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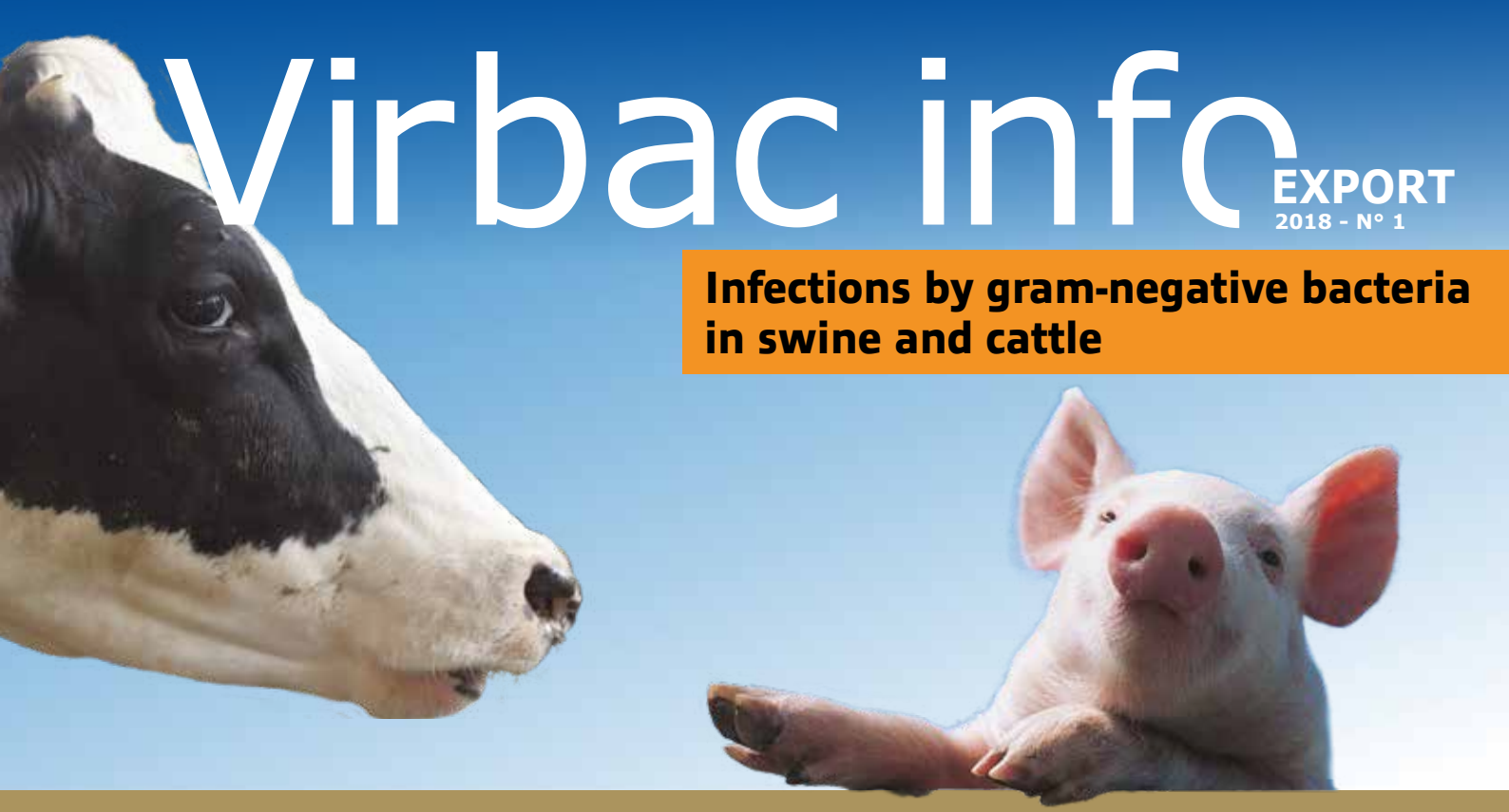
Infections by gram-negative bacteria in swine and cattle



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Counteracting the effects of endotoxin is an important objective for antibiotic treatments against diseases caused by gram-negative pathogens in swine and cattle

Infections by gram-negative bacteria and possible complications with endotoxemia are a common cause of mortality, morbidity and economic damage in cattle and swine. The pathogenic effects of these widespread bacteria are linked to their multiplication in the infected animal body in some circumstances but also to the intense host inflammatory response against a major component of the gram-negative bacteria: endotoxin.
Any antibiotic treatment aims to stop bacterial infection, but should also ideally counteract the effects of endotoxin, as released by gram-negative bacteria during the course of infection or upon the lysis of bacteria, as may happen under bactericidal therapy.

Bacteriostatic versus bactericidal activity:

Antimicrobials may be bacteriostatic (i.e., they inhibit microorganism growth) or bactericidal (i.e., they kill microorganisms). This distinction is partly dependent on the antimicrobial site concentration and duration of contact with the target bacterium, the organism involved, and whether the drug is used in combination with other antimicrobials. As an example macrolides tend to be primarily bacteriostatic, but may be bactericidal against some pathogens. Generally, antimicrobials can be classified as below:

- Bacteriostatic: tetracyclines, macrolides, sulfonamides, trimethoprim
- Bactericidal: beta-lactams, fluoroquinolones, aminoglycosides

What is endotoxin?

Endotoxins, also known as lipopolysaccharides (LPS), are structural parts of the outer membrane of the cell wall of gram-negative bacteria like *Enterobacteriaceae* (coliforms like *Escherichia coli*), or *Pasteurellaceae*, and in general comprise three major regions: the side chain, the core polysaccharides and lipid A (Figure 1, published in Andersen 2003).

The side chain differs widely between gram-negative bacterial strains and is therefore used for serological typing of gram-negative bacteria. Alternatively, lipid A is a highly conserved structure of the endotoxin molecule and is responsible for most of the toxic activity of LPS (Andersen 2003). During bacterial growth in culture, LPS is continuously shed (Prins *et al.* 1994). This phenomenon has been observed in, among other organisms, *Escherichia coli* and other members of the family Enterobacteriaceae (Eng *et al.* 1993, Ishiguro *et al.* 1986, Mattsby-Baltzer *et al.* 1991).

The risk of LPS release is particularly high upon lysis of the gram-negative bacteria during infection, or as a consequence of a bactericidal therapy.

Pathogenicity of endotoxin in bacterial infections

LPS is one of the most potent microbial virulence factors in the pathogenesis of localized and systemic inflammation caused by gram-negative bacteria. As shown in humans and animals, especially ruminants and pigs, the *in vivo* pathogenicity of endotoxin seems to be mostly linked to the release by host of multiple pro-inflammatory cytokines (IL1, IL6, TNF-α…) that are responsible of the "septic shock" or "endotoxic shock" syndrome, leading to a frequent lethal issue (Angus *et al.* 2013, Burvenich *et al.* 2003, Zimmerman *et al.* 2012) (Figure 2).

Endotoxin interacts with cells and molecules of inflammation, immunity and haemostasis. Fever is induced by interleukin-1 (IL1), produced by the liver in response to endotoxin, acting on the temperature-regulating hypothalamus. The action of LPS on platelets and activation of Hageman's factor causes disseminated intravascular coagulation with ensuing ischaemic tissue damage to various organs. Septic shock occurs during severe infections with gram-negative organisms when bacteria or LPS enter the bloodstream and create "endotoxemia".

Therefore, LPS that enters the bloodstream may create a risk of endotoxemia in animals infected by gram-negative bacteria.

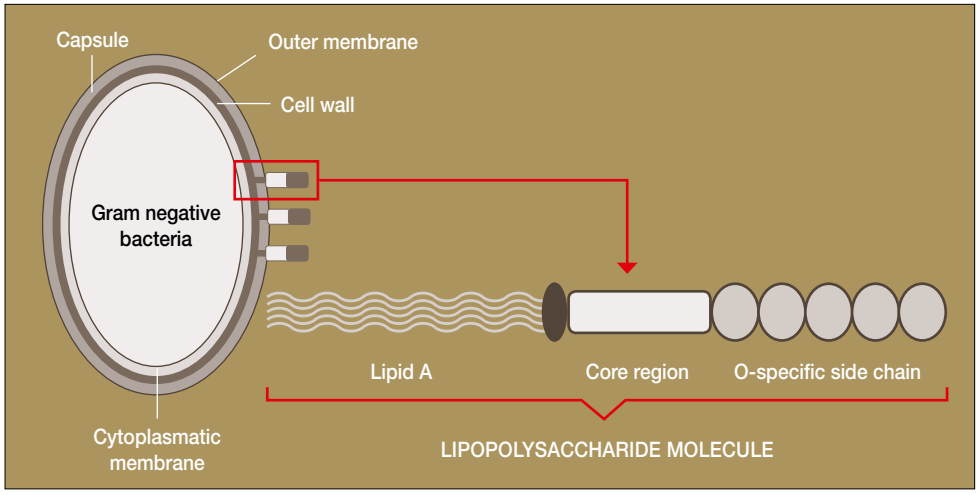

Figure 1: Endotoxins (s. lipopolysaccharides) are structural parts of the bacterial cell wall and in general comprise 3 major regions: the side chain, the core polysaccharides and lipid A (Modified from Rietschel 1996).

Infections associated with a potential risk of endotoxemia in cattle

Calf diseases: neonatal calves deprived of adequate amounts of colostrum are among the most susceptible targets to gram-negative bacterial infections. Even among calves with adequate passive immunity, gram-negative bacterial sepsis remains a substantial problem (Gerros *et al.* 1995). *Escherichia coli* (gram-negative bacterium and part of the normal digestive microflora) is one of the most common causes of diarrhoea and septicemia in calves, affecting dairy as well as beef production (Kolenda *et al.* 2015). Calf scours are the dominant disease in the first month of age, especially in the first two weeks; *Escherichia coli* has been estimated responsible for 51% to 75% total losses during the neonatal period in dairy and cow-calf farms in a US model based on field incidence data (House 1978). Commensalic *E. coli* is a common part of the intestinal microbiota of mammals. Only a small fraction of the *E. coli* population is pathogenic due to virulence mechanisms (intestinal pathogenic *E. coli* or septicemic *E. coli*) or by occurrence and multiplication of *E. coli* in organs and tissues which are sterile in healthy hosts. As an example, navel infection is a potential risk in neonate calves and may turn into septicemia and endotoxemia if hygiene is poorly respected and infection remains untreated.

Septicemic strains of *Escherichia coli* can be isolated from invasive field cases of colibacillosis in calves and show various virulence factors like adhesins that enable the attachment of the bacterium to the intestinal epithelium (F17, CS31A) and specialized toxins (alpha-hemolysin, cytotoxic necrotizing factor etc…).

Enterotoxigenic *E. coli* (ETEC) is the main causative agent of neonatal calf diarrhoea. ETEC are characterized by the presence of specific adhesins and release of exotoxins (enterotoxins), besides LPS that is present in the ETEC cell wall. Therefore LPS that can enter the bloodstream from the gut may create a risk of endotoxemia.

A general antimicrobial therapy in calves is indicated to treat diarrhoea associated to septicemia, to other infections or to immune deficiency and in calves under one week of age. An early therapy is recommended to avoid a massive lysis of bacteria under bactericidal treatment.

Cow mastitis: acute coliform mastitis is a common and usually fatal disease in lactating dairy cows. Endotoxemia and disseminated intravascular coagulation in cows with acute *Escherichia coli* mastitis are generally

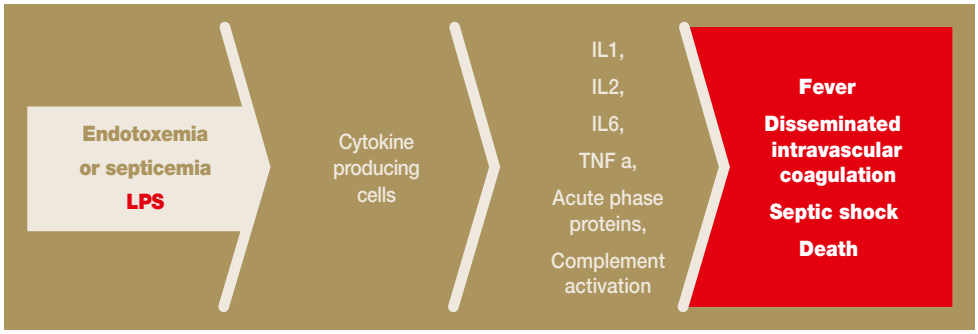

Figure 2: Pathogenicity of endotoxemia in gram-negative infections

recognized as the causes of mortality. In severe *E. coli* mastitis, impaired hepatic functions of dairy cows could lead to difficulty in LPS detoxification and to decreased antithrombin synthesis, putting them at high risk of death (Hagiwara *et al.* 2014). Tumor necrosis factor (TNF) concentrations in plasma, as a consequence of infection and endotoxin release, are associated closely with the manifestation of peracute signs of coliform mastitis and are important factors contributing to morbidity and mortality of endotoxic shock (Sordillo *et al.* 1992).

Bacteremia has been reported to occur in 32% (Wenz *et al.* 2001) to 75% (Katholm *et al.* 1992) of cows with naturally occurring coliform mastitis. There should be a high suspicion of bacteremia in cows with severe systemic disease signs; consequently parenteral antimicrobial therapy may be indicated in such cases.

Endotoxemia-related diseases in swine

Classically perceived as a part of the **mastitis-metritis-agalactia (MMA)** complex, **postpartum dysgalactia syndrome (PPDS)** is a major cause of neonatal problems in swine with multiple manifestations and aetiologies.

Systemic signs of disease in sows, such as fever and anorexia, are widespread, often associated with constipation and depression. The infected mammary glands show typical signs of inflammation, such as severe oedema and skin congestion. The main adverse economic effect is high pre-weaning piglet mortality mainly due to underfeeding, with possible ingestion by piglets of LPS originating from maternal infection. Bacteria most commonly isolated from affected sows are coliforms and the predominant role of these organisms in mastitis of sows has been demonstrated by several infection experiments. Hence, to avoid the confusing terminology and to point out the parallels to coliform mastitis in cows, the term coliform mastitis is commonly used for peripartal mastitis in sows (Gerjets *et al.* 2009).

Evidence suggests that endotoxin plays a central role. Bacterial endotoxin can be absorbed from the uterus (from endometritis or metritis), mammary glands (acute mastitis), or gut (constipation as a consequence of feeding finely ground feed to sows may result in bacterial overgrowth and absorption of endotoxin from the intestines) and lead to endotoxemia. Endotoxin exerts multiple effects even before farrowing and may adversely affect production and secretion of colostrum and milk. Precisely, endotoxin suppresses the secretion of prolactin (hormon for milk production) which leads to a depression in milk. In a field study, endotoxin was detected in the blood from 32.5% sows clinically affected by coliform mastitis (Pejsak *et al.* 1989) and sampled between 24 and 72 hours post-partum.

Risk factors are those associated with stress of the sow and with conditions that lead to bacterial multiplication and subsequent endotoxemia.

Treatment with antibiotics and anti-inflammatory drugs aims to stop bacterial infection and counteract the effects of endotoxins; it should preferably include a broad-spectrum antibiotic or antibiotic association. Nutrition management is a useful tool to minimize the risk of coliform mastitis.

■ **Postweaning diarrhea (PWD):** *Escherichia coli* is one of the most important causes of postweaning diarrhoea in piglets. This diarrhoea is responsible for economic losses due to mortality, morbidity, decreased growth rate and cost of medication. *Escherichia coli* postweaning diarrhoea, also called postweaning enteric colibacillosis, is an important cause of death in weaned pigs. Enteric *E. coli* infection in weaned piglets may also manifest as a diarrhoea which usually occurs during the first week of post-weaning and often results in decreased weight gain. PWD due to E. *coli* is caused primarily by ETEC, a pathotype that is characterized by production of adhesins that mediate bacterial adherence to the intestine and enterotoxins (heat-stable and heat-labile enterotoxin) that cause diarrhoea. During the development of *E. coli* postweaning diarrhoea in pigs, death is generally due to dehydration. However, endotoxin may be absorbed from the intestine or may be released from bacteria that are in the bloodstream, and septicemia and/or endotoxemia may lead to death in the absence of severe dehydration (Fairbrother *et al.* 2005).

Goal of antibiotic treatments against gram-negative bacterial pathogens

The course of diseases induced by proliferation of gram-negative bacteria may be influenced by the pathogenic activity of various bacterial toxins, especially endotoxin.

Therefore, beyond the susceptibility of target bacteria, any antibiotic treatment should ideally induce not only the eradication of the bacterial infection, but also a decrease of endotoxin concentrations in the treated animal. The administration of some antibiotics, especially bactericidal ones (e.g. beta-lactams, fluoroquinolones…) may increase the risk for endotoxin release because of bacterial cell lysis, unless these antibiotics can neutralize endotoxin. This risk should be taken into consideration when treating animals infected by gram-negative pathogens in clinical cases possibly associated to endotoxemia.

Beyond antimicrobial efficacy: effects of antibiotics on endotoxin

The prototype endotoxin-binding antibiotic is polymyxin B. Polymyxin B specifically binds to lipid A, and after binding, LPS particles of bacteria are disintegrated into small fragments (Prins *et al.* 1994). Polymixins are a group of antibiotic peptides produced by *Bacillus polymixa*. Among polymyxins only polymixin B (human use) and polymixin E (colistin) have therapeutic indications.

Colistin binding to LPS has been well documented (Warren *et al.* 1985), with induced reduction of the release of inflammatory cytokines and blocking of the biological activity of theses cytokines. The neutralizing activity of colistin on bacterial endotoxin has been confirmed by *in vivo* challenge trials (Giacometti *et al.* 2003, Dosogne *et al.* 2002) and veterinary clinical studies in naturally occurring cases of septic shock (Senturk 2005); these experiments have demonstrated the ability of colistin to significantly decrease the inflammatory cytokine response to LPS, especially TNF-α, and to alleviate some connected clinical signs (Senturk 2005) as well as lethality in a rat model (Giacometti *et al.* 2003).

Gentamicin and some other aminoglycosides can also bind endotoxin and partially neutralize the effects of endotoxin but their neutralizing effects are less than polymixins. Probably because of their endotoxin-neutralizing abilities, the addition of aminoglycosides to beta-lactams reduced the amount of endotoxin measured compared with that measured after monotherapy with these beta-lactams (Prins *et al.* 1994).

A research project was conducted by scientists from the University of Lublin (Poland), in cooperation with Virbac, to document the neutralizing effect on endotoxin of a combination of polymixin E (colistin) with amoxicillin in an *in-vitro* study mimicking natural infection and antibiotic treatment in calves.

In this recently published study, the amoxicillin/colistin combination was shown

to afford at peak serum concentration a high LPS neutralizing activity as compared to single compounds (colistin, amoxicillin) and to a fluoroquinolone (ciprofloxacin, metabolite from enrofloxacin accounting for 40% enrofloxacin injected dose in treated ruminants), especially after a 24 hour pre-incubation of cells with LPS (Szuster-Ciesielska et al. 2017). In field conditions, animal treatment is usually delayed by 6-24 hours considering the onset of clinical symptoms and detection by the farmer; the duration of contact of LPS with cells prior to treatment in study conditions (6, 12 or 24 hours) could therefore be considered as mimicking an early antibiotic therapy in infected calves (Figures 3-4).

As a conclusion, besides their bactericidal activity, broad antimicrobial spectrum and therapeutic indications, associations of colistin with beta-lactams as amoxicillin (Potencil®) or ampicillin (Multibio®) could be of interest to limit the harmful effects of endotoxin in treated animals.

Interest of dexamethasone in controlling the effects of endotoxin

As a complement to antimicrobial therapy, an anti-inflammatory treatment may be indicated to provide relief from the inflammation triggered by acute infections and stimulated by endotoxin, as in cow coliform mastitis or PPDS in sows. As a potent inhibitor of interleukin-1 (IL1), dexamethasone was proven to specifically modulate the IL-1 related *in-vivo* effects of endotoxin in a mice challenge model (Bertini *et al.* 1989).

A single intramuscular injection of a combination product containing ampicillin, colistin and dexamethasone (Multibio®) to dairy cows experimentally challenged with endotoxin neutralized some of the most severe early systemic reactions to inflammation (Ziv *et al.* 1998).

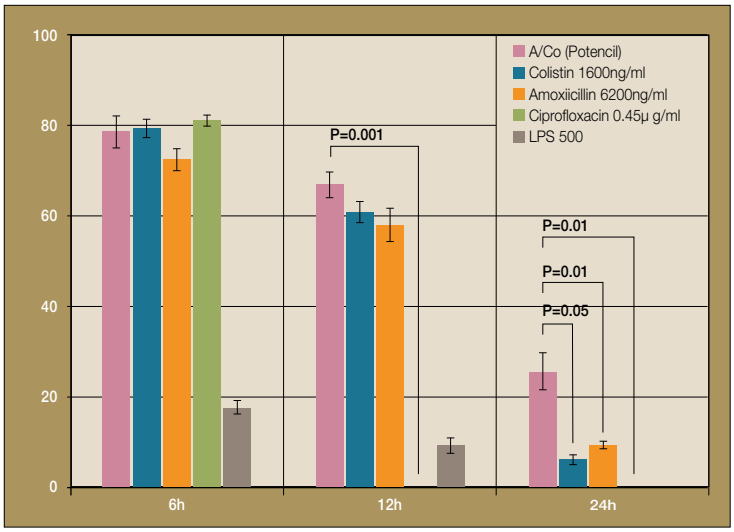

Fig. 3: Comparison of the neutralizing effect of 500µg/ml of LPS by different antibiotics or association of antibiotics (LDH method) (Szuster-Ciesielska et al. 2017)

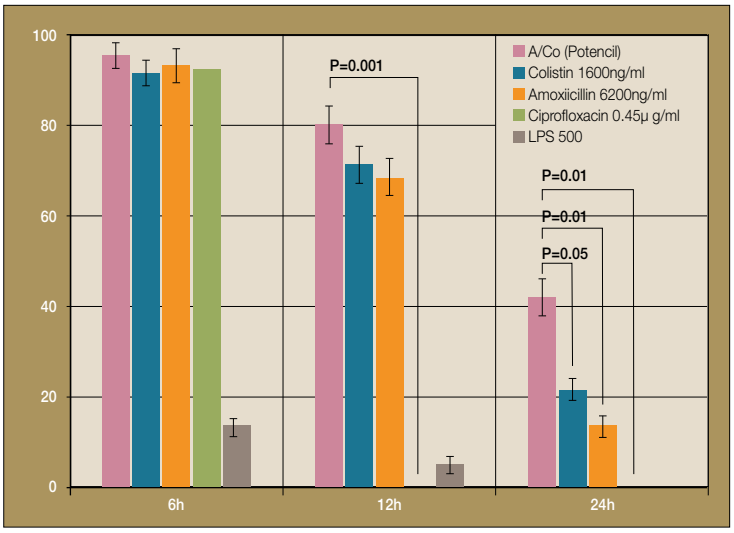

Fig. 4: Comparison of the neutralizing effect of 500µg/ml of LPS by different antibiotics or association of antibiotics (MTT method) (Szuster-Ciesielska et al. 2017)

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