the authors' opinion unwise given the current knowledge level of sepsis in calves. It holds the danger of overusing critically important antimicrobials in the field.

ADDITIONAL BLOOD PARAMETERS AND BIOMARKERS

To confirm severe sepsis and MODS, additional blood parameters need to be tested. Renal failure, liver failure and respiratory failure are generally confirmed with increased renal values (creatinine and blood urea nitrogen), liver values and arterial blood PaO₂ and PaCO₂. The determination of possible organ failure is important for the prognosis, as the mortality risk in humans is 16-20% in sepsis or severe sepsis, compared to 46% in septic shock (Rangel-Frausto et al., 1995).

To improve the diagnostic accuracy of the models for sepsis, several blood parameters have been tested in a single calf study (Lofstedt et al., 1999). Failure of passive transfer, defined as an immunoglobulin G < 10 g/L in a calf < 1 week, is a dominant risk factor for sepsis (OR= 2.7 (1.2-6.5)) (Lofstedt et al., 1999). Also a marked increase in serum creatinine, as would be seen in severe sepsis, and toxic changes in neutrophils are predictive for sepsis. The sensitivity and specificity of this model were 40% and 95.4%, respectively. In the study, this laboratory model only improved specificity by 5% compared to the clinical model. The presence of band neutrophils, increased packed cell volume, decreased total protein and ionized calcium and increased PaCO₂ were univariably associated with sepsis (Lofstedt et al., 1999). In contrast, total white blood cell and neutrophil count, fibrinogen, pH, base excess, bicarbonate, chloride, creatine kinase, gamma-glutamyl transferase, glucose, potassium and sodium could not be associated with septicemia.

Next to these parameters of a standard blood examination, predominantly in human medicine, a search for potent biomarkers to differentiate sepsis from other inflammatory processes is ongoing. Especially procalcitonin, the prohormone of calcitonin, has been put forward as a suitable sepsis marker in infants, with the optimal cut-off being >1.2 ng/mL (Se= 77%; Sp= 79%) (Delevaux et al., 2003; Park et al., 2014). Current advice in human medicine is to initiate broad spectrum antimicrobial therapy before culture results are returned in patients above this cut-off. Acute phase



Figure 3. Sterile blood sampling procedure after clipping and alcoholic chlorhexidine desinfection.

proteins are known to be highly species specific. In calves, PGE₂, malondialdehyde (MDA), IL-8, TNF- α , IFN- γ , neopterin and procalcitonin have been evaluated in a single study (Ercan et al., 2016). The last four were significantly increased in *E. coli* septicemic calves. Ercan et al. (2016) also suggest procalcitonin to be the best marker as it increased four times, had a long half-time life and remained stable at room temperature. In order to be practically useful for on-farm-decision-making, the development of a cow-side test with this molecule will be necessary.

Diagnosis by culture

In humans, blood cultures are among the most commonly submitted microbiological samples, whereas they are only very occasionally used in food animals. Given the very limited information on large animals, extrapolation of human best practices is advisable. The method of choice is peripheral venipuncture, instead of blood collection through in place catheters, as it may harbor several bacteria. In humans, the contamination rate of aseptically collected peripheral blood samples is 3.4-13% (Lamy et al., 2016). In veterinary clinics, similar aseptic conditions can be achieved, but likely, limiting contamination risk will be much more difficult when sampling in a stable environment. After clipping, the use of alcoholic chlorhexidine is recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines for blood culture collection from infants > 2 months on (Lamy et al., 2016) (Figure 3). In most guidelines, sample collection is advised in the absence of antimicrobial therapy and at or around the time of fever spikes (Lamy et al., 2016). However, a recent study has shown that the likelihood of a positive blood culture is independent from the timing in respect of a temperature spike (Riedel et al., 2008). In human guidelines, a minimum of two blood cultures, 30-60 minutes apart is recommended. The likelihood of detecting a blood stream infection depends on the bacterial concentration in blood and the

volume cultured, with the latter being most important (Lamy et al., 2016). Average bacterial concentrations in blood during sepsis (mean: 250 cfu/L blood; range: 100-2000 cfu/L (Lamy et al., 2016)) are much lower than in local infections, making direct blood culture of low volumes very insensitive. The recovery rate increases markedly with the increasing volume of blood cultured. Recovery rates for collected blood volumes of 20, 40 and 60 mL are 65-76%, 80-89% and 96-98%, respectively (Lamy et al., 2016). Current recommendations in humans include 3-4 blood culture sets with 20-30 mL collected per set. To counter the issue of low bacterial concentration in blood stream infections, also specific enrichment procedures (some hold resin or charcoal to neutralize antibiotic substances) with an automated detection system are common practice in hospital settings (e.g. Bactec (Beckton-Dickinson, USA) or BactAlert/Virtuo systems (bioMérieux, France)). The recommended volumes are 8-10 cc in bottles for adults and 1-5 cc in pediatric bottles. These machines automatically report when sufficient bacterial growth is reached to allow standard culture techniques and subsequent antimicrobial susceptibility testing. Point of attention is to also disinfect the top of each vial with a single alcohol swab and allow drying for 60 seconds, before injecting the collected blood. As mentioned, the volume cultured is the crucial factor. Attention should be paid to adequately fill the bottles, as inadequate filling is common and reduces sensitivity. These automated detection systems also result in a faster detection of the bacteria, which is crucial for a disease where every hour counts. It is important to realize that a complete blood culture holds three different media: an aerobic, an anaerobic and a medium for fungi and yeast. If an economic choice needs to be made, aerobic culture should be withheld.

Clinicians can either opt for a multi-sampling strategy or a single-sampling strategy (Lamy et al., 2016). The rationale behind the multi-sampling strategy is that an increased volume is obtained, that contaminants may be distinguished from pathogens and that sensitivity is improved in cases of intermittent bacteremia. The disadvantages for application in veterinary medicine are the need for repeated farm visits and more sampling material resulting in substantial extra costs. Moreover, repeated venipuncture increases the contamination risk (more false positives) (Lamy et al., 2002). A large problem in humans is that frequently, only the first sample is submitted, possibly resulting in false conclusions. Apparently, the concept of intermittent bacteremia or fungemia as generally suspected has never been evidenced. In contrast, most clinical blood stream infections are associated with continuous bacteremia for 24 hours with very low concentrations of circulating microorganisms (Riedel et al., 2008). Hence, the interest in single-sampling strategies, yielding a higher volume (4-6 bottles) at one time has been regained. This method assures enough volume sampled and a reduced risk of contamination due to multiple punctures. It also reduces

workload and the risk of occupational exposure to pathogens. Sensitivity of single sampling is equal to multi-sampling if the sampled blood volume is large enough (35-42 mL) (Lamy et al., 2002). Summarized, using these culture systems, the sensitivity and specificity of large volume single sampling is estimated at 95% and 97.5%, respectively (Lamy et al., 2002). Disadvantages are difficulties with collection of such volumes in certain patients through a single puncture, and the rather limited number of studies to confirm their equality to multi-sampling. A standard blood culture procedure takes 1-7 days, with only a minority of samples (2.7%) still turning positive between 5 and 7 days (Marginson et al., 2014). Alternative technology to rapidly detect bacteria directly on blood samples is being developed. PCR has been used and may identify with high accuracy bacteria involved in blood stream infections within 4-8 hours (Ginn et al., 2017). A disadvantage is the need for multiple primers to cover the most likely organisms. Another example is the use of the Sepsityper kit on the matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometer, which enables species identification within a few hours (Morgenthaler and Kostrzewa, 2015).

In summary, a single-sample strategy appears most achievable in the field and is likely to return an acceptable diagnostic accuracy, if a sufficient volume is cultured. Determination of its Se and Sp is urgently needed. Alternatively, documentation of an infected body site in an animal with signs of sepsis might be considered. For some sites (e.g. lung, udder, bladder), interpretation may be straightforward, whereas it is virtually impossible for intestinal or throat samples.

Antimicrobial susceptibility testing

It is outside the scope of this paper to go into detail on the advantages and disadvantages of the different methods for antimicrobial susceptibility testing. It is however important to realize that there is no 100% agreement between the commonly used disk-diffusion method and the micro-dilution gold standard procedure, as demonstrated for *Pasteurellaceae* (Catry et al., 2007). Very important mistakes resulting in not treating an animal or treating it with an inadequate antimicrobial when treatment is required, were for example present in 3.7% and 7.4% of *P. multocida* and *M. haemolytica* isolates, respectively. Fast diagnostics are crucial to guide rational sepsis treatment. With MBT-ASTRA, a MALDI TOF procedure, an antibiogram could be reached as fast as one to three hours post culture initiation, also for veterinary pathogens like *Pasteurella multocida* (Sparbier et al., 2016; Van Driessche et al., 2017). This technology completely meets the demand for fast diagnostics, which is also crucial in food animal medicine if prudent antimicrobial use needs to be combined with maximization of patient survival chances.

Therapy

Sepsis therapy has three priorities: (1) immediate stabilization of the patient (airway, breathing, circulation); (2) removal of bacteria from the blood stream as fast as possible and (3) treatment of the original focus of infection. The key factor for successful antimicrobial therapy for sepsis is to be early and appropriate. Appropriate antimicrobial therapy (= therapy with an antimicrobial to which the bacteria are susceptible) must be initiated as soon as possible, because delay is associated with increased mortality (for each hour + 6% mortality risk) (Dellinger et al., 2013). Early signifies administration within <30-60 minutes after recognition of severe sepsis or septic shock.

In the absence of culture and antimicrobial susceptibility test results, initial antimicrobial therapy is considered empiric. The primary criterion for empiric antimicrobial drug selection is spectrum. Because of the high mortality associated with septic shock, empiric therapy should cover both gram-positive and gram-negative bacteria (broad spectrum). Based on the probable anatomic site of infection (lungs, umbilicus, intestines, etc), an informed selection of an antimicrobial able to reach this site, needs to be made. Other criteria for antimicrobial selection are local antimicrobial resistance data on the most likely pathogens, earlier antimicrobial therapies and available susceptibility testing results from local infections (Liang and Kumar, 2015). To rapidly and effectively clear the blood stream infection, a bactericidal antimicrobial is recommended (Liang and Kumar, 2015). Antimicrobials are often combined, not only to reach broad spectrum activity, but also to increase potency, reaching higher cidalty by attacking the bacteria by different mechanism of action. Evidence of increased cidalty is available for β -lactam/aminoglycoside and β -lactam/fluoroquinolone combinations, but no marked benefit for clinical outcomes could be evidenced in the available meta-analyses, except for the very critically ill (septic shock) (Liang and Kumar, 2015).

Next to the antimicrobial drug selected, optimization of the dosing regimen plays a crucial role in cidalty. Intravenous administration is generally preferred as it results in the fastest increase in blood concentration. In the critically ill patient, altered pharmacokinetics are present, with an increased distribution volume for β -lactams and aminoglycosides, possibly resulting in subtherapeutic concentrations (Liang and Kumar, 2015). Therefore, current recommendation is that initial antimicrobial therapy in septic shock should begin with the maximum recommended dose. For time-dependent antibiotics, the effective time above the minimum inhibitory concentration (MIC) can be improved by either a more frequent dosing regimen or by continuous infusion therapy (after a high loading dose). To optimize concentration-dependent killing agents (aminoglycosides and fluoroquinolones), the area un-

der the curve (AUC) in 24h/MIC ratio and the peak antimicrobial concentration (C_{max})/MIC ratio need to be maximized; in brief, this signifies the use of the maximum non-toxic dose for a concentration-dependent antibiotic (Liang and Kumar, 2015). In cattle, the renal toxicity of gentamicin is feared, especially in dehydrated patients. Determination of creatinine/blood urea nitrogen and fluid therapy may counteract this issue. An overdose of fluoroquinolones can result in neurological signs (depression, incoordination, muscle fasciculation, transient nystagmus, recumbency and possibly death) (Anonymous, 2007). An advantage of using higher dosages of fluoroquinolones is that the mutant prevention concentration (MPC) is reached, limiting the development of antimicrobial resistance in at least the targeted pathogen (principles of the single injection short acting antibiotic (SISAAB) concept) (Balaje et al., 2013).

Equally important as the fast initiation of antimicrobial therapy is the timely recognition of therapy failure. When culture and susceptibility test results are available (2-4 days minimum, depending on the bacteria), antibiotic reassessment needs to be done. Inappropriate empirical antimicrobial therapy is defined as the use of an antimicrobial demonstrated insensitive for the cultured bacteria, and has been associated with a fivefold increase in mortality in humans (Kumar, 2009). In contrast, effective antibiotic reassessment has been associated with a better outcome in humans (Aillet et al., 2017). De-escalation is the general principle, signifying that a shift to a more narrow-spectrum antimicrobial is made. Research is ongoing, but de-escalation does not appear to adversely impact survival in humans (Leone et al., 2014). A treatment length of seven to ten days is recommended in humans, although the evidence for this advice is considered weak, and more and more, preference is given to the shortest treatment length possible (Rhodes et al., 2017). To achieve this, daily assessment of the possibilities to de-escalate or stop antimicrobial therapy is necessary. The whole of this approach should be part of an antimicrobial stewardship program at the level of the veterinary hospital, practice or farm.

Available formularies in the Netherlands and Belgium suggest sulfonamides-trimethoprim as the primary choice for sepsis in cattle. Secondary choices are broad-spectrum β -lactams (amoxicillin, ampicillin), or the combination of penicillin with an aminoglycoside (neomycine or gentamicin) or dihydrostreptomycin. Tertiary choices consist of the critically important fluoroquinolones and cephalosporins of the third and fourth generation (AMCRA, 2014; KNMVD, 2017). To the authors' knowledge, no randomized clinical trials on the effectiveness of any of these treatments to survive sepsis have been conducted in cattle. Based on the above information and guidelines from human medicine, in severe sepsis or septic shock cases, preference should be given to an intravenous and bactericidal drug in high dosage. Critically important fluo-

roquinolones or cephalosporins comply with this requirements, but this is also the case for sodium amoxicillin/ampicillin (with or without clavulanic acid) or the combination sodium penicillin and gentamicin. It should be remarked that intravenous administration of (amino)-penicillins is off-label antimicrobial use and a cascade application in Belgium.

The focus of this article is on antimicrobial therapy. Patient stabilization and ancillary anti-inflammatory therapy will only be limited to what is achievable in the field and evidenced in cattle. In severe sepsis or septic shock, fluid therapy with isotonic saline is recommended at 30 mL/kg bodyweight in 5-20 minutes to sustain blood pressure (Dellinger et al., 2017). Glucose administration is controversial but common practice in pediatric medicine, and is likely needed in the hypoglycemic calf. As to the use of anti-inflammatory drugs, corticosteroids are controversial in human medicine. The advice of the 'surviving sepsis campaign' is against their use in sepsis or septic shock responding on fluid therapy. If fluid therapy and vasopressors fail to restore blood pressure, corticosteroid administration is suggested (Rhodes et al., 2017). Surprisingly and totally in contrast to animal models where non-steroidal inflammatory drugs (NSAIDs) (cyclooxygenase inhibitors) display beneficial effects for mortality, human studies have failed to demonstrate clinical utility of NSAIDs in sepsis treatment (Aronoff, 2012). To the authors' knowledge, for the use of NSAIDs for sepsis in cattle, no randomized clinical trials are available neither. Work on experimental endotoxemia in calves has shown no effects of corticosteroids, whereas ketoprofen completely alleviated all symptoms (Plessers et al., 2012; Plessers et al., 2016).

PREVENTION

The optimal method to avoid antimicrobial use for sepsis is of course to prevent septicemia from happening. Sepsis in large animals is largely unexplored, and to the authors' knowledge, no substantial epidemiological studies on risk factors for septicemia are available. Avoiding failure of passive transfer by effective colostrum management is to date the most accessible preventive measure that can be taken (Lofstedt et al., 1999). In addition, attention should be paid to the adequate hygiene of colostrum delivery materials. In human medicine, the importance of adequate hygiene in catheter placement or injection procedures in sepsis prevention has been made clear. It is also important to realize that in certain high risk situations in humans, like group B streptococcal infections at parturition, preventive antimicrobial use is considered rational. To date, no evidence for such indications in food animal medicine is available, and preventive antimicrobial use should be discouraged at all times.

CONCLUDING REMARKS

Sepsis is likely a seriously underestimated problem in cattle, previously masked by empirical, broad-spectrum antimicrobial therapy and limited diagnostic efforts. Dealing with sepsis already is a true challenge for the bovine practitioner, and has become more complicated in face of the new legislation. Severe sepsis and septic shock are a typical indication (if not the only one) where the argument of 'urgency', as mentioned in the legislation, appears justified. Adequate sampling to obtain a susceptibility test result is perfectly possible, either by blood culture or alternatively by sampling the local focus of infection (e.g. lung or udder). However, training to improve sample quality might be necessary and easy access to the required laboratory technology might not be covered in the whole of the country. Study priorities in this area are the identification of clinical and biochemical predictors of the different grades of sepsis to provide a clear definition of what is considered 'urgent', and randomized clinical trials comparing different antimicrobial regimens and evidencing the benefit and practical achievability of de-escalation procedures in bovine practice. In order to safeguard their efficacy for human and veterinary medicine, fluoroquinolone and cephalosporin use for animals should be highly exceptional.

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